ÁLLATTENYÉSZTÉS TAKARMÁNYOZÁS

1

CONTENTS — TARTALOM

Preface (Előszó) 1
List of papers (Az előadások jegyzéke) 4
A magyarnyelvű összefoglalók jegyzéke (List of abstracts in Hungarian)... 7
Papers in English (Előadások angolul) 10
Összefoglalók magyar nyelven (Summaries in Hungarian) 153

Szemle

„100 éves az állami gyapjúminősítés". Az Országos Mezőgazdasági Minősítő Intézet jubileumi emlékülése. („The 100 years of wool classification in Hungary". Anniversary Meeting) 189

13th EUROPEAN COLLOQUIUM ON CYTOGENETICS OF DOMESTIC ANIMALS

The first European Colloquiums on Cytogenetics of Domestic Animals was organized by Professor *G.W. Rieck* in 1970 and 1975 in Giessen. Since 1980 we continue to see each other every second year in different countries and our Colloquium became international.

The 13th Colloquium was organized by the Hungarian Academy of Sciences, the Research Institute for Animal Breeding and Nutrition, the Institute for Small Animal Research and was held in the Assembly of the headquarters of the Hungarian Academy of Sciences on 02–05 June, 1998.

The main topic of the Colloquium was *gene mapping and its application.* There were 72 participants mostly from Europe, but also from Algeria, Iran, Japan, Mongolia, Thailand and USA. There were 58 lectures, and the poster exhibitors got also the possibility for short oral presentations. Optical companies *Nikon* and *Opton* demonstrated their products during the meeting.

The material of the lectures and posters (edited but not corrected) together with abstracts in Hungarian are published in this scientific journal. We were waiting for the manuscripts until the end of January 1999 but as there are manuscripts still missing, we are sorry to publish the summaries only.

The 14th European Colloquium will be organized in 2000 by *Jiri Rubes* in Brno (Czech Republic).

The Organization Committee would like to express their thanks to the sponsors for their kind help, to Ms. *Klara Biszkup* for the technical organization and to *Dr. Andras Kovacs and Dr. Andras Hidas* for getting the abstracts ready in Hungarian.

13. EURÓPAI HÁZIÁLLAT CITOGENETIKAI KOLLOKVIUM

Az első Európai Háziállat-Citogenetikai Kollokviumokat, 1970-ben és 1975-ben, *Rieck, G.W.* professzor szervezte Giessenben. 1980. óta, különböző országokban, kétévenként találkozunk és kollokviumaink nemzetközivé váltak.

A 13. Kollokviumot a Magyar Tudományos Akadémia, az Állattenyésztési és Takarmányozási Kutatóintézet és a Kisállattenyésztési Kutatóintézet közösen rendezték, 1998. június 2–5-én, Budapesten, az MTA Dísztermében.

A fő téma a géntérképezés és annak alkalmazása volt. A 72 résztvevő többsége Európai volt, de Algériából, az USA-ból, Iránból, Japánból, Mongóliából és Thaiföldről is érkeztek vendégek. 58 előadás hagzott el, a poszterkiállítók is lehetőséget kaptak rövid előadás tartására. A Nikon és Opton optikai cégek a kollokvium alatt bemutatót tartottak.

Az előadások és poszterek anyagát, nyelvi és szakmai lektorálás nélkül, magyar összefoglalókkal kiegészítve közöljük. Január végéig vártunk a kéziratokra, ha ezek addig sem érkeztek meg, csak az összefoglalókat közöljük.

14. Európai Kollokviumot, *Jiri Rubes* rendezi, 2000-ben, Brnoban.

A szervező bizottság köszönetet mond Biszkupné Nánási Klárának a szervezésért és szponzorainak az anyagi támogatásért, továbbá Kovács András és Hidas András uraknak a magyar nyelvű referálások elkészítéséért.

International Scientific Committee / Nemzetközi Tudományos bizottság

Chairman / Elnök	*Ingemar Gustavsson* (Sweden)
Secretary / Titkár	*András Hidas* (Hungary)
Members / Tagok	*Victoria Arruga* (Sain)
	Anneke Bosma (The Netherlands)
	Leopodo Iannuzzi (Italy)
	András Kovács (Hungary)
	Manfred Schwerin (Germany)
	Gerald Stranzinger (Switzerland)
	Marek Switonski (Poland)

Hungarian Scientific Committee / Magyar Tudományos Bizottság

Chairman / Elnök	*Ferenc Kovács*
Co-chairman / Társelnökök	*László Fésüs*
	István Szalay
Members / Tagok	*András Hidas*
	András Kovács

Organizing Committee / Szervezőbizottság

Chairman / Elnök	*János Dohy*
Co-chairman / Társelnökök	*János Gundel*
	András Hidas
	András Kovács
	Sándor Kukovics
Secretary / Titkár	*Mrs. Klára Biszkup*

Sponsors / Szponzorok

Carl Zeiss Opton (Budapest)
FAO Subregional Office for Central and Eastern Europe (Budapest)
Hungarian Academy of Sciences (Budapest)
Hungarian Artificial Insemination Corporation (Gödöllő)
National Committee for Technological Development (Budapest)
Optoteam GMBH (Vienna – Budapest)
Mr. Dezső Szomor, Rancher of Hungarian Grey cattle (Apajpuszta)
Hungarian Journal of Animal Production (Herceghalom)

OF THE COLLOQUIUM

Budapest, 02–05 June, 1998

LIST OF PARERS

Gustavsson, I.: Time for revival of clinical cytogenetics? 10
Stranzinger, G.F.: The years after Nate Fechheimer 15
Iannuzzi, I.: An easy way to resolve bovid chromosome nomenclature inconsistencies 19
Schwerin, M. — Goldammer, T. — Brunner, R.M.: Mapping of differentially expressed cDNA sequences to bovine metaphase chromosomes by FISH 20
Brunner, R.M. — Fürbass, R. — Goldammer, T. — Vanselow, J. — Schwerin, M.: Fiber-FISH as a tool for resolving genomic structures as demonstrated with the bovine *CYP19* locus 24
Goldammer, T. — Hess, M. — Brunner, R.M. — Bishop, M.D. — Gelhaus, A. — Schwerin, M.: Refine physical mapping of the bovine major histocompatibility complex (MHC) on *Bos taurus* chromosome BTA 23 29
Joerg, H. — Rieder, S. — Suwattana, D. — Stranzinger, G.: Molecular analysis of Robertsonian translocations 33
Lipsi, M.R. — Antonacci, R. — Massari, S. — De Caro, F .— Miccoli, M. — Ciccarese, S Molecular analysis and comparative FISH-mapping of TCR genes *in bovidae* 36
Iannuzzi, L. — Di Meo, G.P. — Perucatti, A. — Bardaro, T. — Ferrara, L.: ZOO-FISH mapping and R-banding revealed an extensive conservation of human chromosome regions in river buffalo chromosomes 40
Lahbib-Mansais, Y. — Dalias, G. — Milan, D. — Yerle, M. — Robic, A.. — Gyapay, G. — Gellin, J.: Use of human ESTs for mammalian comparative mapping: 65 human ESTs localized in pig 41
Yerle, M. — Pinton, P. — Robic, A. — Hawken, R. — Alexander, L. — Beattie, C. — Schook, L. — Milan, D. — Gellin, J.: Generation of a porcine whole-genome radiation hybrid panel: a new tool for mapping the pig genome 43
Musilova, P. — Kubickova, S. — Vozdova, M. — Rubes, J.: An alternative detection method for labeled DNA probes in porcine gene mapping by *in situ* hybridization 47
Komisarek, J. — Szydlowski, M. — Switonski, M. — Kuryl, J.: The use of chromosomal markers for the identification of QTLs controlling fattening, and carcass and meat quality traits in pigs 51
Goldammer, T. — Brunner, R.M — Kühn. C. — Weikard, R. — Schwerin, M.: Bovine chromosome fragment specific libraries — tool for the generation of region specific high density marker maps 52
Iannuzzi, L. — Palomba, R. — Di Meo, G.P. — Perucatti, A. — Ferrara, L.: Chromosomal localization of the prion protein gene (PRNP) in cattle, river buffalo, sheep and goat by FISH and R-banding 56
Castiglioni, B. — Comincini, S. — Drisaldi, B. — Hills, D. — Williams, J.L. — Ferretti, L.: Characterization of the prion gene (PRNP) locus in cattle, sheep and man by physical mapping 57
Lopez-Corrales, N.L. — Sonstegard, T.S — Smith, T.P.L.: Comparative physical mapping of PROC, EN1, ALPI, TNP1 and IL1B genes in cattle and sheep 58
Tabet-Aoul, K. — Saidi-Mehtar, N. — Lantier, I. — Vaiman, D. — Lantier, F.: Sheep gene mapping using somatic cell hybrids: characterization of ovine chromosomes 4, 5 and 6 62
Zijlstra, C. — Korstanje, R. — de Haan, N.A. — van Lith, H.A. — van Zutphen, L.F.M. — Bosma, A.A.: Localization of 18S+28S rDNA in the rabbit (*Oryctolagus cuniculus*) by FISH and an update of the cytogenetic map of this species 63
Lopez-Corrales, N.L. — Mungall, C. — McCarthy, L. — McDowall, S. — Goodfellon, P.N. — Archibald, A.L.: A radiation hybrid map of pig chromosome 4 65
Muramatsu, M. — Miyazaki, H. — Muramatsu, S. — Yoshizawa, M. — Fukui, E.: Spontaneous frequencies of sister chromatid exchanges in the horse 66
Pienkowska, A. — Switonski, M.: Chromosome banding studies of raccoon dog (*Nyctereutes procyonoides procyonoides*) 68
Rabsztyn, A. — Jaszczak, K. — Jaszczak, J. — Kapkowska, E.: Inheritance of two morphological forms of chromosome 4 in Zatorska geese 72

Fillon, V. — Pitel, F. — Morisson, M. — Pouzadoux, A. — Bergé, R. — Zoorob, R. — Auffray, C. — Gellin, J. — Vignal, A.: New markers for the integration of chicken microchromosome cytogenetic and genetic maps ... 75
Hidas, A.: Molecular cytogenetic studies in domestic goose ... 78
Hidas, A. — Várkonyi, E. — Liptói, K. — Sayahzadeh, H. — Lennert, L. — Szalay, I.: Recurrent trisomies in chicken embryos ... 80
Liptói, K. — Hidas, A. — Szalay, I.: Investigation of chromosomal and embryonic abnormalities in early dead goose embryos ... 82
Saz, J. — Arruga, M.V. — Tejedor, M.T. — Villarroel, M. — Savva, D.: Genetic differentiation in *Alectoris rufa* and *A. graeca* from Spain ... 86
Liptói, K. — Újvári, B. — Korsós, Z. — Hidas, A.: Investigation of the karyotype of Vipera ursinii rakosiensis ... 90
Ráczkevi-Radics, J.— Radics, F.: Spontaneous triploidy in the African catfish *Clarias gariepinus* (Pisces: Clariidae) ... 92
Várkonyi, E. — Horváth, L. — Ozouf-Costaz, C. — Billard, R.: Connection between overmaturation of the eggs and chromosomal abnormalities in *Silurus glanis L.* ... 93
Váradi, L. — Tóth, B.: Chromosome researches on Silver crucian carp (*Carassius auratus gibelio)* ... 98
Pardo, B.G. — Bouza, C. — Castro, P. — Martínez, J. — Sánchez, L.: Molecular cytogenetics of flatfishes (Pleuronectidae, Scophthalmidae, Soleidae) ... 101
Hidas, A. — Várkonyi, E.: Application of genomic *in situ* hibridization (GISH) in the identification of fish hybrids ... 101
Switonski, M. — Ładon, D. — Schelling, C. — Schlapfer, J. — Stranzinger, G. — Dolf, G.: Physical mapping of microsatellite markers on the dog (*Canis familiaris*) chromosomes ... 103
Breen, M. — Reimann, N. — Bosma, A.A. — Ładon, D. — Zijlstra, C. —Bartnitzke, S. — Switonski, M. — Long, S.E. — de Haan, N.A. — Binns, M.M. — Bullerdiek, J. — Langford, C.F.: Standardization of chromosomes nos. 22–38 of the dog *(Canis familiaris)* with the use of canine painting probes. Committee for the Standardized Karyotype of the Dog *(Canis familiaris)* ... 104
Ładon, D. — Switonski, M. — Schelling, C. — Schlapfer, J. — Dolf, G.: Physical localization of the canine microsatellite markers on the silver fox *(Vulpes fulvus desm.)* chromosomes. 106
Ciccarese, S. — D'Aiuto, L. — Cserpán, I. — Vonghia, G. — Barsanti, P.: A sequence containing a junction between sheep satellite I and II DNA mediates isochromosome and microchromosome formation in hamster cells ... 107
Tumennasan, Kh. — Tuya, Ts. — Tsendtsesmee, L. — Hotta, Y. — Takase, H. — Speed, R.M. — Chandley, A.C.: Cytogenetic cause of sterility on the hybrids between yak (*Bos grunniens*) and cattle (*Bos taurus*) ... 108
Rejduch, B. — Jazdzewski, J. — Slota, E. — Kozubska-Sobocinska, A. — Danielak-Czech, B.: Evaluation of reproductive performance on the basis of semen analysis in the bulls carrying 60,XX/60,XY chimerism ... 110
Słota, E. — Rejduch, B. — Kwaczynska, A. — Kozubska-Sobocinska, A. — Danielak-Czech, B.: The level of XX;XY lymphocyte population in chimeric bulls ... 111
Mäkinen, A. — Katila, T. — Mäkilä, M.: Sex chromosome analysis as a tool in reproductive studies in mares ... 114
Házas, G. — Kovács, A. — Karakas, P.: Decrease of percentage of carriers of bovine hereditary chromosome abnormalities ... 115
Llambí, S. — Guevara, K. — Rincón, G. — Nunez, R. — Arruga, M.V. — Postiglioni, A.: Aphidicolin-induced fragile sites in *Bos taurus* lymphocyte cultures. (A preliminary study) .. 117
Switonski, M. — Pienkowska, A. — Golinski, P. — Bukowska, D. — Bereszynski, A.: X trisomy in the dog — a case report ... 121
Kovács, B.Zs. — Switonski, M. — Went, D. — Stranzinger, G.: The event of meiotic synapsis in female rabbits ... 121
Christensen, K. — Bruusgaard, K.: Incidence of disomy in mink sperm by strong centromere repeat probes for the chromosome 2, 5, 8, 9, 11 and Y ... 125
Hassanane, M. — Kovács, A. — Laurent, P. — Lindblad, K. — Gustavsson, I.: Simultaneous detection of X- and Y- bearing bull spermatozoa by double colour fluorescence *in situ* hybridization ... 128
Tardy, E.P. — Szalai, G. — Gustavsson, I. — Hassanane, M. — Lindblad, K. — Kovács, A. — Házas, G. — Tóth, A. — Dohy, J.: First results of Sexing bovine spermatozoa by FISH in Hungary ... 128

Rieder, S. — Checa-Cortes, M.L. — Stricker, Ch. — Joerg, H. — Meijerink, E. — Stranzinger, G.: Coat colour and complex traits: a genetic approach to melanoma in grey horses ... 130
Mayr, B. — Reifinger, M. — Alton, K. — Jugl, M. — Brem, G.: Heterogeneous cytogenetic findings in feline fibrosarcomas ... 134
Rubes, J. — Vozdová, M. — Kubícková, S.: Analysis of numerical chromosome abnormalities in boar sperm by means of FISH ... 138
Lechniak, D — Switonski, M.: The incidence of aneuploidy in bovine oocytes matured *in vitro* ... 142
Sosnowski, J. — Waroczyk, M. — Lechniak, D. — Switonski, M.: High incidence of diploid secondary oocytes matured *in vitro* in the pig ... 143
Lechniak, D. — Long, S.E. — Nissen, A.K.: Growth hormone polymorphism (RFLP) in day-10 pig embryos ... 144
Mosse, I.B. — Plotnikova, S.I. — Kostrova, L.N. — Dubovic, B.V. — Molophei, V.P.: Melanin influence on chronic and fractionated irradiation in germ and somatic cells of mice 145
Tap, O.T. — Rutteman, G.R. — Zijlstra, C. — de Haan, N.A. — Bosma, A.A.: Chromosome abnormalities in a canine mammary carcinoma cell line analyzed using canine chromosome-specific paints ... 149
Monteagudo, L.V. — Postiglioni, A. — Llambi, S. — Arruga, M.V.: Detection of chromosome breaks and genetic pathology by molecular genetics ... 150

A MAGYAR NYELVŰ ÖSSZEFOGLALÓK

Gustavsson, I.: Eljött az idő a klinikai citogenetika újjáéledésére? ... 153
Stranzinger, G.F.: *Nate Fechheimer* és az őt követő genetikai kutatások korszaka ... 153
Iannuzzi, I.: A szarvasmarha kromoszómális nómenklatúrájában lévő ellentmondások feloldása ... 154
Schwerin, M. — Goldammer, T. — Brunner, R.M.: Eltérő expressziót mutató cDNS szekvenciák *in situ* hibridizációja szarvasmarha kromoszómákon ... 154
Brunner, R.M. — Fürbass, R. — Goldammer, T. — Vanselow, J. — Schwerin, M.: Példa a fellazított kromatinrostokon végzett *in situ* hibridizáció (fiber-FISH) alkalmazására a genom finomszerkezeti vizsgálatában: a marha CYP19 lokusza ... 155
Goldammer, T. — Hess, M. — Brunner, R.M. — Bishop, M.D. — Gelhaus, A. — Schwerin, M.: A szarvasmarha 23. kromoszómáján elhelyezkedő nagy hisztokompatibilitási komplex (MHC) fizikai térképezése ... 155
Joerg, H. — Rieder, S. — Suwattana, D. — Stranzinger, G.: Robertson transzlokációk molekuláris analízise ... 156
Lipsi, M.R. — Antonacci, R. — Massari, S. — De Caro, F .— Miccoli, M. — Ciccarese, S.: Bovidák TCR génjeinek molekuláris analízise és FISH-térképezése ... 156
Iannuzzi, L. — Di Meo, G.P. — Perucatti, A. — Bardaro, T. — Ferrara, L.: Humán kromoszómális régiók nagymérvű konzervációjának kimutatása bivaly kromoszómákon ZOO-FISH térképezéssel és R-sávozással ... 157
Lahbib-Mansais, Y. — Dalias, G. — Milan, D. — Yerle, M. — Robic, A. — Gyapay, G. — Gellin, J.: Humán expresszálódó szekvencia azonosítók (EST-k) alkalmazása a hatékony összehasonlító géntérképezéshez ... 157
Yerle, M. — Pinton, P. — Robic, A. — Hawken, R. — Alexander, L. — Beattie, C. — Schook, L. — Milan, D. — Gellin, J.: Sertés szomatikus sejthibrid panel előállítása besugárzással: a sertés géntérképezés új eszköze ... 158
Musilova, P. — Kubickova, S. — Vozdova, M. — Rubes, J.: A jelzett DNS-próbák felismerésének alternatív módszere az *in situ* hibridizációval végzett sertés-géntérképezésben ... 158
Komisarek, J. — Szydlowski, M. — Switonski, M. — Kuryl, J.: Kromoszómális markerek alkalmazása a sertés hústermelését és a húsminőségét meghatározó QTL-ek (mennyiségi tulajdonságokat meghatározó lokuszok) azonosítására ... 159
Goldammer, T. — Brunner, R.M — Kühn. C. — Weikard, R. — Schwerin, M.: Marha kromoszóma-fragment specifikus könyvtárak — a nagy feloldású fragment-specifikus markertérképek megalkotásának eszközei ... 159
Iannuzzi, L. — Palomba, R. — Di Meo, G.P. — Perucatti, A. — Ferrara, L.:A prion gén (PRNP) kromoszómális lokalizációja szarvasmarha, bivaly, juh és kecske fajokban, fluoreszcens *in situ* hibridizációval és R-sávozással ... 160
Castiglioni, B. — Comincini, S. — Drisaldi, B. — Hills,D. — Williams, J.L. — Ferretti, L.: A prion gén (PRNP) térképezése szarvasmarha, juh és humán kromoszómákon ... 161
Lopez-Corrales, N.L. — Sonstegard, T.S — Smith, T.P.L.: A szarvasmarha és a juh PRO C, EN-1, ALPI, TNP1 és IL-1b génjeinek összehasonlító térképezése ... 161
Tabet-Aoul, K. — Saldi-Mehtar, N. — Lantier, I. — Vaiman, D. — Lantier, F.: A juh géntérképezése szomatikus sejthibridekkel: a 4., 5. és 6. kromoszóma jellemzése. ... 161
Zijlstra, C. — Korstanje, R. — De Haan, N.A. — Van Lith, H.A. — Van Zutphen, L.F.M.— Bosma, A.A.: A 18S+28S rDNS lokalizációja fluoreszcens *in situ* hibridizációval és a házinyúl kromoszómatérképének kiegészítése ... 162
Lopez-Corrales, N.L. — Mungal, C. — Mccarthy, L — Mcdowall, S. — Goodfellow, P.N. — Archibald, A.L.: A sertés 4-es kromoszómájának térképezése besugárzással előállított sejthibrideken ... 162
Muramatsu, M. — Miyazaki, H. — Muramatsu, S. — Yoshizawa, M. — Fukui, E.: A nővér chromatid cserék spontán gyakoriságai lovakban ... 163
Pienkowska, A. — Switonski, M.: Kromoszómasávozási vizsgálatok nyestkutya kariotípuson 163
Rabsztyn, A. — Jaszczak, K. — Jaszczak, J. — Kapkowska, E.: Morfológiailag különböző 4-es kromoszómák öröklődése a zatorska lúd fajtában ... 163
Fillon, V. — Pitel, F. — Morisson, M. — Pouzadoux, A. — Bergé, R. — Zoorob, R. — Auffray, C. — Gellin, J. — Vignal, A.: Új markerek alkalmazása a házityúk fizikai és kapcsoltsági genetikai térképének összekötésére ... 164

Hidas, A.: A házilúd molekuláris citogenetikai vizsgálata 164
Hidas, A. — Várkonyi, E. — Liptói, K. — Sayahzadeh, H. — Lennert, L. — Szalay, I.: Ismétlődő triszómiák csirke embriókban 165
Liptói, K. — Hidas, A. — Szalay, I.: Kromoszómális és embrionális rendellenességek vizsgálata korán elhalt lúd embriókban 165
Saz, J. — Arruga, M.V. — Tejedor, M.T. — Villarroel, M. — Savva, D.: Két szirtifogoly faj (*Alectoris rufa* és *A. graeca*) megkülönböztetése genetikai vizsgálatokkal 166
Liptói, K. — Újvári, B. — Korsós, Z. — Hidas, A.: A Vipera *ursinii rakosiensis* kariotípusának vizsgálata 166
Ráczkevi-Radics, J. — Radics, F.: Spontán triploidia afrikai harcsában (*Clarias gariepinus*) .. 166
Várkonyi, E. — Horváth, L. — Ozouf-Costaz, C. — Billard, R.: A harcsaikra (*Silurus glanis L.*) túlérésének hatása a citogenetikai rendellenességekre 167
Váradi, L. — Tóth, B.: Az ezüstkárász (*Carassius auratus gibelio*) citogenetikai vizsgálata 167
Pardo, B.G. — Bouza, C. — Castro, P. — Martínez, J. — Sánchez, L.: Lepényhalak (*Pleuronectidae, Scopthalmidae, Soleidae*) molekuláris citogenetikája 168
Hidas, A. — Várkonyi, E.: A genomikus *in situ* hibridizáció (GISH) alkalmazása halfajok hibridjeinek azonosítására 168
Switonski, M. — Ładon, D. — Schelling, C. — Schlapfer, J. — Stranzinger, G. — Dolf, G.: Mikroszatellit markerek fizikai térképezése a kutya (*Canis familiaris*) kromoszómáin 169
Breen, M. — Reimann, N. — Bosma, A.A. — Ładon, D.— Zijlstra, C.— Bartnitzke, S. — Switonski, M. — Long, S.E. — de Haan, N.A. — Binns, M.M. — Bullerdiek, J. — Langford, C.F.: A kutya (*Canis familiaris*) 22–38 számú kromoszómáinak standardizálása canin painting próbákkal 169
Ładon, D. — Switonski, M. — Schelling, C. — Schlapfer, J. — Dolf, G.: A kutya mikroszatellita markerek fizikai lokalizációja az ezüstróka (*Vulpes fulvus Desm.*) kromoszómákon 170
Ciccarese, S. — D'Aiuto, L. — Cserpán, I. — Vonghia, G. — Barsanti, P.: Egy juh szatellita I. és II. DNS közötti kapcsolatot tartalmazó szekvencia izokromoszóma és mikrokromoszóma kialakulást támogat hörcsög sejtekben 171
Tumennasan, Kh. — Tuya, Ts. — Tsendtsesmee, L. — Hotta, Y. — Takase, H. — Speed, R.M. — Chandley, A.C.: A ják (*Bos grunniens*) x szarvasmarha (*Bos taurus*) hibridek sterilitásának citogenetikai oka 172
Rejduch, B. — Jazdzewski, J. — Słota, E. — Kozubska-Sobocinska, A. — Danielak-Czech, B.: 60,XX/60,XY kimérizmust hordozó bikák reprodukciós teljesítményének értékelése ondóvizsgálat alapján 173
Słota, E. — Rejduch, B. — Kwaczynska, A. — Kozubska-Sobocinska, A.. — Danielak-Czech, B.: A két limfocita populáció aránya (XX;XY) kiméra bikákban 175
Mäkinen, A. — Katila, T. — Mäkilä, M.: Szex kromoszóma analízis, mint a kancák szaporodási vizsgálatainak eszköze 176
Házas, G. — Kovács, A. — Karakas, P.: Az öröklődő kromoszóma rendellenességeket hordozó szarvasmarhák arányának csökkenése 176
Llambí, S. — Guevara, K. — Rincón, G. — Nunez, R. — Arruga, M.V. — Postiglioni, A.: Aphidicolin által előidézett törési helyek *Bos taurus* limfocita tenyészetekben (Előzetes közlemény) 177
Switonski, M. — Pienkowska, A. — Golinski, P. — Bukowska, D. — Bereszynski, A.: X triszómia kutyában (Esetismertetés) 178
Kovács, B.Zs. — Switonski, M. — Went, D. — Stranzinger, G.: A meiótikus szinapszis lefolyása nőstény nyulakban 178
Christensen, K. — Bruusgaard, K.: A diszómiák gyakoriságának becslése amerikai nyérc spermiumban, a 2, 3, 5, 8, 9, 11 és Y-kromoszómák erős centromér repeat próbájával 179
Hassanane, M. — Kovács, A. — Laurent, P. — Lindblad, K. — Gustavsson, I.: Az X- és Y-kromoszómát hordozó bikaondósejtek egyidejű felismerése kétszínű fluoreszcens *in situ* hibridizációval 180
Tardy, E.P. — Szalai, G. — Gustavsson, I. — Hassanane, M. — Lindblad, K. — Kovács, A. — Házas, G. — Tóth, A. — Dohy, J.: A bikaondósejtek FISH-szexálásának első eredményei Magyarországon 180
Rieder, S. — Checa-Cortes, M.L. — Stricker, Ch. — Joerg, H. — Meijerink, E. — Stranzinger, G.: A ló (*Equus caballus*), mint a rosszindulatú bőrmelanóma genetikai modellje? 181

Rieder, S. — Checa-Cortes, M.L. — Stricker, Ch. — Joerg, H. — Meijerink, E. — Stranzinger, G.: A ló (*Equus caballus*), mint a rosszindulatú bőrmelanóma genetikai modellje? 181
Mayr, B. — Reifinger, M. — Alton, K. — Jugl, M. — Brem, G.: Heterogén citogenetikai leletek macska fibroszarkómákban 182
Rubess, J. — Vozdová, M. — Kubícková, S.: Számbeli kromoszóma rendellenességek analízise sertés-ondósejtben FISH technikával 182
Lechniak, D — Switonski, M.: Az aneuploidia gyakorisága *in vitro* érlelt szarvasmarha petesejtekben 183
Sosnowski, J. — Waroczyk, M. — Lechniak, D. — Switonski, M.: A diploid másodlagos sertés petesejtek gyakorisága *in vitro* érlelés után 184
Lechniak, D. — Long, S.E. — Nissen, A.K.: Növekedési hormon polimorfizmus (RFLP) 10. napos sertés embriókban 184
Mosse, I.B. — Plotnikova, S.I. — Kostrova, L.N. — Dubovic, B.V. — Molophei, V.P.: Lehetőség a krónikus besugárzás citogenetikai hatásainak csökkentésére egérben 186
Tap, O.T. — Rutteman, G.R. — Zijlstra, C. — De Haan, N.A. — Bosma, A.A.: Canin kromoszóma-specifikus painting próbákkal analizált kromoszóma rendellenességek kutya emlőrák sejtvonalban 187
Monteagudo, L.V. — Postiglioni, A. — Llambi, S. — Arruga, M.V.: Kromoszóma törés és genetikai kór felismerése molekuláris genetikával 188

TIME FOR REVIVAL OF CLINICAL CYTOGENETICS?

GUSTAVSSON, I.

Lately, we have seen a fast development of physical gene mapping in domestic animals. The main physical maps have been established for the most important species. The progress can be ascribed to intemational cooperation, availability of efficient techniques and possibility to avoid those mistakes which were made in the physical mapping of the human genome. The next future will probably concern high resolution maps as well as application of microdissection techniques for establishment of gene libraries in the search for candidate genes of animal diseases, production traits and so on. Molecular cytogenetics has given us new tools, which also can be used for other purposes, and personally I think it is now urgent to apply available techniques for further development of clinical cytogenetics. It is definitely time for a revival of clinical cytogenetics in veterinary medicine!

Some historical notes: When we started with domestic animal cytogenetics in the early sixties, we hoped to find associations between chromosome aberrations and animal disease. At that time the observations of trisomy 21 causing Down's syndrome, the sex chromosome aberrations XXY and XO causing Klinefelter's and Turner's syndrome, respectively, were described in human cytogenetics. Syndromes caused by sex chromosome aberrations such as XXY in cat (*Thuline and Norby*, 1961) and XO in pig (*Nes*, 1968), were soon found, but it took long time before we found the correspondence to Down's syndrome in a domestic animal, and then we didn't know it was that specific chromosome aberration we had found. Trisomy 28 in horse (*Power*, 1987, *Power et al.*, 1992) apparently more or less corresponds to trisomy 21 in man, since comparative gene mapping has shown homology between HSA 21 and ECA 28 (*Raudsepp et al.*, 1996). It is quite apparent that the new knowledge of gene maps will make possible a better understanding of the pathology of so-called chromosomal diseases in domestic animals. It is now also necessary to use the *in situ* hybridization techniques for increasing the possibilities to identify individual chromosomes and to complement other techniques in the evaluation of possible chromosome aberrations.

In this brief communication I will concentrate on clinical cytogenetics of cattle and pigs, but the considerations can be applied to all domestic animals.

Effects and distribution of chromosome aberrations: It is a well known fact that reproductive performance is the most important characteristic of our domestic animals. Each small disturbance of a breeding animal's reproductive performance has great economical importance. Problems can occur on different levels and of course be due to different factors. Such a type of factors are chromosomal aberrations (mutations). Today, we know that chromosome aberrations particularly influence reproductive performance. The most common effect of unbalanced karyotype in a zygote is early embryonic mortality, but there are exceptions some of which will be mentioned below. It is therefore important to keep breeding animals under cytogenetic control. Particularly in animal populations applying artificial insemination (A.I.), inherited aberrations can quickly become distributed and achieve great economical importance. Such species are cattle, in which A.I. historically has been used very extensively, and pigs, in which species A.I. now is rapidly increasing in many countries. The methods for freezing gametes become more and more efficient and by use of A.I., the effective population size of many animal stocks has become considerably smaller. The avoidence of crossing different breeds was another factor, which earlier prevented unrestricted distribution of genetic material. Lately, the farmers' interest to keep breeds separated has decreased. Consequently, chromosome mutations can become worldwide spread in short time.

Aberration frequencies in cattle and pigs and present use of preventive chromosome investigation.

Our knowledge of mutation rates and aberration frequencies in domestic animals is very poor. We have to assume, however, that spontaneous chromosome mutations are relatively rare in domestic animals and give, most often, if they are not lethal, sporadic cases of malformation and sterility. Such mutations are of less economical importance, since they can not be spread by breeding work. Our own investigations - you can see our poster at this meeting — demonstrate that for instance the frequency of sex chromosome disomy in the germ cells of bulls is 0.1% or four times less common than sex chromosome disomy in man.

Aberrations like translocations and inversions, which occur in balanced form, are inherited and at least translocations give in each generation disturbances such as early embryonic mortality and more rarely fetal death, stillbirth and malformations. According to observations in man (*Boué and Boué*, 1981), the mutation rate should be low (less than half a per cent), and observations point to low frequencies also in domestic animal populations, although there can be exceptions. The first population studies in pigs (*Golisch et al.*, 1982) demonstrated low frequencies (less than half a percent), of reciprocal translocations. A recently observed high frequency (3% or more) of inheritedaberrations — reciprocal translocations and inversions — in the population, made the breeding organization of France take a decision about routine chromosomal investigation of boars used for A.I. purposes (*Ducos*, 1995). In many pig populations there are also observations, which indicate that the frequency of structural aberrations probably is higher than the expected rate according to experience from other species. The conclusion that chromosome aberrations are more common in pigs than earlier believed is supported by observations of the common occurrence of reciprocal translocations in boars producing decreased litter size (e.g. *Gustavsson*, 1990), observations of two translocations in the same family (*Gustavsson et al.*, 1983) as well as the occurrence of two three-breakpoint translocations (*Mäkinen et al.*, 1987; 1997) in small numbers of translocations described in Sweden and Finland, respectively. Aberrations also appear to be very easy to induce in pigs (*Zartman et al.*, 1969, *Fries and Stranzinger*, 1982).

All of us know too well, that several domestic animals have chromosomes which are very difficult to distinguish individually. Such a species is cattle, and the identification problems are reflected by several failures to create a reliable international standard. Preventive chromosome investigations of breeding animals have hitherto particularly been used for eradication of bulls having the 1/29 translocation. On national basis decisions have been taken about eradication strategies (for a bibliography of the 1/29 translocation, see *Popescu and Pech*, 1991). The centric fusion type of translocation is easy identifiable by counting conventionally stained chromosomes and looking at their main morphology. Although some other aberrations influencing chromosome number and/or main morphology also have been found, it is quite apparent that many aberrations have escaped our attention. Few autosomal reciprocal translocations (*Mayr et al.*, 1979, 1983; *deSchepper et al.*, 1982; *Kovács et al.*, 1992; *Ansari et al.*, 1993; *Villagómez et al.*, 1993) and inversions (*Popescu*, 1972; *Roldan et al.*, 1984; *Langhammer and Rex*, 1987; *Guo and Chen*, 1989) have so far been found in cattle, a fact which probably to a large extent is due to identification problems. Identification of reciprocal translocations not producing unusually small or large chromosomes is difficult even in preparations with well banded chromosomes. In Austria, collegues have routinely banded cattle chromosomes in the search for aberrations (e.g. *Mayr and Schleger*, 1977). Such work is very troublesome and most attempts are probably more or less partly doomed to fail, because of the problems to identify individual chromosome pairs. In our laboratory we have identified two autosomal reciprocal translocations (*Kovács et al.*, 1992; *Villagómez et al.*, 1993). This was, however, possible after the translocations had been proven by synaptonemal complex analysis of meiotic chromosomes. In our material from recent years, before the *in situ* hybridization technique became available, we had cases with suspected reciprocal translocations. One cow with reduced fertility had an autosome considerably smaller than the smallest normal chromosome. We made great efforts to identify the aberration, but we failed. Personally I think this small chromosome was a product of a translocation between one small and one large chromosome. In our material we also had a malformed calf with an extra small chromosome having banding patterns not in accordance with chromosomes of similar size. Probably it was a case of tertiary trisomy. There are the wellknown trisomies for chromosomes 17 (*Herzog and Höhn*, 1968; *Mori et al.*, 1969; *Dunn and Johnson*, 1972) and 18 (*Herzog et al.*, 1982) causing complex malformations with the classical features brachygnathia inferior and nanismus, respectively, but trisomies of cattle chromosomes probably most often cause early embryonic mortality. There are also single cases of trisomies, some of them described in literature by unbanded or poorly banded chromosomes. That makes me ask if all cases of trisomy described really are "pure" trisomies. Probably some of them were tertiary trisomies. A recently found A.I. bull, which after insemination of chromosomally normal cows demonstrated a fairly normal rate of early embryonic mortality but considerably increased rate of stillbirth, was carrying one very small chromosome, which presumably was the product of a reciprocal translocation. In metaphases with not too contracted chromosomes the latter chromosome could be identified as considerably smaller than the smallest normal chromosome. Although we are still struggling with identification by banding techniques of the chromosomes involved in the presumptive translocation, we are quite sure to come to a happy end. If necessary we can of course, as a complementary technique for the identification, apply chromosome painting by use of chromosome scraping, PCR-amplification and *in situ* hybridization (*Guan et al.*, 1992). It is

quite reasonable to assume that the stillborn calves are carrying the small chromosome as extra chromosomal material in addition to their balanced genome, and we can probably expect that other reciprocal translocations producing small centric fragments cause late effects such as stillbirth and/or malformation due to tertiary trisomy.

Why are not preventive chromosome investigations already in general use? Nowadays chromosome investigation is made when people think it is justified, which means that the cytogeneticist is consulted when fertility is reduced in a breeding animal or there is a high incidence of malformations observed in its offspring. Personally I think that all breeding animals extensively used — and particularly male individuals in populations making use of A.I. — should be under cytogenétical control. In many countries people working with breeding claim that their animal populations are free from aberrations. This concerns the cattle population in the Netherlands as well as the pig population in Finland. All evidence indicates, however, that chromosome mutations occur in all countries and in all breeds of domestic animals, despite people often deny those facts! It is important that we repeatedly inform about these facts.

Since several years the pig karyotype techniques and the observations can r chromosome painting. It is therefore can be accurately analysed by banding ow also be complemented by use of surprising that routine cytogenetical investigations particularly of pigs used for breeding purposes are not applied to a larger extent. The reasons can be discussed. It is more understandable that in cattle there are only routine controls for eradication of specific aberrations like 1/29. As mentioned above, there are still great problems in cattle to identify aberrations like reciprocal translocations and inversions. Only high resolution chromosomes with very good bandings can give a positive result of the analysis. That means that routine investigations at present will be incomplete, uncertain and definitely very expensive.

It can be our fault not being able to clearly and distinctly explain the negative aspects of chromosome aberrations. In this context the advantages with the cytogenetical diagnosis as being defnite should be stressed! Besides problems, discussed above, to identify aberrations, there have also been disagreement concerning the phenotypic effects of certain aberrations. This can sometimes be due to the fact that the effects are very small, the records are poor or the animals studied have been selected according to the traits of interest. Consider all time we have spent discussing the possible fertility reductions of centric fusion translocations! If there are hesitations about the effects of aberrations like centric fusions in cattle and inversions in pigs, we have today possibility to draw conclusions about chromosome segregation after *in situ* hybridization on germ cells using markers for the chromosomes involved.

In general, it is important that the costs for cytogenetic control are less than the economical losses which arise when animals with aberration are used for breeding. It is quite possible that owners of breeding animals have thought the price for a chromosome examination has been too high. Today, however, many of our laboratories have computerized systems for karyotyping, which, besides increasing the accuracy of chromosome diagnosis, also save time and make the analysis cheaper.

Future work and strategy for increasing the impact o f clinical cytogenetics. In pigs available banding techniques can successfully be used for accurate identification of individual chromosomes and possible aberrations, and preventive chromosome analysis of populations can immediately be introduced. In cattle we still have to carry out some developmental work. It is now urgent to establish techniques, which easily can identify all individual cattle chromosomes and possible chromosome aberrations. One way to solve the problems would be to apply multicolour fluorescence *in situ* hybridization (*Schröck et al.*, 1996). Who will be the first one to develop such a system? Would it also be possible to apply synaptonemal complex analysis on meiotic cells in sperm ejaculates? These two issues for detecting chromosome aberrations in cattle should be carefully investigated.

Although I hope we all agree about the reduced fertility of 1;29 carriers, there is a large number of centric fusion translocations for which we have no information at all about reproductive performance. In the same way there appear to be large variations in the phenotypic effects between carriers of different translocations. I think it is important to clarify possible chromosomal causes for such variations. The problems mentioned can be solved by the use of chromosome markers for *in situ* hybridizing experiments with germ cells. By this technique it is possible to get direct measurements of the disjunctional rates. We should also investigate the effects of inversions. All pig inversions hitherto investigated, including one comprising all chromosome 4 between its telomeric segments (*Ducos et al.*, 1997) have demonstrated normal fertility in their carriers. Even those

inversions involving segments comprising as much as a third of a chromosome (*Switonski et al*,. 1998), have by synaptonemal complex analysis not shown the classical feature of a chromosomal loop. The rare occurrence of a loop with crossing over resulting in production of abnormal semen would be possible to detect after *in situ* hybridization with suitable chromosome markers.

The problem of not knowing frequencies of chromosome aberrations and their effects means that we have no idea about the costs of chromosome mutations. Since A.I. to a very large extent is used in farm animals like cattle and pigs, preventive chromosome analyses are probably economically very motivated in such populations. Rough calculations show that in Sweden an annual finding of one young buli and a few boars with reciprocal translocation, will economically motivate routine investigation of all A.I. bulls (with use of multicolour fluorescence *in situ* hybridization techniques) and A.I. boars, respectively. In this communication I have mentioned the economical losses of decreased reproductive performance due to chromosome aberrations, but there are also other aspects to consider. Geneticists think that the delay in breeding progress, which the aberrations cause, is more important. Another aspect is the ethical one. A.I. should particularly stand for high quality breeding and it is ethically not right to use breeding animals with chromosome aberrations, which produce reduced number of offspring, increased number of offspring with fertility problems, high rates of stillbirth and malformation. The farmers themselves have to request the use of high quality breeding bulls and boars. In future it is important that the announcement "free from chromosome aberration" always be included in the information when semen of a breeding buli or boar is offered to the farmers or will be exported.

ACKNOWLEDGEMENT

The investigations have been supported by the Swedish Council of Agricultural Research.

REFERENCES

Ansari, H.A. – Jung, H.R. – Hediger, R. – Fries, R. – König, H. – Stranzinger, G.(1993): A balanced autosomal reciprocal translocation in an azoospermic buli. Cytogenet. Cell Genet., 62. 117–123.p.

*Boué, A. – Boué, J.(*1981): Chromosome structural rearrangements and reproductive failure. In Chromosomes Today (eds. Bennett, M.D. – Bobrow, M. – Hewitt, G.). 7. 281–290.p.

Ducos, A.(1995): Evolutions de 1'objectif de sélection dans 1'espéce porcine en France. Conséquences sur les progrés génétiques attendus. Rev. Med. Vet., 146. 715–722.p.

Ducos, A. – Pinton, A. – Séguela, A. – Berland, H.M. – Blanc, M.F. – Darré, A. – Pinton, P. – Yerie, M. – Darré, R.(1997): A pericentric inversion of chromosome 4 in pigs. Genet. Sel. Evol., 29: 383–394.p.

Dunn, H.O. – Johnson, R.H.(1972): A 61 XY cell line in a calf with extreme brachygnathia. J. Dairy Sci., 55. 524–526.p.

*Fries, R. – Stranzinger, G.(*1982): Chromosomal mutations in pigs derived from X-irradiated semen. Cytogenet. Cell Genet., 34. 55–66.p.

Guan, X.Y. – Meltzer, P.S. – Cao, J. – Trent, J.M.(1992): Rapid generation of region specific genomic clones by chromosome microdissection: Isolation of DNA from a region frequently deleted in malignant melanoma. Genomics, 14. 680–684.p.

*Golish, D. – Ritter, E. – Schwerin, M.(*1982): Zytogenetische Untersuchungen von Spontanaberrationen bei Ebern. Arch. Tierz., 25. 537–547.p.

Guo, A.Z. – Chen, W.F.(1989): Chromosome abnormalities in infertile cows. J. Nanjing Univ., 12. 66–70.p.

*Gustavsson, I.(*1990): Chromosomes of the Pig. In "Domestic Animal Cytogenetics" (ed. R.A. McFeely), Advances in Veterinary Science and Comparative Medicine, Academic Press Inc, 34. 73–107.p.

Gustavsson, I. – Settergren, I. – King, W.A.(1983): Occurrence of two different reciprocal translocations in the same litter of domestic pigs, Hereditas, 99. 257–267.p.

Herzog, A. – Höhn, H.(1968): Autosomale trisomy bei einem Kalb mit Brachygnathia inferior and Ascites congenitus. Dtsch. Tierärtzl. Wochenschr., 75. 604–606.p.

Herzog, A. – Höhn, H. – Olyschlager, F.(1982): Autosomal trisomy in calves with dwarfism. Dtsch. Tierärztl. Wochenschr., 89. 400–403.p.

Kovács, A. –Villagómez, D.A.F. – Gustavsson, I. – Lindblad, K. – Foote, R.H. – Howard, T.H.(1992): Synaptonemal complex analysis of a three-breakpoint translocation in a subfertile bull. Cytogenet. Cell Genet., 61. 195–201.p.

Langhammer, H. – Rex, *E.*(1987): The occurrence of an inversion in beef cattle. Arch. Tierz., 30. 115–120.p.

Mayr, B. – Schleger, W.(1977): Zytogenetische Untersuchungen im österreichischen Erbhygieneprogramm beim Rind. Züchtungskunde, 49. 343–346.p.

Mayr, B. – Krutzler, H. – Auer, H. – Schleger, W.(1983): Reciprocal translocation 60, XY, t(8;15)(21;24) in cattle. J. Repr. Fert., 69. 629–630.p.

Mayr, B. – Themsessel, H. – Wöckl, F. – Schleger, W.(1979): Reziproke Translokation 60, XY, t(10;11)(41;14) beim Rind. Z. Tierzucht. Zuchtgsbiol., 96. 44–47.p.

Mori, M. – Sasaki, M. – Makino, S. – Ishikawa, T. – Kawata, K.(1969): Autosomal trisomy in a malformed newborn calf. Proc. Japan. Acad. Sci., 45. 955–959.p.

Mäkinen, A. – Kuokkanen, M.T. – Nüni, T. – Perttola, L.(1987): A complex three breakpoint translocation in the domestic pig. Acta Vet. Scand., 28. 189–196.p.

Mäkinen, A. – Pitkänen, T. – Andersson, M.(1997): Two cases of reciprocal translocations in domestic pigs producing small litters. J. Anim. Breed. Genet., 114. 377–384.p.

Nes, N.(1968): Betydningen av kromosomaberrasjoner hos dyr. Forsk. Fors. Landbruket, 19. 393–410.p.

Popescu, C.P.(1972): Un cas possible d'inversion péricentrique chez les bovins. Ann. Genet., 15. 197–200.p.

Popescu, C.P. – Pech, A.(1991): Une bibliographie sur la translocation 1;29 de bovins dans le monde (1964–1990). Ann. Zootech., 40. 271–305.p.

Power, M.M.(1987): Equine half sibs with an unbalanced X;15 translocation or trisomy 28. Cytogenet. Cell Genet., 45.163–168.p.

Power, M.M. – Gustavsson, I. – Switonski, M. – Plöen, L.(1992): Synaptonemal complex analysis of an autosomal trisomy in the horse. Cytogenet. Cell Genet., 61. 202–207.p.

Raudsepp, T. – Frönicke, L. – Scherthan, H. – Gustavsson, I. – Chowdhary, B.P.(1996): ZOO-FISH delineates conserved chromosomal segments in horse and man. Chrom. Res., 4. 218–225.p.

Roldan, E.R.S. – Meran, M.S. – Lawzewitsch, I.(1984): Two abnormal chromosomes found in a cell line of mosaic cow with low fertility. Genet. Sel. Evol., 16. 135–142.p.

Schepper, G.G. de – Aalbers, J.G. – Te Brake, J.H.A.(1982): Double reciprocal translocation heterozygosity in a bull. Vet. Rec., 110. 197–199.p.

Schröck, E. – du Manoir, S. – Veldman, T. – Schoell, B. – Wienberg, J. – Ferguson Smith, M.A. – Ning, Y. – Ledbetter, D.H. – Bar-Am, I. – Soenksen, D. – Garini, Y. – Ried, T.(1996): Multicolor spectral karyotyping of human chromosomes. Sci., 273. 494–497.p.

Switonski, M. – Danielak-Czech, B. – Slota, E. – Sysa, P.(1998): Lack of pairing loop formation in synaptonemal complex preparation of a boar carrying an inversion. Hereditas, 128. 83–85.p.

Thuline, H.C. – Norby, D.E.(1961): Spontaneous occurrence of chromosome abnormality in cats. Sci., 134. 554–555.p.

Villagómez, D.A.F. – Andersson, M. – Gustavsson, I. – Plöen, L.(1993): Synaptonemal complex analysis of a reciprocal translocation, rcp(20;24)(q17;q25), in a subfertile bull: Cytogenet. Cell Genet., 62. 124–130.p.

Zartman, D.L. – Fechheimer, N.S. – Baker, L.N.(1969): Chromosomal aberrations in cultured leukocytes from pigs derived from X-irradiated semen. Cytogenetics, 8. 355–368.p.

Author's address: Centre for Reproductive Biology, Department of Animal Genetics, Swedish University of Agricultural Sciences, Uppsala, Sweden

THE YEARS AFTER NATE FECHHEIMER

STRANZINGER, G.F.

One of the most prominent cytogenetists in farm animals passed away in 1992. He held the position of Professor at the Ohio State University in Columbus, USA and was a frequent participant of our European Colloquium on Cytogenetics of Domestic Animals. For all the young scientists who never met this outstanding man, I would like to summarize his research activities and also mention his remarkable teaching capacity.

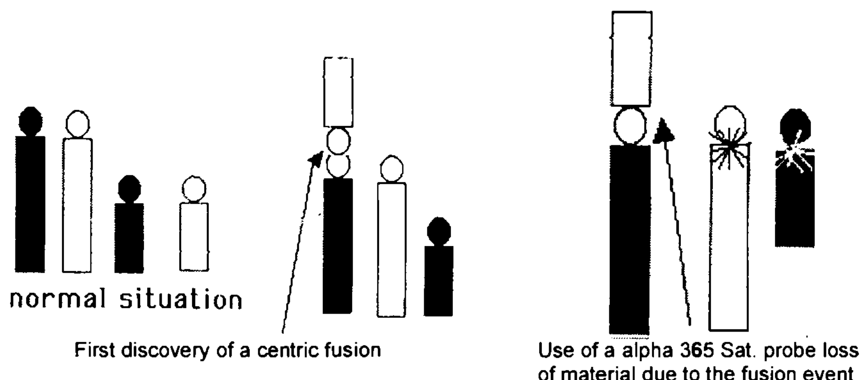
In research, his primary interests were in genetics, beginning with quantitative and ending with qualitative genetics, getting also involved with molecular genetics.

For young scientists it would be worthwhile to read all his publications and to get an idea what genetics is all about. Pigmentation genetics in cattle, intrauterine and premature death of animals, heteroploidy in gametes and zygotes in mammals and fowl, the consequences of structure rearrangements of chromosomes and polymorphism have been his main interests. More than 150 scientific papers and his outstanding reviews are significant for his expert knowledge.

But he was also one of the very few scientists who was able to combine history, science and agriculture so that the students got aware of the general interactions. To demonstrate some of the historic interactions between Fechheimer's work and ours, four examples of scientifically related experiments will be presented (*Fig. 1.– Fig. 4.*).

In 1960 Fechheimer demonstrated that chromosome preparations (*Fig. 1.*) in farm animals are possible and that centromeric fusions occur. After *Gustavsson and Rockborn* (1964) confirmed that these fusions are possible, fruitful research activity followed worldwide. At that time neither banding techniques nor molecular analysis were possible.

Fig. 1.: **First discovery of a centric fusion (1960) and the molecular consequences found (1998)**



In a recently published dissertation (*Joerg*, Diss. ETH No. 12479) in the field of molecular cytogenetics Robertsonian translocations 1;29 and 14;20, the most common chromosomal mutations in Bovidae, were investigated. The alpha 365 satellite probe, hybridizing in the centromeric region of all 29 bovine acrocentric chromosomes, was used. The signal on the fused chromosome 14;20 was very strong and confirmed the cytogenetic state of a dicentric chromosome. No signal was observed on either the X-, Y- or the fused chromosome 1;29. Due to the absence of a signal on the chromosome 1;29 this is a dicentric chromosome. Hereby, an evolutionary similarity can be drawn between the appearance of the sex chromosomes and the different fusion events in the autosomes. Using this microsatellite technique, these findings are of further interest in identifying the fusion events in conserved haplotypes.

Color coat inheritance in cattle (*Baldwin et al.*, 1954 and 1956) was a very prominent issue in the early days of genetics (*Fig. 2.*). In a continuation of this work on a molecular level we were able to show a deletion in the melanocyte stimulating hormone receptor gene (*Joerg et al.*, 1996) causing the change from the dominant black to the recessive red color in Holstein Friesian cattle. A frameshift in the first loop of the outer transmembrane domaine sequence has been identified as the second loop in mice.

Irradiation experiments performed in the 1950s and 1960s were also carried out on large animals as it was published by *Zartmann et al.* (1969) (*Fig. 3.*). Valuable information as to the effects of different dose amounts of irradiation on sperm behaviour, could be drawn from these experiments. When gene mapping in farm animals was first carried out, one of the first assignments was to establish the blood group loci in pigs. Chromosome mutations in the sperm of pigs, which had been produced artificially, were used for this purpose (*Fries and Stranzinger*, 1982).

Fig. 2.: **Time sequence in the investigations of the coat colour phenotypes (1954) and their molecular genetic background (1996)**

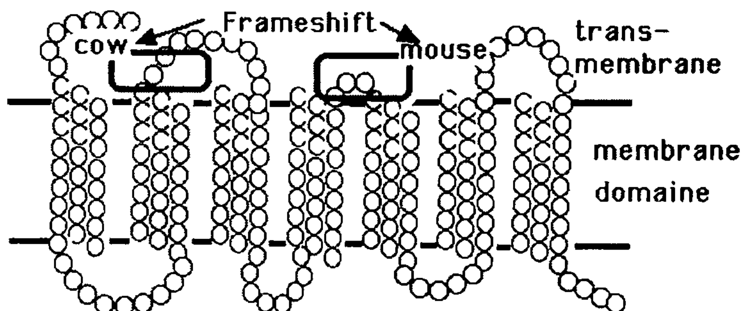
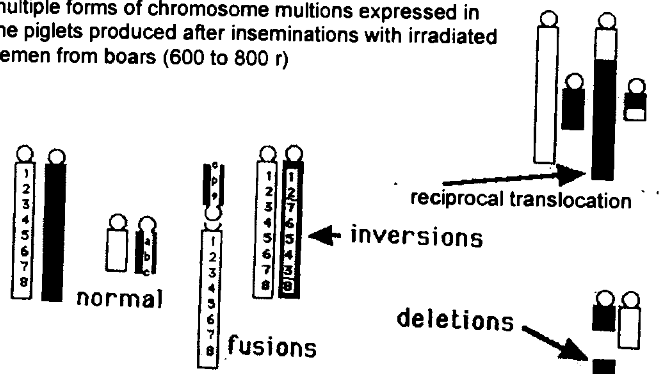
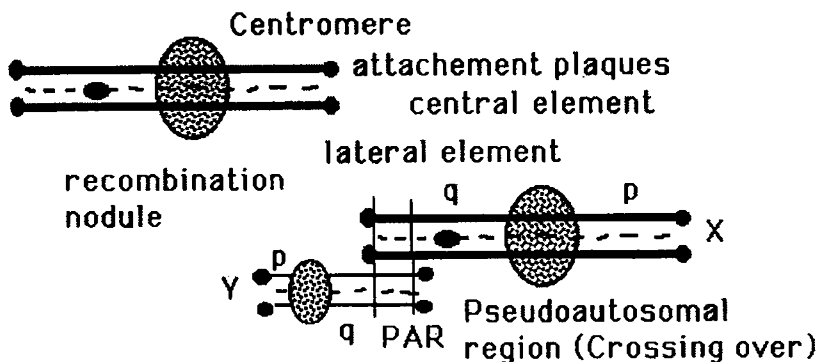


Fig. 3.: **The use of radiation experiments for mutation research (1969) and the use for gene mapping (1982)**



One of Fechheimer's special interests was the pairing behaviour of autosomes and sex chromosomes in farm animals, viewing the genetically relevant meiotic events. He concentrated his efforts mainly on fowl and discovered many important phenomena (*Kaelbling and Fechheimer*, 1983). In a continuation to his work, this technique was applied in the preparation of the synaptonemal complex in cattle (*Switonski et al.*, 1990) and rabbits (*Kovács et al.*, 1998). The female meiosis is of particular interest in the rabbit (*Fig. 4.*), as the pachyten stage occurs postnatally and can be analysed by collecting the ovaries 10–12 days after birth, when compared to cattle, who reach this stage 90–100 days post fertilization.

Fig. 4.: **The use of synaptonemal complex preparations (SC) to determine chromosomal sturctures (1983), the sex chromosome pairing (1990) and female meiosis (1998)**



Fechheimer helped to establish and develop statistic and biometric issues. Now many of his calculations and estimates can, with the help of newer methods and technology, be updated. Newly incorporated molecular genetic techniques and information gained from gene mapping techniques allow us to answer many questions raised already 50 years ago.

The years after Nate Fechheimer have been revolutionary in our field what cytogenetics in combination with molecular genetics is concerned. FISH and ZOO FISH, microsatellite mapping and many marked chromosome regions allow cytogenetic assumptions to be proved. One of the most fascinating areas will be the possibility to study the recombination event within animals, breeds, sexes or environments, to have a better prediction of recombined gametes to be used for breeding techniques.

The statistical assumptions used for the estimation of breeding values are to be tested and, if necessary, changed. Genomics has developed to an extent that a complete new area in science will be opened where all the discoveries, including those from Fechheimer, can be integrated and constructively used.

To understand the years after 1992, you have to study Nate Fechheimer before 1992 and you will understand his philosophy which was published in 1986 in the J. Dairy Sci. 69: 1743–1751. This paper caused intense discussions, before and after publication, and shows the need of bridging the different disciplines in science and practice. Everything what Fechheimer predicted came true and his visions were realistic when judging them retrospectively.

REFERENCE

Baldwin, C.S. – Gilmore, L.O. – Fechheimer, N.S. – Rife, D.C.(1954): Inheritance of black hair patterns in cattle lacking the extension factor for black (E) I. Blackish. J. Dairy Sci., 37: 1368–1375.p.

Baldwin, C.S. – Gilmore, L.O. – Fechheimer, N.S. – Rife, D.C.(1956): Inheritance of black hair patterns in cattle lacking the extension factor for black (E) II. Blackish pattern. J. Dairy Sci., 39. 435–439.p.

Fechheimer, N.S.(1960): Mammalian cell chromosome counts: A simple method of making preparations. Nature, 188. 246–247.p.

Fechheimer, N.S.(1986): Interrelationship between recent developments in molecular genetics and cytogenetics and animal breeding. J. Dairy Sci., 69. 1743–1751.p.

Fries, R. – Stranzinger. G.F.(1982): Chromosomal mutations in pigs derived from X-irradiated semen. Cytogenet. Cell Genet., 34. 55–66.p.

Gustavsson, I. – Rockborn, G.(1964): Chromosome abnormality in three cases of lymphatic leukaemia in cattle. Nature, 203. 990.p.

Joerg, H. – Rieder, S. – Suwattana, D. – Stranzinger, G.(1999): Molecular analysis of Robertsonian Translocations. 13th Europ. Coll. on Cytogen. of Dom. Animals, 1998, Budapest, Állattenyésztés és Takarmányozás, 48. 1. 33–35.p.

Joerg, H. – Fries, H.R. – Meijerink, E. – Stranzinger, G.(1996): Red coat color in Holstein cattle is associated with a deletion in the MSHR gene. Mammalian Genome, 7. 317–318.p.

Jörg, H.(1998): Analysen von Chromosomenabnormalitäten in Rinderspermien. Diss. ETH No. 12479.p.

Kaelbling, M. – Fechheimer, N.S.(1983): Synaptonemal complexes and the chromosome complement of domestic fowl (Gallus domesticus). Cytogenet. Cell Genet., 35. 87–92.p.

Kovács, B.Zs. – Switonski, M. – Went, D. –Stranzinger, G.(1999): The event of meiotic synapsis in female rabbits. 13th Europ. Coll. on Cytogen. of Dom. Animals, 1998, Budapest, Állattenyésztés és Takarmányozás, 48. 1. 121–124.p.

Switonski, M. – Ansari, H.A. – Mathew, A. – Jung, H.R. – Stranzinger, G.F.(1990): Synaptonemal complex analysis in primary spermatocytes of cattle x zebu hybrids (Bos taurus x Bos indicus). J. Anim. Breed. Genet., 107. 229–238.p.

Zartmann, D.L. – Fechheimer, N.S. – Baker, L.N.(1969): Chromosomal aberrations in cultured leukocytes from pigs derived from X-irradiated semen. Cytogenetics, 8. 355–368.p.

Author's address: Department of Animal Science, Breeding Biology, Swiss Federal Institute of Technology
CH-8092 Zürich, Switzerland

AN EASY WAY TO RESOLVE BOVID CHROMOSOME NOMENCLATURE INCONSISTENCIES

IANNUZZI, L.

Many studies have demonstrated that several errors were made during the construction of cattle, sheep and goat standard karyotypes (ISCNDAB9,1990) on the basis of the previous Reading Conference (1976) standards. These errors were in part due to the poor G-banding pattern resolution performed in the Reading Conference (1980) standards, especially in sheep and goat, and the high degree of banding resolution achieved in the ISCNDAB9 (1990), especially in the GTG-, QFQ- and RBA-standards (cattle). The chromosomes involved in these errors were (a) BTA/OAR/CHI 4 and 6 (inverted position in the two systems), (b) OAR 8 and 9 (inverted position in the Reading GTG- and ISCNDAB9 RBA-standards), (c) BTA 25, 27, 28 and 29 (the position of ISCNDA89 R-banded BTA2S and 29 has been inverted when comparing with GTG-banded BTA2S and 29; the position of BTA27 and 28 has been inverted when comparing the ISCNDA89 GTG-standard with both Reading and ISCNDA89 R-banded standards). The banding pattern resolution achieved in the small sheep and goat chromosomes reported in the Reading GTG-standards is too poor to make any serious banding comparison with the ISCNDA89 R-banded standards.

One of the problems we have is to know if G-banding patterns agree with the R-banding ones during the construction of standard karyotypes.

In this study I show an easy way to resolve these nomenclature inconsistencies by using (a) high resolution G- and R-banding comparisons between disputed cattle standard chromosomes and bovid marker chromosomes (both river buffalo and cattle biarmed pairs); (b) FISH-mapping of bovine U25, U29, U8 and U7 in marker river buffalo chromosomes; (c) sequential FISH-mapping of a U8 marker and Ag-NOR-staining in BTA27 and homeologous BBU24; (d) ZOO-FISH mapping with human painting probes in BBU1p, BBU4p, BBU5p and BBU24. All these comparisons show the G- and R-banding patterns of disputed chromosomes very well, allowing an easy decision to be made about the order to be assigned to them in standard karyotypes on the basis of the previous G- and R-banded standards (Reading Conference, 1976; ISCNDA89, 1990) and Texas nomenclature (1996).

ACKNOWLEDGMENTS

We thank Mr. D. Incarnato for technical assistance and microscope image processing. This study has in part been supported by INC, Biologia e Produzione Agraria del CNR, P.S. Biologia e Produzione Agraria per un'agricoltura sostenibile.

Author's address: National Research Council (CNR), I.A.B.B.A.M., *Naples, Italy*

MAPPING OF DIFFERENTIALLY EXPRESSED CDNA SEQUENCES TO BOVINE METAPHASE CHROMOSOMES BY FISH

SCHWERIN, M. — GOLDAMMER, T. — BRUNNER, R.M.

SUMMARY

Pools of cDNA of endometrium epithelium generated by RT-PCR showing differentially expressed cDNA bands in differential mRNA display between two trait differentiated animals (a milk type and a meat/milk type cow) being compared, were amplified, biotin labelled, and used in FISH. Alternatively, the cDNA pool of the one animal was applied as biotin labelled probe using the unlabelled cDNA pool of the other animal as competitor in sequential subtractive hybridisation (cot value 10^{-4}–7×10^{-1}), prior to FISH of bovine metaphase chromosomes. GTG-banded metaphase spreads were digitised with MacKtype karyotype analysis software (PSI, Perceptive Scientific Instruments, Inc.). Chromosomes were karyotyped according to the international standard, ISCNDA 1989, for bovine GTG-banded chromosomes.

Based on counting of doublet symmetrical chromosomal spots a high locus specificity of both cDNA pools with decreasing number of detectable loci with prolonged subtractive hybridisation was observed. Of 15 fluorescence labelled chromosome regions 8 and 3 regions were specific for cDNA pool 1 and 2, respectively, whereas 4 regions were labelled by both probes. Those loci are suggested as candidate loci for QTL regions underlying production traits.

INTRODUCTION

Major efforts in animal genetics are presently being devoted to the genetic mapping of QTL underlying production efficiency in the different livestock species. Results of first whole genome scans in pigs and cattle demonstrate that this approach will very likely be successful and yield a large number of mapped loci in the near future. But, within the constraints of the present experimental designs, QTL are often mapped to support intervals that are several tens of centimorgan large, corresponding to several hundreds or thousands of possible positional candidate genes. Efficient utilisation of the mapped QTL in breeding programs, and the ultimate positional cloning or identification of the corresponding genes, requires a significantly higher mapping resolution. An alternative way for the identification of positional candidate genes underlying phenotypic variance of traits could be the simultaneous identification of genes that are, on the one side, up- or down-regulated in the corresponding response organs or tissues of founder animals (e.g. *Aiello et al.*, 1994; *Li et al.*, 1993; *Nishio et al.*, 1994)., and that map, on the other side, to the QTL region. The present study demonstrates identification and physical mapping of cDNAs which are differentially expressed in a selected tissues of lactating cows differing in metabolic type (milk type, meat/milk type).

MATERIAL AND METHODS

mRNA differential display

The technique of differential display of messenger RNA species essentially as described by *Liang and Pardee* (1992) was used for detection of genes that are differentially expressed. Deviating from this method Expand^{TM} Long Range Polymerase was used for polymerase chain reaction (PCR), the gels were silver stained and the GeneExScreen Primer Kit (Biometra, Göttingen) was used. Tissue were sampled each at day 120 of lactation immediately after slaughtering. Total RNA was extracted using the RNeasy Total RNA Kit (Quiagen, Hilden) following the protocol of the manufacturer. The cDNAs were amplified under the following cycling conditions: initial denaturation at 94 °C for 5 min. and 40 cycles for 30 sec. at 94 °C, 60 sec. at 40 °C, 30 sec. at 72 °C. PCR products were resolved on a 4% native polyacrylamide sequencing gel and the bands visualised by conventional silver staining.

Fluorescence in situ hybridisation (FISH)

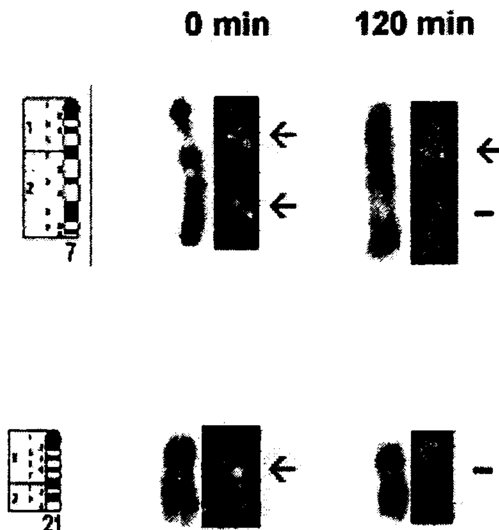
Metaphase spreads were prepared from fibroblast cultures of *Bos taurus* by standard cytogenetic techniques. Chromosomes were GTG-banded by trypsin and Giemsa as described by *Seabright* (1971) prior to their use for FISH. GTG-banded metaphase spreads were digitised with Mac K Type karyotype analysis software (PSI-Perceptive Scientific Instruments, Inc.). Chromosomes were karyotyped according to the ISCNDA (1990) for bovine GTG-banded chromosomes. The cDNA pools generated by mRNA differential display method described probe was labelled with biotin-16-dUTP via PCR (Boehringer Mannheim). Subtractive hybridisation was done for 0 and 120 min, respectively, using the alternative cDNA pool as competitor. Hybridisation of the probe to bovine chromosomes was performed essentially as described by *Pinkel et al.* (1988). The hybridisation sites on metaphase chromosomes were detected with a Nikon Microphot FXA microscope. Images were digitised with a CCD-camera and analysed with a Mac Probe FISH software (PSI). Ten metaphase spreads were analysed per hybridisation experiment counting the doublet symmetrical chromosomal spots.

RESULTS AND CONCLUSIONS

cDNA pools of endometrium epithelium of two cows differ in metabolic type and showing differentially expressed cDNA bands in differential mRNA display were succesfully applied as biotin labelled probe using the alternative unlabelled cDNA pool as competitor in subtractive hybridisation in FISH of bovine metaphase chromosomes.

As illustrated in *Fig. 1*, prolongation of subtractive hybridisation from 0 to 120 min decreases the number of detectable loci but does not change chromosomal location of high specific signals. Using labelled cDNA pool 1, of 22 chromosome regions with at least 5 double dots in metaphase spreads analysed after 0 min subtractive hybridisation, 12 identical map positions remain after 120 min subtractive hybridisation.

Fig. 1.: **Number of detectable loci decreases with prolonged subtractive hybridisation**



A high locus specificity of both cDNA pools was observed. As shown in *Fig.* 2 and summarised in *Table 1*, of 15 fluorescence labelled chromosome regions 8 and 3 regions were specific for cDNA pool 1 and 2, respectively, whereas 4 regions were labelled by both probes.

Fig. 2.: **Fluorescence *in situ* of excessively expressed cDNA after subtractive hybridisation of alternative labelled and non-labelled cDNA pools**

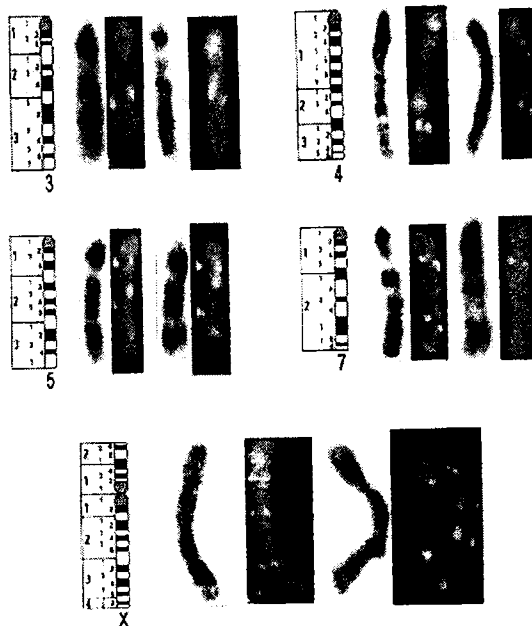


Table 1.

Chromosomal location of differentially expressed cDNas after subtractive hybridisation (120 min) of labelled and non-labelled cDNA pools and subsequent FISH

Biotin-labelled probe	cDNA pool 1	cDNA pool 2
Unlabelled competitor	cDNA pool 2	cDNA pool 1
Map position	2q33-36 3q22–24 4q24–31 5q14–21 5q26–32 7q15–21 12q12–14 16q21–24 25q12–14 Xp13–14 Xq21–22 Xq32–34	 3q22–24 4q24–31 12q12–14 16q16–17 18q13–21 22q11–12 25q12–14
No. of probe specific hybridisation signals	8	3

Summarising the present results, it could be shown that subtractive hybridisation (cot 10^{-4}–$7x10^{-1}$ sec x mol/L) of differently expressed cDNA pools is an useful tool for generation of excessively expressed cDNAs as hybridisation probes. FISH with cDNA probes generated by alternative subtractive hybridisation of labelled and non-labelled cDNA pools allows physical localisation of differently expressed cDNA sequences. Differently expressed cDNAs physically mapped to a QTL region potentially represent candidate gene loci underlying QTL effect.

REFERENCES

Aiello, L.P. – Robinson, G.S. – Lin, Y-W. – Nishio, Y. – King, G.L.(1994): Identification of multiple genes in bovine retlnal pericytes altered by exposure to elevated levels of glucose by using mRNA differential display. Proc. Natl. Acad. Sci., USA, 91. 6231–6235.p.

ISCNDA(1989): International System for Cytogenetic Nomenclature of Domestic Animals. Di Berardino D, Hayes H, Fries R, Long S (eds) (1990) Cytogenet Cell Genet., 53. 65–79.p.

Liang, P. – Pardee, A.B.(1992): Differential display of eukaryotic messenger RNA by means of the polymerase chain reaction. Science, 257. 967–971.p.

Li, F. – Barnathan, E.S. – Kariko, K.(1994): Rapid method for screening and cloning cDNAs generated in differential mRNA display: application of Northern blot for affinity capturing of cDNAs. Nucleic Acids Res., 22. 1764–1765.p.

Nishio, Y. – Aiello, L.P. – King, G.L.(1994): Glucose induced genes in bovine aortic smooth muscle cells identified by mRNA differential display. FASEB, J., 8. 103–106.p.

Pinkel D. – Landegent, J. – Collins, C. – Fuscoe, J. – Segraves, R. – Lucas, J. – Gray, J.W.(1988): Fluorescence *in situ* hybridization with human chromosome specific libraries: Detection of trisomy 21 and translocations of chromosome 4. Proc Natl Acad Sci., USA, 85. 9138–9142.p.

Seabright, M.A.(1971): A rapid banding technique for human chromosomes. Lancet, 2. 971.p.

Authors' address: Forschungsbereich Molekularbiologie, Forschungsinstitut für die Biologie landwirtschaftlicher Nutztiere
18196 Dummerstorf, Wilhelm-Stahl-Allee 2, Germany

FIBER-FISH AS A TOOL FOR RESOLVING GENOMIC STRUCTURES AS DEMONSTRATED WITH THE BOVINE *CYP19* LOCUS

BRUNNER, R.M. — FÜRBASS, R. — GOLDAMMER, T. — VANSELOW, J. — SCHWERIN, M.

SUMMARY

Fluorescence *in situ* hybridization (FISH) applied to G- or R-banded metaphase chromosomes of moderate resolution provides a resolution of about one band. For higher resolution mapping as necessary for ordering of clones in positional cloning or in resolving gene structures, hybridization to extended chromatin (fiber-FISH) has been developed. We used this technique to resolve the structure of the *CYP19* gene locus at BTA 10. In contrast to exon 2 to 10 the exon 1 have been isolated on separated clones only and could not connected to any of the other *CYP19* clones by conventional restriction mapping. Furthermore the chromosomal arrangement of the closely linked gene and pseudogene and the distance between them was yet unresolved. The results demonstrate: 1) The exon 1 of *CYP19* is located 20 kb, upstream from the remainder of the coding region of the gene. 2) Within the chromosomal region 10q26 *CYP19* and *CYP19*φ are arranged "tail-to-head", being separated by 24 kb of DNA. 3) The physical size the bovine aromatase locus amounts to a minimum of 130 kb.

INTRODUCTION

Fluorescence *in situ* hybridization (FISH) is an important tool for physical mapping. The resolution of FISH to banded metaphase chromosomes of moderate resolution, however, is limited to one or a few bands. During the last few years, several attempts have been made to improve the resolution of FISH. The most powerful was the hybridization to extended DNA fibers. There are several methods to prepare extended chromatin fibers as: chemical resolution of DNA-fibers from interphase nuclei (*Wiegant et al.*, 1992; *Parra and Windle*, 1993), mechanically extended chromatin fibers (*Haaf and Ward*, 1994), agarose embedded DNA molecules stretched mechanically on coated slides (*Heiskanen et al.*, 1994) and DNA molecules stretched during drying by the hydrodynamic action of a receding liquid surface (*Weier et al.*, 1995), respectively. All techniques gave slightly different but useful preparations of DNA fibers for FISH experiments. They were successfully used to map stretches of DNA ranging from 1 up to 500 kb in human *(Florijn et al.*, 1995, 1996; *Heiskanen et al.*, 1995a; *Raap et al.*, 1996; *Klockars et al.*, 1996).

A previous effort to isolate the bovine *CYP19* gene including its regulatory regions revealed overlapping clones representing only exons 2 to 10 and an additional, separated clone containing exon 1.1. Despite extensive screening of two libraries using conventional cloning strategies the connecting DNA could not be isolated. Additionally, several clones deriving from a homologous pseudogene (*Fürbass and Vanselow*, 1995) were found to be closely linked to *CYP19* pseudogene. Both, *CYP19* gene and pseudogene, were assigned to BTA10q26 (*Goldammer et al.*, 1994), which is referred to as *CYP19* locus. However, the precise physical structure of the entire locus remained unresolved. We applied the technique of hybridization to mechanically stretched DNA fibers to analyze the structure of the bovine *CYP19* locus and to proof fiber-FISH for high resolution mapping in cattle.

MATERIAL AND METHODS

Probes: Five λ clones served as hybridization probes (*Table. 1.*). The clones were labeled either by biotin-16dUTP or digoxygenin-11dUTP (Boehringer Mannheim) using the Gibco-BRL nick translation kit. Various probe combinations were used for several FISH experiments ((1)λ4/3+λ3/1.1; (2)λ2/10+λ4/3; (3)λ3/1.1+λ2/10; (4)λ2/10+λ1/18; (5)λ3/1.1+λ1/18; (6)λ2/10+λ6/18+λ1/18; (7)λ2/10+λ6/18+λ1/18 (biotin label underlined)).

Fiber preparation: Bovine peripheral lymphocytes were isolated by standard density gradient centrifugation procedure and embedded in 1% (w/v) NuSieve low melting point agarose in PBS at about 10^7 cells per ml. The agarose blocks were digested under permanent shaking in fresh made 2% (w/v) Proteinase K in 0.5 M EDTA, pH 8.0 containing 0.25% (w/v) Na-lauroyl-sarcosine at 50 °C for 3 days. After digestion the blocks were clear, not fragmented and sank to the bottom of the tube. Tubes were cooled on ice for 10 min to harden the blocks. To proof the digestion results, 1/3 of a block was placed on one end of a poly-L-lysin coated slide, covered with 20 µl H_2O and heated on a warming plate at 80 °C for 90 sec. The melted drop was then spread slowly over the slide using a second slide as drawing spatula, air dried and mounted with DABCO-antifade containing 0.2 µg/ml DAPI. If no nuclei were observable, the blocks were washed 4 times in TE-buffer, pH 8.0 and covered with 40 ml TE containing 40 µl fresh made Phenylmethylsulfonylfluoride (PMSF – 40% w/v in Ethanol). The blocks were incubated at 50 °C for 1 h under permanent shaking, washed 4 times in TE and stored at 4 °C in TE until use. Preparation of fiber slides for hybridization was exactly the same as described above for control of digestion. Only slides with long, straight and nearly parallel fibers were used for hybridization.

The coverslips were removed by washing in 2xSSC, and the slides were dehydrated by passing through an ethanol-series.

In situ hybridization and detection: The *in situ* hybridization protocol followed *Trask* (1991). Biotin labelled DNA was detected with two layers Texas-red conjugated avidin and anti-avidin (Vector). Digoxigenin labeled probes were detected by applying 1 layer each of monoclonal mouse anti-digoxin antibody (Dianova), sheep anti-mouse-DIG antibody (Boehringer Mannheim) and sheep anti-DIG-FITC antibody (Boehringer Mannheim), respectively.

The slides were covered with DABCO-antifade containing 0.2 µg/ml DAPI and examined under an NIKON FXA epifluorescence microscope equipped with an Texas-red/FITC double band pass filter (Chroma Technologies).

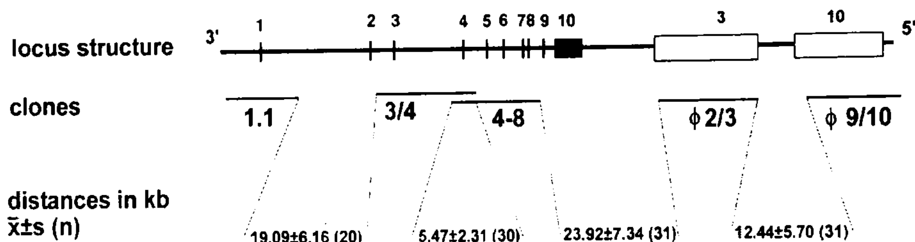
Image analysis: Image analysis and measuring of signal distances was performed with the McProbe fluorescence image analysis system (PSI). We first calculated the relative length of signal and gaps as described by *Sjöberg et al.* (1997) to avoid differences from the pixel shape. Afterwards, we estimated the width of the gap between the red and green signal in kb by comparison of the measured TR or FITC signal width and the known length of the probe from restriction mapping as probe size standard (PSS). To prove the accuracy of our estimation we also used to calculate the size of the TR and FITC signal from the measured with of the opposite colored signal and it's known probe length from restriction mapping, respectively.

RESULTS AND CONCLUSIONS

To analyze the organization and size of the entire *CYP19* locus two-color fiber FISH experiments were performed. All hybridization's gave clear and distinct signals with low background. Using the length of the labeled DNA fiber after hybridization in comparison to the known size of probe as standard, the gaps between the hybridization signals were estimated. The results are summarized in *Fig. 1*. The exon 1.1 (clone λ3/1.1) was found to be separated by about 20 kb of DNA from the coding region. This agrees with findings of *Fuerbass et al.* (1997) who found exon 1.1 to be more than 15 kb away from the rest of the gene. Also, the observed overlap of clone λ4/3 and λ2/10 of about 5 kb agrees with the findings of restriction mapping.

The gene and the pseudogene were found to be in a tail-to-head orientation, being separated by about 24 kb of DNA. According to the combined data of restriction analysis and of fiber FISH, the physical size of the entire bovine *CYP19* locus amounts to a minimum of 130 kb of DNA. The high resolution physical map shown in *Fig. 1.* features the summarized results of the present fiber FISH study.

Fig. 1: **The physical organization of the bovine CYP19 locus as revealed by fiber FISH analysis. Probes are depicted by lines, the included exons are indicated. White boxes indicate the pseudogene. The distance between the probes as calculated after fiber FISH (mean ± standard deviation; n in brackets) are given**



Measurements of probe and gap sizes revealed a variation in the degree of DNA stretching of factor 2.2. All distances measured were shown to have a coefficient of variability (CSV%) of about 25% if calculated from the known length of both clones used. This agrees with the results of *Heiskanen et al.* (1995a,b) which also found a CSV% of about 20%. Possibly, the reported variability is due to suboptimal DNA preparation from bovine cells by the used technique of mechanically stretching as recently reported by *Wang et al.* (1996) and *Duell et al.* (1997a). It might also reflect differences in stretching within the same fiber as reported by *Nishio et al.* (1996). However, as shown in *Table 1.* the DNA fragment sizes derived from fiber FISH experiments were in a fairly good agreement with measurements of restriction fragment lengths. Thus, we are confident, that the technique was appropriate for an estimate of the size of DNA fragments on the base of labeled and unlabeled chromosomal areas and to establish a high resolution map of the bovine *CYP19* locus. Unfortunately, within this experiments the orientation relative to centromere and telomere cannot be resolved by fiber-FISH experiments.

At any time, by enhancing the double colored fiber signals we found a red signal covered by the green one but not in the opposite direction. However, this signal is only detectable if the green channel is reduced to zero (no green signal). Under normal capture and enhancement conditions, this red signal is not visible. To proof, whether this is a true hybridization signal or an optical aberration we hybridized only a FITC labeled clone to DNA fibers and captured it with our default double bandpass filter. The results showed a red signal covered by the green one even in this experiment without a red label (data not shown). Therefore, we concluded that this signal derives from an optical aberration caused by the used equipment. In fact, we are using a double band pass filter and a color camera. Each signal consists of all three color portions (RGB – red-green-blue). Therefore, also a pure green FITC signal may contain some red wavelength portions not blocked by the camera filter. Thus, we cannot find that this signal is a true cross-hybridization signal. This finding has to be taken into consideration if cross hybridization is expected within a fiber-FISH experiment and if the equipment used don't separate well between blue (FITC) or green (TR) excitation.

Interestingly, we found differences in the length of labeled DNA fiber in dependence of differently labeled probe (biotin, digoxygenin) used as standard. In general, biotin labeled probes seems to generate larger labeled DNA fiber signals than the digoxygenin labeled probes (*Table 1.*). These finding is comparable to *Duell et al.* (1997b) who reported a smaller signal from the biotinylated probes than that from the digoxygenated by using AMCA for the detection of biotin and rhodamin for the detection of digoxygenin, respectively. The reason for these results are unknown but may due to optical aberrations concerning the red and green signal wavelength during the microscopic process or the image analysis. Therefore, it is recommended to calculate mean distances derived from calculation of both signals as probe size standard if possible.

Table 1.

Used clones for fiber FISH experiments, exons comprised, lengths of the λ clones insert (restriction analysis) in comparison to the length as measured by the FISH signal (either red, green or both signals used as PSS). Mean ($\bar{x}$) and standard deviation (s) of n analyzed fluorescence images are presented for each probe

Probes	Exons comprised	Insert size (kb)	Size calculated from fiber measurements in kb $\bar{x}$ ±s (n)		
			Red signal	Green signal	Both signals
CYP19					
λ3/1.1	1.1	13.7	14.6±4.3 (21)	18.3±4.6 (20)	16.4±4.7 (41)
λ4/3	3 and 4	16.0	13.5±3.8 (20)	21.9±8.7 (40)	19.1±8.4 (60)
λ2/10	4 to 8	16.1	15.8±7.3 (68)	— (—)	15.8±7.3 (68)
CYP19φ					
λ6/18	2 and 3	16.2	12.8±4.2 (24)	19.6±7.5 (19)	15.8±6.7 (43)
λ1/18	9 and 10	16.0	15.4±6.3 (10)	16.3±5.3 (59)	16.2±5.4 (69)

ACKNOWLEDGEMENT

We appreciate the expert technical assistance of R. Schüler and B. Schwitulla.

REFERENCES

Duell, T. – Nielsen, L.B. – Jones, A. – Young, S.G. – Hug, W.(1997b): Construction of two near-kilobase resolution restriction maps of the 5' regulatory region of the human apolipoprotein B gene by quantitative DNA fiber mapping (QDFM). Cytogenet Cell Genet., 79. 64–70.p.

Duell, T. – Wang, M – Wu, J.– Uj, K. – Hug, W.(1997a): High-resolution physical map of the immunoglobulin λ variant gene cluster assembled by quantitative DNA fiber mapping. Genomics, 45. 479–486p.

Florijn, R.J. – Bonden, L.A. – Vrolijk, H. – Wiegant, J. – Vaandrager, J.W. – Baas, F. – Den Dunnen, J.T. – Tanke, H.J. – Van Ommen, G.J. – Raap, A.K.(1995): High-resolution DNA Fiber-FISH for genomic DNA mapping and colour bar- coding of large genes. Hum Mol Genet., 4. 831–836.p.

Florijn, R.J. – Van De Rijke, F.M. – Vrolijk, H. – Blonden, L.A. – Hofker, M.H. – Den Dunnen, J.T. – Tanke, H.J. – Van Ommen, G.J. – Raap, A.K.(1996): Exon mapping by fiber-FISH or LR-PCR. Genomics, 38. 277–282.p.

Fürbass, R. – Kalbe, C. – Vanselow, J.(1997): Tissue-specific expression of the bovine aromatase encoding gene uses multiple transcriptional start sites and alternative first exons. Endocrinology, 138. 2813–2819.p.

Fürbass, R. – Vanselow ,J.(1995): An aromatase pseudogene is transcribed in the bovine placenta. Genet., 154. 287–291.p.

Goldammer, T. – Guérin, G. – Brunner, R.M. – Vanselow, J. – Fürbass, R. – Schwerin, M.(1994): Chromosomal mapping of the bovine aromatase gene (CYP19) and an aromatase pseudogene to chromosome 10 and syntenic group U5. Mamm. Genome, 5. 822–823.p.

Haaf, T. – Ward, D.C.(1994): Structural analysis of alpha-satellite DNA and centromere proteins using extended chromatin and chromosomes. Hum. Mol. Genet., 3. 697–709.p.

Heiskanen, M. – Hellsten, E. – Kallioniemi, O.P. – Makela, T.P. – Alitalo, K. – Peltonen, L. – Palotie A.(1995b): Visual mapping by fiber-FISH. Genomics, 30. 31–36.p.

Heiskanen, M. – Kallioniemi, O. – Palotie, A.(1996a): Fiber-FISH: experiences and a refined protocol. Genet. Anal., 12. 179–184.p.

Heiskanen, M. – Karhu, R. – Hellsten, E. – Peltonen, L. – Kallioniemi, O.P. – Palotie, A.(1994): High resolution mapping using fluorescence *in situ* hybridization to extended DNA fibers prepared from agarose-embedded cells. Bio. Techniques, 17. 928–933.p.

Heiskanen, M. – Peltonen, L. – Palotif, A.(1996b): Visual mapping by high resolution FISH. Trends Genet., 12. 379–382.p.

Heiskanen, M. – Saitta, B. – Palotie, A. – Chu, M.L.(1995a): Head to tail organization of the human COL6A1 and COL6A2 genes by fiber-FISH. Genomics, 29. 801–803.p.

Klockars, T. – Savukoski, M. – Isosomppi, J. – Laan, M. – Jarvela, I. – Petrukhin, K. – Palotie, A. – Peltonen, L.(1996): Efficient construction of a physical map by fiber-FISH of the CLN5 region: refined assignment and long-range contig covering the critical region on 13q22. Genomics, 35. 71–78.p.

Nishio, H. – Heiskanen, M. – Palotie, A. – Belanger, L. – Dugaiczyk, A.(1996): Tandem arrangement of the human serum albumin multigene family in the sub-centromeric region of 4q: evolution and chromosomal direction of transcription. J. Mol. Bio., 259. 113–119.p.

Parra, I. – Windle, B.(1993): High resolution visual mapping of stretched DNA by fluorescent hybridisation. Nat. Genet., 5. 17–21.p.

Raap, A.K. – Florijn, R.J. – Blonden, L.A.J. – Wiegant, J. – Vaandrager, J.W. – Vrolijk, H. – Den Dunnen, J. – Tanke, H.J. – Van Ommen, G.J.(1996): Fiber FISH as a DNA Mapping Tool. Methods, 9. 67–73.p.

Sjöberg, A. – Peelman, L.J. – Chowdhary, B.P.(1997): Application of three different methods to analyse fibre-FISH results obtained using four lambda clones from the porcine MHC III region. Chromosome Res., 5. 247–253.p.

Trask, B.J.(1991): DNA sequence localization in metaphase and interphase cells by fluorescence *in situ* hybridization. Meth. Cell. Biol., 35. 3–35.p.

Wang, M. – Duell, T. – Gray, J.W. – Weier, Hug(1996): High sensitivity, high resolution physical mapping by fluorescence *in situ* hybridization (FISH) on to individual straightened DNA molecules. Bioimaging, 4. 73–83.p.

Weier, Hug – Wang, M. – Mullikin, J.C. – Zhu, Y. – Cheng, J.F. – Greulich, K.M. – Bensimon, A. – Gray, J.W.(1995): Quantitative DNA fiber mapping. Hum. Mol. Genet., 4. 1903–1910.p.

Wiegant, J. – Kalle, W. – Mullenders, L. – Brookes, S. – Hoovers, J.M. – Dauwerse, J.G. – van Ommen, G.J. – Raap, A.K.(1992): High-resolution *in situ* hybridization using DNA halo preparations. Hum. Mol. Genet., 1. 587–591.p.

Authors' address: Forschungsinstitut für die Biologie landwirtschaftlicher Nutztiere
Wilhelm-Stahl-Allee 2, 18196 Dummerstorf, Germany

REFINE PHYSICAL MAPPING OF THE BOVINE MAJOR HISTOCOMPATIBILITY COMPLEX (MHC) ON *BOS TAURUS* CHROMOSOME BTA 23

GOLDAMMER, T. — HESS, M. — BRUNNER, R.M. —
BISHOP, M.D. — GELHAUS, A. — SCHWERIN, M.

SUMMARY

Different investigations in several species, including genetic and physical mapping suggest a high homology in chromosomal location and order of the MHC in mammals. However, the MHC of cattle was found to be divided into two distinct regions as already shown in one physical and several genetic mapping experiments. The present study contributes to a better understanding of the organization of the bovine MHC on BTA 23 by physical mapping of yeast artificial chromosome (YAC) probes containing 300 to 1600 Kb bovine genomic DNA which were identified by 14 MHC specific genes of cattle.

INTRODUCTION

The critical role of the mammalian MHC in immunity is known. So the identification and chromosomal localization of the genes inside of the MHC region is of specific interest. Previously mapping studies in cattle genome have clarified that the bovine MHC is located on BTA 23 (*Fries et al.*, 1986; *Iannuzzi et al.*, 1993). The chromosomal loci of several MHC specific genes were identified (*Gallagher et al.*, 1993; *Skow et al.*, 1996). By different genetically mapping experiments it was determined that there is a recombination distance of about 17 cM between the class II genes DNA, DYA, DIB, DOB, and LMP2, named class IIb genes, on the one side and the main MHC containing the class I, class III, and the classical class II genes of the DQ and DR families, named class IIa genes on the other side (*Andersson et al.*, 1988; *Georges et al.*, 1990; *van Eijk et al.*, 1993; *Stone et al.*, 1993; *Park et al.*, 1995; *Shalhevet et al.*, 1995). Because of the existing differences in the gene order and the genetic distances for the MHC genes in the available gene maps of BTA 23 (e.g. *Creighton et al.*, 1992; *Bishop et al.*, 1994; *Barendse et al.*, 1994; *van Eijk et al.*, 1995) additional mapping studies are require to resolve the structure of the bovine MHC. In fact this will be useful for positional cloning of genes underlying phenotypic variance. In this study the physical mapping of 14 MHC specific genes on BTA 23 by fluorescence *in situ* hybridization (FISH) is presented.

MATERIAL AND METHODS

YACs containing MHC specific genes were identified by PCR screening of a YAC library containing bovine genomic DNA using primers derived from known selected MHC gene sequences. Positive clones were characterized by determining the genes that they contain, using PCR with primers specific for class I, class IIa, class IIb, or class III genes. The PCR products were sequenced, and their identities were confirmed by comparison of their homology with published sequences.

The isolated YACs were prepared as probes for FISH on bovine metaphase chromosomes. Therefore, 1 µg of each bovine genomic DNA containing YAC-probe was labelled by nick translation with biotin-16-dUTP or alternatively biotin-14-dATP. After ethanol precipitation at –80 °C for 2 hours each probe was prehybridized in a final volume of 10 µl hybridization mix (50% formamid, 10% dextrane sulphate, 2xSSC, pH 7.0) containing the complete labeled YAC probe (1µg), 20 µg salmon sperm DNA, 10 µg genomic cattle DNA, and 10µg bovine Cot-1 DNA at 37 °C for 40 minutes prior to FISH. The probe hybridization mix was than put on prephotographed slides (two probes side by side), covered with 18x18 mm cover slips, and hybridized overnight. After posthybridization washing steps slides were overlaid with FITC using the Streptavidin/Antiavidin system. Following, the slides were stained with PI, counterstained with DABCO-antifade solution, and than analyzed. Detection of hybridization signals minimal on 10 metaphase spreads was carried out with an epifluorescence

microscope of type Nikon FXA equipped with a dual band pass filter for FITC and Texased. Metaphase chromosomes for FISH were prepared from bovine embryonic fibroblasts and G-banded for identification by standard procedures. Metaphases were digitized and karyotyped with the software package Mac K-Type (Perceptive Scientific Instruments).

RESULTS AND DISCUSSION

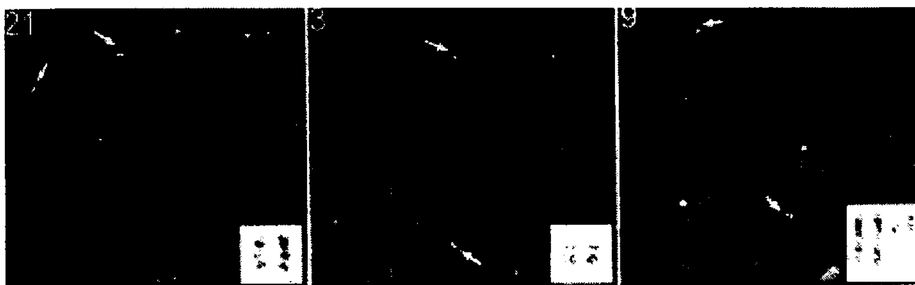
By PCR screening of the bovine YAC library with MHC gene specific primers twenty four YACs were identified. Until now the characterization of these YAC clones with 14 primer pairs of the MHC specific genes DMA, DMB, DNA, DIB, DYA, DOB, LMP2, LMP7, DQB, DQA, DRB3, DRA, TNFα, and BoLA-A have shown that the bovine MHC genes detected in the individual YACs divide the YACs in three groups as shown in *Table 1*. The first group contains the MHC class IIb genes, the second group the MHC class IIa genes, and the third group the class III and class I genes. All YACs were proved by FISH for their chimerical status. It was found that 7 of the 24 YACs are not chimerical.

Table 1.

Characterization of identified MHC specific YACs. Size of YACs was proved by RFLP analysis. (e.g. the YACs 1, 2, and 13 represent all genes of the corresponding MHC class analyzed until now; n.d. = not detected)

YAC	Size	MHC IIb								MHC IIa				MHC III	MHC I
clone	kb	DMA	DMB	DNA	DIB	DYA	DOB	LMP2	LMP7	DQB	DQA	DRB3	DRA	TNFα	BoLA-A
1	960													+	+
2	1200									+	+	+	+		
3	1300									+	+	+	+		
4	365									+	+	+			
5	470									+	+	+			
6	600									+	+	+			
7	1600									+	+				
8	1000									+					
9	580											+	+		
10	500												+		
11	750												+		
12	n.d.	+	+	+	+	+	+	+	+						
13	n.d.	+	+	+	+	+	+	+	+						
14	580		+	+	+	+	+	+	+						
15	500		+	+	+	+	+	+	+						
16	580		+	+	+	+	+	+	+						
17	590				+	+	+	+	+						
18	470				+	+	+	+	+						
19	n.d.					+		+	+						
20	n.d.				+	+	+								
21	450				+	+	+								
22	780					+	+								
23	350				+										
24	800				+										

Fig. 1.: **Examples for FISH of MHC specific YAC probes on bovine metaphase chromosomes. YAC probe 21 on BTA 23q12-13 (left), YAC probe 3 on BTA 23 q21 (middle), and the chimera YAC probe 9 with FITC signals on BTA 23q21 and BTA 1**



The probes explained very clear fluorescence signals either to the centromere or to the center of BTA 23 as shown for selected YAC probes in *Fig. 1.* YAC probes 13, 15, and 21 containing class IIb genes (DMA, DMB, DIB, DYA, DOB, LMP2, and LMP7) were assigned to chromosome region BTA 23q12-13, whereas YAC probes 2, 3, 4, and 10 including class IIa genes (DQA, DQB, DRB3 and DRA) were localized on BTA 23q21. The YAC probe 1 containing a class III gene (TNFα), and a class I gene (BoLA-A), hybridized to chromosome band BTA 23q21–22. The mapping results of the non chimerical YACs and of the chimerical YAC clone 1 which represents alone the MHC classes III and I by the previously mapped genes BoLA-A and TNFα are summarized in *Table 2,* and the physical loci of these YAC probes are given in an ideogram of BTA 23 in *Fig. 2.* The identified loci demonstrate the clear physical distance of the MHC class IIb from the main MHC. Additional this become support by the double FISH of YAC probes 13 and 2, also shown in *Fig. 2.*

Table 2.: **Mapping results of all non chimerical YACs and the chimera YAC 1**

YAC clone	chimera	analyzed metaphases	FL_{cen} values	G-band locus
1	yes	14	0.632±0.050	23q21-q22
2	no	15	0.576±0.038	23q21
3	no	14	0.616±0.041	23q21
4	no	10	0.606±0.044	23q21
10	no	10	0.622±0.025	23q21
13	no	11	0.325±0.027	23q12-q13
15	no	12	0.328±0.046	23q12-q13
21	no	12	0.322±0.040	23q12-q13

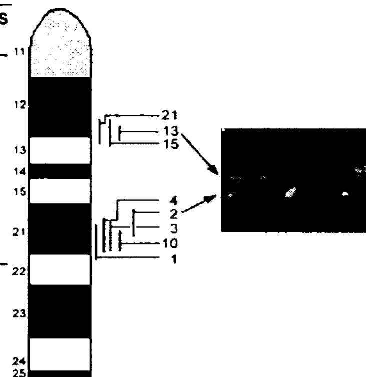


Fig. 2: **Summary of YAC loci in an ideogram of BTA 23, and double FISH of YAC probes 2 and 13.**

Information's to all mapped genes inside of the YAC probes like chromosomal localization and previously physical mapping data are summarized in *Table 3,* and both the loci of the genes and simultaneously with it of the different MHC classes are shown in an ideogram in Fig. 3. All presented results confirm the physical location of the bovine MHC class IIb region on the crossing region of the G-bands q12 to q13 on BTA 23. The chromosomal location of the classical class II genes of the DQ and DR families was clarified to be on BTA 23q21. This location is more proximal as assumed until now, and also the proved location of BoLA-A on BTA 23q21-q22 that represents the MHC class I was found more proximal as detected in an earlier mapping experiment by *Iannuzzi et al.* (1993). The close physical proximity of the classical class IIa genes to the class I and class III genes in the bovine MHC, as well as the large physical separation of the class IIb genes from the main MHC in cattle was confirmed. The BTA 23 specific YACs are now an useful tool for both the generation of a MHC specific DNA contig and the construction of a physical fine map of MHC specific genes.

Table 3.: **Physical mapping data of MHC specific genes**

locus name	locus symbol	present mapping results locus	previously physical mapping results
BoLA-DI β-chain	DIB	23q12-13	
BoLA-DM α-chain	DMA	"	
BoLA-DM β-chain	DMB	"	
BoLA-DN α-chain	DNA	"	
BoLA-DO β-chain	DOB	"	
BoLA-DY α-chain	DYA	"	23q12-13 (*Skow et al.*, 1996)
large multifunctional protease 2	LMP2	"	
large multifunctional protease 7	LMP7	"	
BoLA-DQ α-chain	DQA	23q21	
BoLA-DQ β-chain	DQB	"	
BoLA-DR α-chain	DRA	"	
BoLA-DR β-chain	DRB3	"	
tumor necrosis factor α	TNFα	23q21-22	
Bovine lymphocyte antigen A	BoLA-A	23q21-22	23q22 (*Iannuzzi et al.*, 1993)

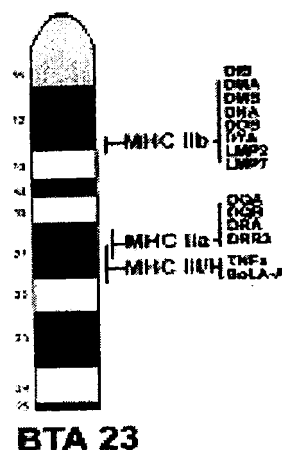


Fig. 3.: **Loci of MHC specific genes in an ideogram of BTA 23**

REFERENCES

Andersson, L. – Lunden, A. – Sigurdardottir, S. – Davies, C.J. – Rask, L.(1988): Linkage relationships in the bovine MHC region. High recombination frequency between class II subregions. Immunogenet., 27. 4. 273–280.p.

Barendse, W. – Vaiman, D. – Kemp, S.J. – Sugimoto, Y. – Armitage, S.M. – Williams, J.L. – Sun, H.S. – Eggen, A. – et al.(1997): A medium-density genetic linkage map of the bovine genome. Mamm. Genome, 8. 21–28.p.

Bishop, M.D. – Kappes, S.M. – Keele, J.W. – Stone, R.T. – Sunden, S.L. – Hawkins, G.A. – Toldo, S.S. – Fries, R. – Grosz, M.D. – Yoo, J. – et al.(1994): A genetic linkage map for cattle. Genetics., 136. 2. 619–639.p.

Creighton, P. – Eggen, A. – Fries, R. – Jordan, S.A. – Hetzel, J. – Cunningham, E.P. – Humphries, P.(1992): Mapping of bovine markers CYP21, PRL, and BoLA DRBP1 by genetic linkage analysis inreference pedigrees. Genomics, 14. 2. 526–528.p.

Fries, R. – Hediger, R. – Stranzinger, G.(1986): Tentative chromosomal localization of the bovine major histocompatibility complex by *in situ* hybridization. Anim. Genet., 17. 4. 287–294.p.

Gallagher, D.S. Jr. – Grosz, M.D. – Womack, J.E. – Skow, L.C.(1993): Chromosomal localization of HSP70 genes in cattle. Mamm. Genome, 4. 388–390.p.

Georges, M. – Lathrop, M. – Bouquet, Y. – Hilbert, P. – Marcotte, A. – Schwers, A. – Roupain, J. – Vassart, G. – Hanset, R.(1990): Linkage relationships among 20 genetic markers in cattle. Evidence for linkage between two pairs of blood group systems: B-Z and S-F/V respectively. Anim. Genet., 21. 95–105.p.

Iannuzzi, L. – Gallagher, D.S. Jr. – Womack, J.E. – Di Meo, G.P. – Skow, L.C. – Ferrara, L.(1993): Chromosomal localization of the major histocompatibility complex in cattle and river buffalo by fluorescent *in situ* hybridization. Hereditas, 118. 187–190.p.

Park, C. – Russ, I. – Da, Y. – Lewin, H.A.(1995): Genetic mapping of F13A to BTA 23 by sperm typing: differences in recombination rate between bulls in the DYA-PRL interval. Genomics, 27. 113–118.p.

Shalhevet, D. – Da, Y. – Beever, J.E. – van Eijk, M.J.T. – Ma, R. – Lewin, H.A. – Gaskins, H.R.(1995): Genetic mapping of the LMP2 proteosome subunit gene to the BoLA class IIb region. Immunogenet., 41. 44–46.p.

Skow, L.C. – Snapples, S.N. – Davis, S.K. – Taylor, J.F. – Huang, B. – Gallagher, D.S. Jr.(1996): Localization of bovine lymphocyte antigen (BoLA) DYA and class I loci to different regions of chromosome 23. Mamm. Genome, 7. 388–389.p.

Stone, R.T. – Muggli-Cockett, N.E.(1993): BoLA-DIB: species distribution, linkage with DOB, and northem analysis. Anim. Genet., 24. 41–45.p.

Van Eijk, M.J.T. – Beever, J.E. – Da, Y. – Stewart, J.A. – Nicholaides, G.E. – Green, C.A. – Lewin, H.A.(1995): Genetic mapping of BoLA-A, CYP21, DRB3, DYA, and PRL on BTA23. Mamm. Genome, 6. 151–152.p.

Van Eijk, M.J.T. – Russ, I. – Lewin, H.A.(1993): Order of bovine DRB3, DYA, and PRL determined by sperm typing. Mamm. Genome, 4. 113–118.p.

Authors' address: *Goldammer, T. – Brunner, R.M. – Schwerin, M.:* Forschungsbereich Molekularbiologie, Forschungsinstitut für die Biologie landwirtschaftlicher Nutztiere, D 18196 Dummerstorf, W.-Stahl-Allee 2, Germany, http://www.fbn-dummerstorf.de/fb3/tom/tom1de.htm

Hess, M. – Gelhaus, A.: Molekularbiologie, Bernhard-Nocht-Institut für Tropenmedizin, D 20359 Hamburg, Germany

Bishop, M.D.: American Breeders Service, Research and Technology, DeForest, Wisconsin 53232, USA

MOLECULAR ANALYSIS OF ROBERTSONIAN TRANSLOCATIONS

JOERG, H. — RIEDER, S. — SUWATTANA, D. — STRANZINGER, G.

SUMMARY

The chromosomal fusion of acrocentric chromosomes, known as the Robertsonian translocation, is the most common chromosome rearrangement in Bovidae. In general, a Robertsonian translocation has no visible phenotypic effect on the carrier and segregates through generations. Fertilization differences, embryonic mortality and selective forces in all stages of development can contribute to the altering ratio of offspring from heterozygote carriers. Different fusion carriers were crossed within and between different breeds to study segregation patterns and effects of the fusion on fertility in one pedigree. Cytogenetic studies using different banding techniques showed the presence of rob(1;29) and rob(14;20) in the same animal. Molecular and *in situ* hybridization studies revealed repetitive sequences, known as alphoid satellite DNA, that are tightly associated with the centromeric regions. Fluorescence *in situ* hybridization (FISH) with bovine alphoid satellite probes was used to detect differences in Robertsonian translocations. The bovine 1.715 satellite DNA probe hybridized to all acrocentric chromosomes. No signal was observed on the X-,Y- and the fused chromosome 1;29. The signal on chromosome 14;20 appeared like a double signal and confirmed the cytogenetic state of a dicentric chromosome. The chromosome 1;29 due to the absence of both alphoid sequences, a monocentric chromosome. Alphoid DNA is not necessary for the functioning of the centromere, but the varying number of attachment places for the centromere proteins could be a reason for increased non-disjunctions and reduced fertility.

Microsatellite markers in the linkage group originating from the fusion are able to recognize a buli as a putative carrier of the Robertsonian translocation 1;29. An informative allele combination in all homozygote and heterozygote carriers of the Robertsonian translocation 1;29 from Brown Swiss and Simmental breeds confirm the hypothesis of a common origin of chromosome 1;29. The fusion appears to have taken piace before the origination of current breeds.

INTRODUCTION

Genetic monitoring of inherited diseases is carried out in many breeding countries. Among other things this also includes cytogenetic analysis to examine whether centromeric fusions are present, particularly in bulls used for artificial insemination. The "Institute of Animal Science ETH Zurich" carries out cytogenetic analysis on bulls bred in Switzerland. Approximately 340 bulls are analysed per year, with positive findings in several offspring of two imported Brown Swiss bulls. As these two bulls are not related, an hypothesis was made that there are two different fusions of chromosomes 1 and 29. Since the first publishing by *Gustavsson and Rockborn* (1964), the Robertsonian translocation 1;29 had been reported worldwide in approximately 50 breeds (*Popescu*, 1990). This worldwide distribution can be explained by a de novo mutation as well as by a common origin. Heterozygote carriers were studied to determine the breakpoints involved in the merging of these chromosomes. The segregation between microsatellite markers near the centromere of chromosomes 1 and 29 and the fusion were analysed. Fluorescence *in situ* hybridization using alphoid DNA probes, C-banding and typing of microsatellite markers were used to analyse the Robertsonian translocations.

MATERIALS AND METHODS

Three different groups of Robertsonian translocation carriers were chosen for this study. The first group were Brown Swiss descendants of the bull Jester, who is known to be a carrier of the Robertsonian translocation 1;29. The second group were bulls whose pedigree did not include Jester in the last six generations. The third group were carriers of the Robertsonian translocation 14;20.

Bovine genomic DNA and primers specific to the sequence of the bovine 1.715 satellite DNA (*Plucienniczak et al.*, 1982) were used for the PCR amplification. The microsatellite markers were selected from the "cattle genome map" of MARC, USDA. On chromosome 1, AGLA17, BM6438 and TGLA49 and on chromosome 29, BM4602, BMC2228 and BMS1857 were analysed. The PCR was carried out in a reaction volume of 25 ul containing 100 ng of bovine and human genomic DNA, respectively, 10 mM Tris-HCl pH 8.9, 50 mM KCl, 1.5 mM $MgCl_2$, 200 uM of each deoxynucleotide, 0.4 µM of each primer and 2.5 U of Taq DNA polymerase (Boehringer Mannheim) in a thermal cycler (Hybaid). After an initial denaturation of 5 min at 95 °C, the PCR profile consisted of a denaturation step at 94 °C for 30 s, an annealing step at 55 °C for 30 s and an elongation step at 72 °C for 30 s for a total of 30 cycles, followed by a final extension of 7 min at 72 °C. Using the QIAEX II kit (QUIAGEN Inc.) the PCR product was extracted from the agarose gel. The purified DNA was then labeled by random priming with biotin-16-dUTP. Efficiency of this labeling was determined by a dot biot assay (BluGene Kit BRL). Fluorescence *in situ* hybridization was performed on metaphase chromosomes as described by *Solinas-Toldo et al.* (1993). The probes were hybridized at a final concentration of 1ng/µl (10µl/18x18mm), without a suppression of repetitive DNA sequences. Pictures were taken by digital imaging microscopy using a cooled CCD camera system (Photometrics). The images were processed using IPLab and GeneJoin software package (*Ried et al.*, 1992). An ABI 373 Sequencer in combination with ABI 672 Genescan software was used to evaluate the size of the PCR-fragments of the microsatellite markers.

RESULTS AND CONCLUSIONS

Fluorescence *in situ* hybridization was used to characterize the Robertsonian translocations 1;29 and 14;20 for the presence of DNA derived from the short arms of the translocated acrocentric chromosomes and the centromeres. With the bovine 1.715 satellite DNA all 1;29 translocation chromosomes of six different animals were without signal. This result strongly suggests that the chromosomal rearrangements leading to Robertsonian translocations 1;29 occur in or next to the centromeric alpha-repeat region. This indicates that both breakpoints were on the proximal long arm

of acrocentric chromosomes. By contrast, all 14;20 translocation chromosomes gave a distinct hybridization signal. The results provide direct molecular cytogenetic evidence that Robertsonian translocations can take place in different regions in both the short arm and proximal long arm of acrocentric chromosomes. The DNA and protein composition of the centromeric domains in mammalian chromosomes is now relatively well characterised (*Mitchell*, 1996). Major repetitive DNA sequences, i.e. alphoids in man and satellite sequences (both major and minor) in the mouse have been sequenced and long-range maps using pulse-field gels of some centromeres have been produced. Autoimmune antibodies have provided an insight into some of the proteins which interact with these DNA sequences. Although the individual components of the mammalian centromere may have been identified, how they interact with each other to give the functional structure is yet to be determined. The segregation of the Robertsonian translocation 1;29 and studies of the human chromosomes (*DuSart et al.*, 1997) have shown that a functional centromere does not have to contain alphoid DNA. *Schmutz et al.* (1997) showed that fertility is severely impaired in carriers of the 14;20 translocation, as was observed with the 1;29 translocation, with most loss due to embryo mortality rather than a lowered conception rate. We hypothesised that due to the loss or the addition of centromere sequences, the different number of attachment places for centromere proteins could be the reason for increased non-disjunctions and reduced fertility.

Typing of the microsatellite markers AGLA17, BM6438, TGLA49, BM4602, BMC2228 and BMS1857 in Brown Swiss bulls with or without Jester blood resulted in an allel combination: 116, 259, 220, 113, 172 and 161, respectively, which segregates with the Robertsonian translocation 1;29. In an additional random sample from Brown Swiss and Simmental cattle the informative allele combination was found in all homozygote and heterozygote carriers of Robertsonian translocation 1;29. This finding confirms the hypothesis of a common origin of chromosome 1;29, which was strongly supported by the fact that despite the significant number of animals studied, not a single case of de novo appearance of the translocation has been reported (*Popescu*, 1990). Furthermore chromosome 1;29 appears to be very stable because it has segregated through many generations. The use of conventional cytogenetic in combination with molecular studies have allowed a more precise evaluation of the breakpoints involved in Robertsonian translocations than either approach alone might have done.

REFERENCES

Du-Sart, D. – Cancilla, M.R. – Earle, E. – Mao, J.I. – Saffery, R. – Tainton, K.M. – Kalitsis, P. – Martyn, J. – Barry, A.E. – Choo, K.H.A.(1997): A functional neo-centromere formed through activation of a latent human centromere and consisting of non-alpha-satellite DNA. Nature Genetics,16. 2. 144–153.p.

Gustavsson, I. – Rockborn, G.(1964): Chromosome abnormality in three cases of lymphatic leukemia in cattle. Nature, 203. 990.p.

Mitchell, A.R.(1996): The mammalian centromere: Its molecular architecture. Mutation Research, 372. 2. 153–162.p.

Popescu, P.C.(1990): Cattle chromosomes. In: Domestic animal cytogenetics. Ed. R.A. McFeely, Academic Press, San Diego, 41–71.p.

Ried, T. – Baldini, A. – Rand, T.C. – Ward, D.C.(1992): Simultaneous visualization of seven different DNA probes by *in situ* hybridization using combinatorial fluorescence and digital imaging microscopy. PNAS, 89. 4. 1388–1392.p.

Schmutz, S.M. – Moker, J.S. – Pawlyshyn, V. – Haugen, B. – Clark, E.G.(1997): Fertility effects of the 14;20 Robertsonian translocation in cattle. Theriogenology, 47. 4. 815–823.p.

Toldo, S.S. – Fries, R. – Steffen, P. – Neibergs, H.L. – Barendse, W. – Womack, J.E. – Hetzel, D.J.S. – Stranzinger, G.(1993): Physically mapped, cosmid-derived microsatellite markers as anchor loci on bovine chromosomes. Mamm. Genome, 4. 12. 720–727.p.

Authors' address: Institut für Nutztierwissenschaften der ETH, Gruppe Züchtungsbiologie,
TAN 1, ETH Zentrum
CH-8092 Zürich, Switzerland

MOLECULAR ANALYSIS AND COMPARATIVE FISH-MAPPING OF TCR GENES *IN BOVIDAE*

LIPSI, M.R. — ANTONACCI, R. — MASSARI, S. —
DE CARO, F .— MICCOLI, M. — CICCARESE, S.

SUMMARY

We have isolated and characterized five genomic clones representing sheep TCRA, TCRD and TCRG regions. All genomic clones were used in FISH experiments on sheep chromosomes obtained from peripheral blood lymphocytes. When λLVAI7 (TCRA) and λMCD (TCRD) clones were co-hybridized, specific signals were found on sheep chromosome band 7q14–22. The co-mapping of the two genomic clones on sheep chromosome 7 is the first evidence by FISH indicating the co-localization of TCRA and TCRD genes *in Bovidae*. Moreover, when λ6A3 (TCRG I) and λ17A5 or λ18A6 (TCRG2) clones were used in co-hybridization experiments two distant signals on the same sheep chromosome 4 were observed. Furthermore, we demonstrated that TCRG 1 and TCRG2 genes are divided also on homologous chromosomes of goat, cattle and river buffalo. Such result suggests a possible recombinative event that could have occurred in chromosome 4 before *Bovidae* family radiation.

INTRODUCTION

The T-cell receptor (TCR) complex has been shown to consist either of a disulfde-linked αβ (TCRA/TCRB) or γδ (TCRG/TCRD) heterodimer that is noncovalently associated with an invariant set of molecules called CD3 complex (*Danska,* 1989). Functional α, β, γ and δ variable regions originate from somatic rearrangements of variable (V), diversity (D) and joining (J) gene segments, with the exception of α and γ genes which lack the D segments. After transcription the variable sequence is spliced to a constant (C) region.

The chromosomal localization of TCRG, TCRB, TCRA and TCRD genes is well known in human. While TCRB and TCRG are encoded by genes located on different portions on chromosome 7 (7q35 and 7p14–15 respectively), TCRD genes are embedded in TCRA locus on chromosome 14 (*O'Brien,* 1993).

In artiodactyls, an order that represents the most dramatic radiation of large mammals, little information is known about the chromosomal localization of these loci. Particularly, in sheep TCRB locus has been subregionally mapped on chromosome 4 (*Pearce et al.*, 1995) while TCRA/TCRD locus has been recently assigned to chromosome 7 (*Broad et al.*, 1995).

In cattle TCRG and TCRB loci have been assigned to chromosome 4 (*Eggen et al.,* 1995) and TCRA/TCRD locus has been mapped on chromosome 10 (*Solinas-Toldo et al.*, 1995). In this paper we report the physical assigment of TCRA/TCRD and TCRG loci on sheep chromosomes using specific ovine genomic clones one of them corresponding to a new Vα element that shows homology with a human Vα segment, the others containing the sheep C, Cλ1 and Cλ2 constant genes.

MATERIAL AND METHODS

Four different probes were used to screen, under high stringency hybridization conditions, a sheep genomic library from lung DNA in λDASHII vector (Stratagene, La Jolla, CA). The first probe was a KpnI/PstI 260 bp long fragment corresponding to a Va sheep element (pBVT49) found in sheep foetal thymus DNA (data not shown). The other probes were cDNA consisting of the sheep Cδ, Cλ1 and Cλ2 genes (gift from Dr. W. R. Hein, Basel Institute for Immunology, Basel, Switzerland). Positive clones were recovered and further characterized by Southern blot analysis. Selected sections of the inserts were subcloned and sequenced. Sheep, goat, cattle and river buffalo chromosome preparations were obtained from peripheral blood lymphocytes according to standard procedures. Detailed procedures for FISH experiments and image processing have been reported previously (*Massari et al.*, 1997).

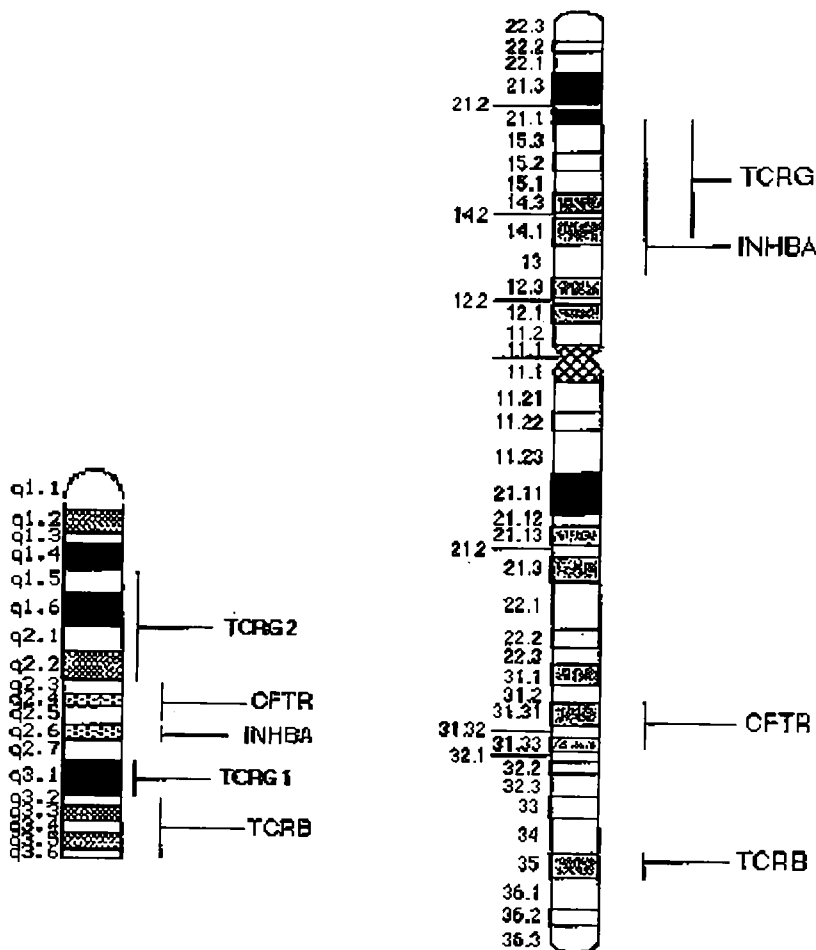
RESULTS AND DISCUSSION

Two genomic clones, with inserts of approximately 14 Kb, representing the sheep TCRA/TCRD region have been isolated. The first clone (λLVAI7) contains a new V gene, never described in sheep before, that shows a high degree of amino acid identity (76%) with a human Vα segment (*Guglielmi et al.,* 1988). The other phage clone (λMCD) referring to TCRD locus, corresponds to the entire Cδ gene. When digoxigenin-11-dUTP labelled λLVAI7 and biotin-16-dUTP labelled λMCD were hybridized on sheep metaphase spreads, specific signals were found on band 7q14-22. The co-mapping of our genomic clones on sheep chromosome 7, presented here, is the first evidence by FISH indicating the co-localization of TCRA and TCRD genes *in Bovidae*. Until now, only two genes have been regionally assigned to sheep chromosome 7 by isotopic *in situ* hybridization: GMBTI9 (D7S1) probe, which was regionally mapped to 7q15-q22 (*Georges et al.*, 1991) and the hexosaminidase A (alpha polypeptide) (HEXA), which was mapped to 7q 12–13 (*Broad et al.*, 1995). Therefore, the TCRA/TCRD mapping to sheep chromosome 7 is the first regional assignment to this chromosome by fluorescence *in situ* hybridization.

Moreover, *in Bovidae* the only regional mapping of TCRA locus has been reported on bovine at 10q15 position (*Solinas-Toldo et al.,* 1995). Considering that 10q15 bovine region presents conserved synteny with human 14q112 chromosome band (*Solinas-Toldo et al.*, 1995), our data imply that ovine 7q 14–22 region, bovine I Oq 15 region and the human 14q 11.2 region belong to the same group of synteny.

For sheep TCRG locus three genomic clones have been isolated and characterized (*Massari et al.*, 1998). Two overlapping clones, λ17A5 and λ18A6, retain the entire gene for the constant region Cγ2. The other clone, λ6A3, contains the first exon for the constant gene Cγ1. When digoxigenin-11-dUTP labelled λ6A3 and biotin-16-dUTP labelled λ17A5 or λ18A6 probes were co-hybridized on sheep metaphase spreads, specifc signals were detected on one pair of chromosomes in two distant positions. For each probe the regional cytological position was determined. λ6A3 clone referring to Cγ1 gene maps at 4q3.1 while λ17A5 or λ18A6 clones referring to Cγ2 gene map at 4q1.5–2.2 bands. The localization of Cγ1 and Cγ2 constant genes in different regions of the same chromosome suggests that TCRG locus is split in sheep giving rise to TCRG1 and TCRG2 clusters. As in sheep ideogram (*Fig. 1.*), TCRG 1 genes are close to the 4q2.6 inhibin beta A (INHBA) gene (*Ansari et al.,* 1994) and both these loci are located in between the 4q23–25 CFTR (*Tebbutt et al.*, 1996) and 4q3.2-qter TCRB (*Pearce et al.*, 1995) loci. In human TCRG locus, which contains both Cy1 and Cy2 constant genes, maps close to inhibin beta A (INHBA) gene on the short arm of chromosome 7 (*O'Brien,* 1993). This localization is therefore outside the chromosomal segment whose boundaries are the CFTR and TCRB markers which map in the long arm of chromosome 7. The sheep TCRG 1 cluster chromosomal location, presented here, closely connected to INHBA gene, is consistent with the remarkable conservation of linkage organization of TCRG and INHBA genes found in human. This linkage conservation is likely to reflect an ancestral gene organization inherited through evolution radiation of the mammalian orders. In addition, the location in sheep of TCRG I genes close to TCRB cluster, as a typical example of gene duplication (*Lundin,* 1993), would support the hypothesis that the arrangement of CFTR, INHBA, TCRG1 and TCRB genes in the distal portion of sheep chromosome 4 reflects an ancestral situation. Thus, in human chromosome 7, a rearrangement might have taken piace, which would explain the separation of TCRG from TCRB genes. On the other hand, the different location of TCRG2 with respect to TCRG1 cluster on the same chromosome could have been caused by an intrachromosomal rearrangement which took TCRG2 locus away from TCRGI. As a consequence of the unexpected split of the TCRG locus in sheep we extended the FISH study to other species of the *Bovidae* family i.e. goat, cattle and river buffalo. On all these examined species, a similar hybridization pattern of the Cγ1 and Cγ2 probes was found on chromosomes homologous to sheep chromosome 4. FISH results map TCRG genes on goat and cattle chromosome 4 (*ISCNDAB9* 1990; *Texas nomenclature*, 1996) and on river buffalo chromosome 8 (*Committee for Standardized Karyotype of Bubalus bubalis,* 1994; *Texas nomenclature,* 1996). This chromosomal rearrangement common to the *Bovidae* family may be associated to the peculiarity of TCRG genes which are involved in a series of duplication events along the ruminant lineage, culminating in a cluster of at least fve groups of genes in sheep and four groups of genes in cow. Finally, we believe the distal part of sheep chromosome 4 could represent an evolutionary "hot spot" in the *Bovidae* chromosomal evolution.

Fig. 1.: **Diagrammatic representation of sheep chromosome 4 (a) and human chromosome 7 (b). Loci markers from previous works and the new localization (boldface type) on the sheep map have been insered. On human chromosome 7 only orthologous genes were reported. I-luman and sheep idiogram were extracted from GDB and SheepBase**



REFERENCES

Ansari, H.A. – Pearce, P.D. – Maher, D.W. – Broad, T.E.(1994): Regional assignment of conserved reference loci anchors unassigned linkage and syntenic groups to ovine chromosomes. Genomics, 24. 451–455.p.

Broad, T.E. – Burkin, D.J. – Cambridge, L.M. – Pearce, P.D. – Maher, D.W. – Ansari, H.A. – Jones, C.(1995): Physical assignment of loci to sheep chromosome 7 confirms its homology to cattle chromosome 10. Mamm. Genome, 6. 749–750.p.

Committee for Standardized Karyotype of Bubalus bubalis(1994): Standard karyotype of the river buffalo (*Bubalus bubalis* L., 2n=50). Report of the committee for the standardization of banded karyotypes of the river buffalo (L. Iannuzzi, coordinator). Cytogenet Cell. Genet., 67. 102–113.p.

Danska, J.S.(1989): The T cell receptor: structure, molecular diversity and somatic localization. Curr. Opin. Immunoi., 2. 81–86.p.

Eggen, A. – Fries, R.(1995): An integrated cytogenetic and meiotic map of the bovine genome. Animal. Genet., 26. 215–236.p.

Georges, M. – Gunawardana, A. – Threadgill, D.W. – Lathrop, M. – Olsaker, I. – Mishra, A. – Sargeant, L.L. – Schobcrlein, A. – Steele, M.R. – Terry, C. – Threadgill, D.S. – Zhao, X. – Holm, T. – Fries, R. – Womack, J.E.(1991): Characterization of a set of variable number of tandem repeat markers conserved in Bovidae. Genomics, 11. 24–32.p.

Guglielmi, P. – Davi, F. – d'Auriol, L. – Bories, J.C. – Dausset, J. – Bensussan, A.(1988): Use of a variable alpha region to create a functional T-cell receptor delta chain. Proc. Natl. Acad. Sci., USA, 85. 5634–5638.p.

ICSNDA 1989(1990): International System for Cytogenetic Nomenclature of Domestic Animals, 1989. Cytogenet. Cell Genet., 53. 65–79.p.

O'Brien (ed.)(1993): "Genetics maps". Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Lundin, L.G.(1993): Evolution of the vertebrate genome as reflected in paralogous chromosomal regions in man and the house mouse Genomics, 16. 1–19.p.

Massari, S. – Antonacci, R. – De Caro, F. – Lipsi, M.R. – Ciccarese, S.(1997): Assignment of the TCRA/TCRD locus to sheep chromosome bands 7q1.4–>q2.2 by fluorescence *in situ* hybridization. Cytogenet Cell Genet., 79. 193–195.p.

Massari, S. – Lipsi, M.R. – Vonghia, G. – Antonacci, R. – Ciccarese, S.(1998): T-cell receptor TCRGI and TCRG2 clusters map separately in two different regions of sheep chromosome 4. Chromosome Research, in press

Pearce, P.D. – Ansari, H.A. – Maher, D.W. – Broad, T.E.(1995): Five regional localizations to the sheep genome: frst assignments to chromosomes 5 and 12. Animal Genet., 26. 171–176.p.

Solinas-Toldo, S. – Lengauer, C. – Fries, R.(1995): Comparative genome map of human and cattle. Genomics, 27. 489–496.p.

Tebbutt, S.J. – Broom, M.F. – van Stijn, T.C. – Montgomery, G.W. – Hill, D.F.(1996): Genetic and physical mapping of the ovine cystic fibrosis gene. Cytogenet Cell Genet., 74. 245–247.p.

Texas nomenclature(1996): Standardization of cattle kayotype nomenclature: report of the committee for the standardization of the cattle kayotype. (Popescu, C.P. – Long, S. – Riggs, P. – Womack, J. – Schmutz, S. – Fries, R. – Gallagher, D. eds). Cytogenet Cell Genet., 74. 259–261.p.

Authors' address: Institute of Genetics, University of Bari
Via Amendola 165/A 70126 Bari, Italy

ZOO-FISH MAPPING AND R-BANDING REVEALED AN EXTENSIVE CONSERVATION OF HUMAN CHROMOSOME REGIONS IN RIVER BUFFALO CHROMOSOMES

IANNUZZI, L. — DI MEO, G.P. — PERUCATTI, A. — BARDARO, T. — FERRARA, L.

Commercially available specifc human chromosome libraries (Painting kit 1089- KB, Cambio, England) were used for this study. Chromosome preparations were from blood lymphocyte cultures treated for the late-incorporation of both BrdU (10 µg/ml) and Hoechst 33258 (20 µg/ml). Slides and human probe DNA denaturation, as well as probe signal detection, followed normal protocols. Metaphases with both hybridization signals and RBH-banding were separately captured by using a CCD-camera. To improve banding patterns, the slides were later counterstained with acridine orange. At least 15 early-metaphases and prometaphases were studied for each probe. River buffalo chromosome identification and banding were according to the standard karyotype (CSKBB, 1994). Comparisons were performed with previous gene localizations made in river buffalo chromosomes, cattle standard karyotypes and ZOO-FISH data reported in cattle. The clear banding pattern achieved allowed not only the easy identifcation of chromosomes but also of chromosome regions and bands painted by human probes. Our results demonstrate a high degree of human chromosome region conservation in river buffalo chromosomes, even if complex chromosome rearrangements occurred during the karyotype evolution which differentiated the artiodactyls and primates. Only BBU13 (homoeologous to BTA 12) shows a banding pattern similar to that of HSA 13. The data we obtained fully agree with previous molecular marker assignments made in this species and are essentially in agreement with previous ZOO-FISH mapping made in cattle.

The ZOO-FISH mapping data achieved on BBUlp (HSA 8), BBU4p (HSA10), BBUSp (HSA11) and BBU24 (HSA16+7) confrm our previous FISH-mapping data obtained in river buffalo and further support that errors were made in cattle (and homoeologous species) standard nomenclatures.

ACKNOWLEDGMENTS

We thank Mr. D. Incarnato for technical assistance and microscope image processing. This study has in part been supported by INC, Biologia e Produzione Agraria del CNR, P.S. Biologia e Produzione Agraria per un'agricoltura sostenibile.

Author's address: *National Research Council* (CNR)
I.A.B.B.A.M., *Naples, Italy*

USE OF HUMAN ESTS FOR MAMMALIAN COMPARATIVE MAPPING: 65 HUMAN ESTS LOCALIZED IN PIG

LAHBIB-MANSAIS, Y. — DALIAS, G. — MILAN, D. — YERLE, M. — ROBIC, A.. — GYAPAY, G. — GELLIN, J.

SUMMARY

A collaboration with Généthon (ComparEST project) allowed us to develop a comparative mapping strategy and to complete our systematic work on cDNAs localization in pig. Généthon localized human ESTs with human radiated hybrid panel and tested them by heterologous PCR on pig, bovine and dog DNAs. We tested 344 human ESTs selected to amplify porcine DNA: 186 primer pairs allowed us to generate a specific pig amplification. We localized 65 human ESTs (among 107 tested) by PCR on somatic cell hybrid panel. Identity of the PCR products was controlled by sequencing: 60 ESTs matched significantly with the expected human sequences. The distribution of the localizations in pig was over all chromosomes (except Sscr18). Fifty one of these localizations are in agreement with the comparative mapping data between human and pig based on heterologous painting. In 6 cases, the localisation of ESTs had completed the data obtained by the painting (gene order modified or precision of limits of homologous chromosomal segments). Seven ESTs localized in a non expected region revealed new chromosomal correspondences. Our first analysis demonstrated that human ESTs can be considered for comparative mapping, particularly useful for the search of candidate genes in QTL regions. This approach will continue within the framework of the european project GENETPIG.

INTRODUCTION

To approach genes of interest in domestic animal, we must locate as many coding sequences as possible and focus on regions where QTL have been found. In pig, the number of genes localized was still relatively low with 158 genes listed in the last compilation of the cytogenetic map published in 1997 (*Yerle et al.*, 1997). High chromosome homologies have been found between pig and human maps by using heterologous chromosome painting experiments (*Goureau et al.*, 1997). This work has provided a first global comparative map. More generally, using the large amount of information provided in the human species, it is now possible to refine the correspondences between human and pig chromosomes.

Recently, a general approach has been developed: the isolation and characterization of porcine ESTs (Expressed Sequences Tags) from different tissue specific cDNA libraries (porcine small intestine, muscle and granulosa). About one hundred of porcine ESTs have been recently mapped by PCR on somatic cell hybrid panel (*Jörgensen et al.*, 1997; *Fridolfsson et al.*, 1997; *Winterö et al.*, 1998). To develop the comparative mapping strategy and to complete our systematic work about cDNA isolation, we have initiated a complementary approach that consists to localize in pig some human ESTs isolated for the human genome project. Généthon localized human ESTs with human RH panel (Radiated Hybrid panel) and, in the same time, tested them by PCR methods with pig, bovine and dog DNAs. This ZOO-PCR showed that about 10% on 10000 ESTs tested can amplify the DNA of at least one other species than human. A collaboration with Généthon allowed different partners of a French INRA project *ComparESTs* to use the original human primers to map these ESTs in different species (mouse, bovine, pig and dog). We reported the subregional localization in pig of 65 human ESTs by PCR on porcine cell hybrid panel.

MATERIALS AND METHODS

Human oligonucleotides provided by Généthon were essentially designed in the 3' untranslated regions (3'UTR) of human cDNA sequences. Hot start and stepdown PCR (annealing temperature from 61 to 55 °C) conditions were used to reduce the formation of primer dimers and spurious PCR products. A single set of standardized PCR conditions was used for both ZOO-PCR tests and chromosomal assignment analyses on porcine somatic cell hybrid panel. PCR reactions were carried out in 15 pl reaction mixture containing 50 ng of genomic DNA, 0.5 μM of each oligonucleotide primer, 2mM MgCl2, 0.2mM of each dNTP, the buffer suplied by the manufacturer and 1U Taq DNA polymerase (Gibco BRL). PCR amplification in 96 well microtiter plates, was performed using a Hybaid thermocycler. After Stepdown PCR, 30 additional cycles at 55 °C were realized.

After a ZOO-PCR screening realized in our laboratory, primers that produced a distinct PCR product from total porcine DNA were selected to be tested for mapping in pig. Regional assignments in pig were realized by PCR on a pig/rodent somatic cell hybrid panel (*Yerle et al.*, 1996). Additional informations on the panel can be found on the WWW INRA server (http://www.toulouse.inra.fr/lgc/lgc.html). The analysis of the segregation of PCR products in interspecific somatic cell hybrids was done on 2% agarose or single strand conformation analysis (SSCA) gels. Regional assignment in pig was achieved through hybrid cells analysis using the statistical rules as defined by *Chevalet et al.* (1997).

Direct sequencing of PCR products from both directions was performed by the fluorescent dye terminator methodology and automatic analysis with 377 apparatus (Perkin-Elmer) and ABI Prism dRhodamine Terminator cycle sequencing kit.

RESULTS

In pig, we have tested 344 human ESTs by using heterologous standardized PCR. Among them, 186 primer pairs were selected to generate a pig specific amplification. Then 65 ESTs in 107 tests were regionally localized on pig chromosomes by using PCR amplification on somatic pig/rodent cell hybrids. The analysis of the segregation of PCR products in interspecific somatic cell hybrids was done either in agarose gel or in Single Strand Conformation Polymorphism gel. Direct sequencing of pig PCR product was performed and their sequences were compared for similarities with DNA sequences available in Genbank data: sixty ESTs matched significantly with the expected human sequences. Among these 65 ESTs localized in pig, 37 corresponded to human genes and 28 were non identified. Forty three of these 65 ESTs were regionally located in human that increased the links between human and pig maps and, fifteen were only assigned. The distribution of these localizations in pig was over all autosomes (except Sscr18) and X. Most of these localizations (51) in pig were in agreement with the comparative mapping data between human and pig. Among them, 3 cases revealed that gene order was modified and 3 others precised the limits of chromosomal correspondences between both species. Interestingly, 7 ESTs presenting a similar sequence to the corresponding human EST revealed new chromosomal correspondences.

Our first analysis demonstrates that human ESTs can be considered very useful for comparative mapping strategy. We will continue this approach in the framework of the *GÈNETPIG* european project to accelerate the mapping and to identify GENes controlling Economic Traits in PIG.

REFERENCES

Chevalet, C. – Gouzy, J. – San Cristobald-Gaudy, M.(1997): Regional assignment of markers using a somatic cell hybrid panel: a WWW interactive program for the pig genome. Cabios, 13. 69–73.p.

Goureau, A. – Yerle, M. – Schmitz, A. – Riquet, J. – Milan, D. – Pinton, P. – Frelat, G. – Gellin, J. (1996): Human and porcine correspondence of chromosome segments using bidirectional chromosome painting. Genomics, 36. 252–262.p.

Fridolfsson, A.K. – Hori, T. – Winterö, A.K. – Fredholm, M. – Yerle, M. – Robic, A. – Andersson, L. – Ellegren, H.(1997): Expansion of the pig comparative map by expressed sequence tags (EST) mapping. Mamm. Genome, 8. 907–912.p.

Jörgensen, C.B. – Winterö, A.K. – Yerle, M. – Fredholm, M.(1997): Mapping of 22 expressed sequence tags isolated from a porcine small intestine cDNA library. Mamm. Genome, 8. 423–427.p.

Winterö, A.K. – Jörgensen, C.B. – Robic, A. – Yerle, M. – Fredholm, M.(1998): Further improvment of the porcine transcription map: Localization of 33 genes, of which 24 are orthologous. Mamm. Genome (in press)
Yerle, M. – Echard, G. – Robic, A. – Mairal, A. – Dubut-Fontanat, C. – Riquet, J. – Pinton, P. – Milan, D. – Lahbib-Mansais, Y. – Gellin, J.(1996): A somatic cell hybrid panel for pig regional gene mapping characterized by molecular cytogenetics. Cytogenet. Cell Genet., 73. 194–202.p.
Yerle, M. – Lahbib-Mansais, Y. – Pinton, P. – Robic, A. – Goureau, A. – Milan, D. – Gellin, J.(1997): The cytogenetic map of the domestic pig (*Sus scrofa domestica*). Mamm. Genome, 8. 592–607.p.

Authors' address: *Lahbib-Mansais, Y. – Dalias, G. – Milan, D. – Yerle, M. – Robic, A. – Gellin, J.*:
Laboratoire de Génétique Cellulaire INRA
BP 27 31326 Castanet-Tolosan, France
Gyapay, G.: Généthon, Evry, France

GENERATION OF A PORCINE WHOLE-GENOME RADIATION HYBRID PANEL: A NEW TOOL FOR MAPPING THE PIG GENOME

YERLE, M. — PINTON, P. — ROBIC, A. — HAWKEN, R. —
ALEXANDER, L. — BEATTIE, C. — SCHOOK, L. — MILAN, D. — GELLIN, J.

SUMMARY

We have developed a whole genome radiation hybrid (WG-RH) panel for high resolution gene mapping in pigs. One hundred fifty two radiation hybrids were produced. The number and the size of the porcine chromosome fragments retained in each hybrid clone were checked by fluorescent *in situ* hybridization with a SINE probe or by primed *in situ* labelling (PRINS) with SINE specific primers. A strategy based on the interspersed repetitive sequence polymerase chain reaction (IRS-PCR) was developed on selected clones to determine if the large fragments painted by the SINE probe corresponded to fragments from one or different pig chromosomes. Genome retention frequency was estimated for each clone. The first data indicated that, depending on the region investigated, comparison of the centiRay distances between loci on the WG-RH maps with genetic distances in centiMorgans revealed that 1cM was approximatively equivalent to 10 to 20 cR_{6500}.

INTRODUCTION

Porcine somatic cell hybrid panels have been produced and used particularly to increase the links between genetic and cytogenetic maps. Although the porcine map is not at the same definition than the human one, in some chromosomal regions, as the region bearing RN gene, it appears necessary to have a more performant tool to determine finely the position and order of genes and markers. Much progress has been made recently in humans in establishing radiation hybrid panels (RH). The presence or absence of specific DNA sequences in RHs is mainly determined by PCR and the relative order and distance between markers is calculated by a statistical algorithm that estimates the frequency of breakage between markers. We have constructed a porcine radiation hybrid panel by fusing irradiated pig lymphocytes or fibroblasts to recipient cells. One hundred and

fifty two clones were obtained. A cytogenetic characterization was realized using various techniques: 1) FISH with SINE probe to determine the number and the size of the porcine chromosome fragments retained, 2) reverse painting for some hybrids using their DNA as probes after interSINE amplification (and reverse hybridization on pig metaphases) to determine the porcine content of these clones, 3) double PRINS with centromeric alpha satellite primers specific of two different groups of chromsomes to determine if porcine centromeres have been retained in small fragments and in rearranged chromosomes. In addition, 32 markers chosen for their repartition on different chromosomes, have been used in PCR to estimate the genome retention frequency of each hybrid clone. Taking into account all the informations gathered by the cytogenetic and molecular studies, 126 clones were retained to produce an efficient gene mapping tool for the porcine genome.

MATERIALS AND METHODS

Generation of whole genome radiation hybrids: Fusion experiments were carried out using porcine diploid fibroblasts or lymphocytes irradiated with either 6000 or 7000 rads. Porcine cells were then fused with HPRT deficient Wg3hCl2 hamster cells in the presence of polyethylene glycol for 1 minute at room temperature. After fusion, cells were plated in complete RPMI medium plus HAT selection. One colony was picked into a separate flask, between 10 to 15 days after fusion, depending on the experiment, to establish a panel of independent hybrid clones. For each hybrid clone selected, cells were produced at the same transfer for cytogenetic studies, DNA extraction and storage in liquid nitrogen.

SINE sequence detection by FISH: The pSR1C plasmid containing a 300-bp porcine SINE (*Frengen et al.*, 1991) was used as probe for FISH experiment as described elsewhere (*Yerle et al.*, 1996). Three microliters of labeled products (out of 25) were dissolved in 22 µl of hybridisation mixture and hybridised on each hybrid slide. Hybridisation was performed at 37 °C for 24 hours. Post hybridisation washes and probe detection were also standard.

Single and double PRINS: The sequence of the two SINE primers that were used are: SINE B (5'-ATATGGAGGTTCCCATAGG-3'; consensus SINE sequence X64127 pos 195) and SINE - (5'-GTTCCCGTTGTGGCTCAGTGG-3', for more information see *Yerle et al.*, 1996) . The reaction mixture contained: 2 µM of each oligonucleotide; 100 µM each of dATP, dGTP, and dCTP; 2 µM dTTP; 25 µM fluorescein-12-dUTP (Boerhinger Mannheim) in Taq buffer (Life Technology); 5 U Taq DNA pomymerase (Life Technology) and 2 mM $MgCl_2$. The slides were incubated at 94 °C for 1 min, 55 °C for 25 min and at 70 °C for 35 min in a Hybaid Omnislide thermocycler.

Detection of α centromeric satellite sequences: Two primers: SSCSR2A and AC6 (*Rogel-Gaillard et al.*, 1997a, 1997b) were used in double PRINS experiment to detect the presence of centromeric regions in porcine fragments retained in the hybrids. The double PRINS experiment was carried out according to *Pellestor et al.* (1995).

Porcine interSINE-PCR analysis (pIRS-PCR): The interSINE amplification of hybrid DNA was carried out as previously described (*Yerle et al.*, 1996). Fifty nanograms of hybrid DNA was used in 25 µi of 1 x Taq DNA polymerase buffer (Life Technology), 0.2 mM each dNTP, 1 µM SINE+ primer, 1.5 mM $MgCl_2$, 1 U DNA Taq polymerase (Life Technology). After initial denaturation step at 94 °C for 4 min, 30 cycles of PCR were carried out as follows: 94 °C for 1 min, 60 °C for 1 min, and 72 °C for 1 min. The quality of amplification was analysed by agarose gel electrophoresis. The labeling was realized in 15 additional PCR cycles (4 min denaturation 94 °C, 1 min 60 °C, 1 min 72 °C, 1 min 94 °C) carried out on 1 µl of amplified product. The final volume of the labeling reaction was 25 µl and contained 0.1 mM of biotin-16-dUTP (Boerhinger Mannheim), 0.06 mM dTTP and 0.2 mM each of dATP, dCTP, and dGTP.

Estimation of the genome retention frequency by PCR using microsatellites: Thirty two porcine markers: 31 microsatellites and one expressed sequence tag (*Clouscard-Martinato et al.*, 1998) were used to estimate retention frequency of all hybrid clones produced. Each primer pair was tested for specificity in PCR with porcine and hamster total genomic DNA.

RESULTS AND DISCUSSION

Cytogenetic analysis: A total of 152 independent whole genome radiation hybrids were produced. The hybrids were analysed by FISH or PRINS (*Koch et al.*, 1989; 1992) for porcine DNA content using a SINE probe or SINE specific primers. In all cases, the pig DNA contained in the hybrids was clearly painted by the SINE sequence. Among the 152 hybrids, 140 retained porcine fragments. The 12 remaining clones have probably retained small pieces of porcine DNA not detectable by these techniques. The number of porcine fragments retained was variable: 12 hybrids out of 152 (7%) did not retain any fragment detectable by FISH/ PRINS, 68 out of 152 (45%) retained 1 to 4 porcine DNA fragments, 57 (38%) 5 to 8 fragments and 15 (10%) retained more than 8 fragments. Fragments were retained either as fragments of only porcine origin, were integrated in rodent chromosomes as very small, or as large fragments translocated onto hamster. As a relatively high dose of irradiation (6000–7000 rads) was used to break the pig chromosomes before fusion, it was surprising to observe that the SINE probe painted large fragments in many hybrid clones. We analysed an additional 12 hybrids to determine if these large fragments corresponded to regions of a single pig chromosome or to pieces of different chromosomes fused together. For this purpose, pIRS-PCR amplification was performed on the DNA of these hybrids that were then used as probes in reverse painting experiments on normal porcine metaphases (*Yerle et al.*, 1996). For example, SINE analysis of RH32 indicated that it had retained a large acrocentric chromosome and 2 small chromosomes. The pIRS-PCR indicated that fragments of 12 different chromosomes were present in this hybrid and that no acrocentric chromosome has been retained in its entire form. Consequently the large acrocentric chromosome observed in this hybrid corresponded to fragments of different chromosomes fused together. Additional data favouring this conclusion was given by a double PRINS experiment produced with primers specific for α satellite sequences. Centromeric sequences of three different chromosomes were detected on this abnormal chromosome.

Analysis of RH clones by PCR screening of molecular markers: An additional analysis using thirty-one microsatellites plus one EST selected according to their position on different chromosomal regions (*Table 1*) was run on the hybrid panél to estimate the genome fraction retained in each hybrid. The number of markers retained in each hybrid from the 32 markers tested and scored for each clone allowed us to estimate the genome retention frequency for each hybrid. Among the 152 hybrids produced, 31 hybrids had a genome retention frequency <9% (0 or 1 marker retained/32), 48 ranged between 9% and 25% (2 to 8 markers retained/32) and for 73 hybrids, it was > 25% (9 to 28 markers retained/32).

Selection of an informative RH panel: Twenty-six hybrids of 152 produced were eliminated because they had retained only few porcine material. This allowed us to select 126 hybrid clones among the 152 produced.

In parallel to this analysis, the hybrid panel was also used to construct a radiation map of a region of interest on SSC15 bearing the acid meat or RN gene (*Milan et al.*, 1995, 1996) with a 13 fold higher resolution than the one obtained on the linkage map (*Milan et al.*, 1996; *Robic et al.*, in preparation).

In summary, our initial cytogenetic and molecular analyses, allowed us to select 126 radiation hybrids among the 152 produced to construct an RH panel. An extensive PCR screening of this panel has recently been undertaken in a collaborative project with the University of Minnesota (Drs L. Schook, L. Alexander, C. Beattie) to order > 1000 markers, principally microsatellites selected from the most current swine genetic map (*Rohrer et al.*, 1996), and EST to produce a set of framework markers that will allow future mapping of additional markers on the pig genome. This effort will also significantly improve: 1) integration of linkage and physical maps and 2) linkage map reliability through the unambiguous determination of marker order. Altogether, the points detailed above demonstrate that this panel will provide a powerful tool for fine mapping the swine genome in the future.

Table 1.

Estimation of the marker retention frequency with 32 markers

Microsatellites (reference)	Localisations (reference)	Marker retention frequency* (%)
S0008 (*Archibald et al.*, 1995)	1p22-p23 (*Robic et al.*, 1996)	34.1
Sw780 (*Rohrer et al.*, 1994)	1p21-p27 (*Robic et al.*, 1996)	28.6
Sw781 (*Rohrer et al.*, 1994)	1p13 (*Yerle et al.*, 1997)	32.8
S0082 (*Archibald et al.*, 1995)	1q21-q27 (*Robic et al.*, 1996)	38.1
Sw240 (*Rohrer et al.*, 1994)	2p14-p17 (*Robic et al.*, 1996)	30.2
S0010 (*Archibald et al.*, 1995)	2q24-q29 (*Robic et al.*, 1996)	24.6
Sw72 (*Rohrer et al.*, 1994)	3p (*Robic et al.*, 1996)	31.0
S0379 (*Robic et al.*, 1997)	3q21-q27 (*Robic et al.*, 1997)	24.8
Sw314 (*Rohrer et al.*, 1994)	3q21-q27 (*Robic et al.*, 1996)	34.9
S0073 (*Archibald et ai.*, 1995)	4q15-q16 (*Robic et al.*, 1996)	29.4
S0092 (*Archibald et al.*, 1995)	5p (*Robic et al.*, 1996)	33.3
S0018 (*Archibald et al.*, 1995)	5q25 (*Robic et al.*, 1996)	30.2
Sw824 (*Rohrer et al.*, 1994)	6q31-q35 (*Robic et al.*, 1996)	21.4
S0121 (*Archibald et al.*, 1995)	6q31-q35 (*Robic et al.*, 1996)	33.1
S0064 (*Archibald et al.*, 1995)	7p11-p12 (*Robic et al.*, 1996)	22.2
S0399 (*Robic et al.*, 1997)	7q14 (*Robic et al.*, 1997)	26.4
Sw29 (*Rohrer et al.*, 1994)	8 (*Rohrer et al.*, 1994)	33.3
S0086 (*Archibald et ai.*, 1995)	8q11-q12 (*Robic et al.*, 1996)	37.3
Sw911 (*Rohrer et al.*, 1994)	9p13-p21 (*Robic et al.*, 1996)	34.9
Sw511 (*Rohrer et al.*, 1994)	9 (*Rohrer et al.*, 1994)	33.3
Sw830 (*Rohrer et al.*, 1994)	10p (*Robic et al.*, 1996)	20.6
S0070 (*Fredholm et al.*, 1993)	10q11-q12 (*Robic et al.*, 1996)	38.1
Sw1041 (*Rohrer et al.*, 1994)	10q17 (*Robic et al.*, 1996)	36.0
Swr811 (*Rohrer et al.*, 1994)	11 (*Rohrer et al.*, 1996)	49.2
Sw151 (*Rohrer et al.*, 1994)	11q (*Robic et al.*, 1996)	42.1
Sw769 (*Rohrer et al.*, 1994)	13q41-q49 (*Robic et al.*, 1996)	23.8
EstB11 (*Clouscard et al.*, 1998)	14q15-q29 (*Clouscard et al.*, 1998)	25.4
S0088 (*Archibald et al.*, 1995)	15q15-q22 (*Robic et al.*, 1996)	54.0
Sw936 (*Rohrer et al.*, 1994)	15q21-q22 (*Milan et al.*, 1996)	23.0
Swr1002 (*Rohrer et al.*, 1994)	15 (*Rohrer et al.*, 1994)	24.6
Sw335 (*Rohrer et al.*, 1994)	17q11-q14 (*Robic et al.*, 1996)	26.2
S0022 (*Archibald et al.*, 1995)	X (*Archibald et al.*, 1995)	65.1

* The marker retention frequency was estimated (in percentage) for each marker in the 126 selected hybrid clones (number of hybrids that retained the marker studied/total number of hybrids screened).

REFERENCES

Archibald, A. – Haley, C. – et al.(1995): The PiGMaP Consortium linkage map of the domestic pig (Sus scrofa domestica. Mamm. Genome, 6. 157–175.p.

Clouscard-Martinato, C. – Mulsant, P. – Robic, A. – Bonnet, A. – Gasser, F. – Hatey, F.(1998): Characterization of FSH-regulated genes isolated by mRNA differential display from pig ovarian granulosa cells. Anim. Genet. (in press)

Frengen, E. – Thomsen, P. – Kristensen, T. – Kran, S. – Miller, R. – Davies, W.(1991): Porcine SINEs: characterization and use in species-specific amplification. Genomics, 10. 949–956.p.

Koch, J.E. – Kolvraa, S. – Petersen, K.B. – Gregensen, N. – Bolund, L.(1989): Oligonucleotide-priming methods for the chromosome-specific labelling of alpha satellite DNA *in situ*. Chromosoma, 98. 259–265.p.

Koch, J. – Mogensen, J. – Pedersen, S. – Fisher, H. – Hindkjaer, J. – Kolvraa, S. – Bolund, L. (1992): Fast one-step procedure for the detection of nucleic acids *in situ* by primer-induced sequence-specific labeling with fluorescein-12-dUTP. Cytogenet. Cell Genet., 60. 1–3.p.

Milan, D. – Le Roy, P. – Woloszyn, N. – Caritez, J.C. – Elsen, J.M. – Sellier, P. – Gellin, J.(1995): The RN locus for meat quality maps to pig chromosome 15. Genet. Sel. Evol., 27. 195–199.p.
Milan, D. – Woloszyn, N. – Yerle, M. – Le Roy, P. – Bonnet, M. – Riquet, J. – Lahbib-Mansais, Y. – Caritez, J.C. – Robic, A. – Sellier, P. – Elsen, J.M. – Gellin, J.(1996): Accurate mapping of the «acid meat» RN gene on genetic and physical maps of pig chromosome 15. Mamm. Genome, 7. 47–51.p.
Pellestor, F. – Girardet, A. – Lefort, G. – Andréo, B. – Charlieu, J.P.(1995): Selection of chromosome-specific primers and their use in simple and double PRINS techniques for rapid *in situ* identification of human chromosomes. Cytogenet. Cell Genet., 70. 138–142.p.
Robic, A. – Seroude, V. – Yerle, M. – Jeon, J.T. – Wasungu, L. – Andersson, L. – Gellin, J. – Milan, D.(1998): A radiation hybrid map with 19 loci covering the region q22-q26 of the porcine chromosome 15 and ordered comparative mapping with mouse chromosome 1. Mamm. Genome (submitted)
Rogel-Gaillard, C. – Hayes, H. – Coullin, P. – Chardon, P. – Vaiman, M.(1997): Swine centromeric DNA repeats revealed by Primed IN Situ (PRINS) labelling. Cytogenet. Cell Genet., 79. 79–84.p.
Yerle, M. – Echard, G. – Robic, A. – Mairal, A. – Dubut-Fontana, C. – Riquet, J. – Pinton, P. – Milan, D. – Lahbib-Mansais, Y. – Gellin, J.(1996): A somatic cell hybrid panel for pig regional gene mapping characterised by molecular cytogenetics. Cytogenet. Cell Genet., 73. 194–202.p.

Authors' address: *Yerle, M. – Pinton, P. – Robic, A. – Milan, D. – Gellin, J.:*
INRA, Laboratoire de Génétique Cellulaire,
BP 27, 31326 Castanet-Tolosan, France
Hawken, R. – Alexander, L. – Beattie, C. – Schook, L.:
Program in Comparative Genomics, College of Veterinary Medicine,
University of Minnesota, St Paul, MN, USA

AN ALTERNATIVE DETECTION METHOD FOR LABELED DNA PROBES IN PORCINE GENE MAPPING BY *IN SITU* HYBRIDIZATION

MUSILOVA, P. — KUBICKOVA, S. — VOZDOVA, M. — RUBES, J.

SUMMARY

Non-radioactive, non-fluorescence *in situ* hybridization as a method of choice for pig gene mapping is described here. This approach is based on detection of hybridized biotinylated probe by peroxidase conjugated extravidin and reaction of peroxidase with its substrate diaminobenzidine resulting in dark precipitate. For increasing the sensitivity of the method in single copy gene mapping two amplifications of peroxidase signal were used: immunological amplification by biotinylated antiavidin, and peroxidase-catalyzed deposition of biotinylated tyramide. Using this method, two 2-kb-long probes for the porcine genes cyclin-dependent kinase 4 (CDK4) and proto-oncogene c-MOS were mapped to pig chromosomes 5p12 and 4q14–15, respectively. This non-radioactive, non-fluorescence method of *in situ* hybridization has the advantages of being rapid, safe, sensitive, and accurate.

INTRODUCTION

Several approaches for the detection of labeled DNA probes exist. Radioactive and fluorescent methods are most frequently used in gene mapping by means of *in situ* hybridization. Radioactive *in situ* hybridization (RISH) is a very sensitive and effective method for the mapping of short probes. Statistical analysis is required for the assessment of results. The benefits of fluorescent *in situ* hybridization (FISH) and other non-radioactive ISH include safety, better spatial resolution and speed. As an alternative to fluorochromes, some enzymes can be used for the detection of non-radioactively labeled probes. Target sequences are then visualized by the addition of an appropriate substrate as colorimetric, chemiluminescent or fluorescent signals (*Kagiyama et al.*, 1995, *Speel et al.*, 1995). In order to increase the sensitivity of non-radioactive *in situ* hybridization, signal amplification is required. This is most commonly achieved using an immunological method based on antibody layering. In another strategy called catalyzed reporter deposition (CARD), the ISH signal amplification is achieved by peroxidase-catalyzed deposition of biotinylated tyramide (*Bobrow et al.*, 1992).

In our experiments horseradish peroxidase and its substrate diaminobenzidine (DAB) were used for the mapping of two 2-kb-long probes for the porcine genes cyclin-dependent kinase 4 (CDK4) and proto-oncogene c-MOS. Signals were amplified by both immunological and CARD methods.

MATERIALS AND METHOD

Chromosome preparation and banding: Metaphase chromosomes were obtained from cultures of pig periferal blood lymphocytes according to standard procedures. Chromosomes were G-banded, and the best metaphases were selected and photographed prior to hybridization. Metaphase chromosomes were classified according to standardized karyotype (Committee for the Standardized Karyotype of the Domestic Pig; *Gustavsson* 1988). Prior to hybridization the slides were destained in 50% methanol and dehydrated.

Probes and signal labeling: Probes for both genes were prepared by means of PCR using primers designed on the basis of gene sequences from the gene bank (CDK4: SSU68478, c-MOS: X78318). The length of the CDK4 and c-MOS probes were 1982 bp and 1868 bp, respectively. PCR products were cloned in plasmid vectors. DNA from recombinant plasmids was labeled with biotin-11-dUTP using nick translation.

In situ hybridization, signal detection and interpretation of results: In situ hybridization was performed according to standard procedures (*Trask,* 1991). The final concentration of probe DNA in the hybridization mixture was 2 μg/ml. Hybridization was carried out overnight at 37 °C. After washing, the biotin-labeled probes were detected and amplified by immunological and CARD methods. Prior to each layering, the slides were blocked in TNB (0.05% blocking buffer in TNT: 0.1 M Tris-HCL, pH 7.5 in 0.15 M NaCl) for 10 min at room temperature. Immunological amplification was performed by subsequent layering of extravidin-peroxidase (Sigma, 1:200 dilution), antiavidin-biotin (Sigma, 1:300 dilution), and again extravidin-peroxidase, for 30 min at room temperature. Excess conjugates were removed by washing 3x for 5 min in TNT at 42 °C. Peroxidase-catalyzed deposition of biotinilated tyramide (NEN Life Science Products) was carried out for 15 min and was followed by washing and additional incubation with extravidin-peroxidase.

For visualization of hybridized probes, peroxidase substrate DAB (Sigma Fast DAB with metal enhancer; Sigma) was used. The addition of DAB 2x for 40 min resulted in the formation of a dark precipitate. After washing in TNT, the DAB signal was enhanced by immersing slides in 0.5% $CuSO_4$/0.9% NaCl for 5 min. Finally, the slides were rinsed in distilled water and restained with Wright solution for 5 min.

After *in situ* hybridization, the chromosomes were measured using computer-assisted image analysis. Statistical evaluation of the number of dots per unit of chromosome length was done assuming the Poisson distribution of signals.

RESULTS AND DISCUSSION

For the localization of **CDK4**, a total of 19 metaphases were scored. Of 91 dots counted, 11 (12.1%) were present on chromosome 5. Of these 11 dots, 7 (63.6%) were clustered over the region 5p12 (P<0.001). Although only a limited number of metaphases were investigated the results indicate the most probable location of the CDK4 gene (*Fig. 1*).

For the localization of c-MOS, a total of 53 metaphases were scored. Of 217 dots counted, 33 (15.2%) were present on chromosome 4. Of these 33 dots, 15 (45.5%) were clustered over the region 4q14-15 (P<0.001), indicating the most probable location of the c-MOS gene (*Fig. 2*). As these genes were mapped to human chromosomes 12q14 (*Mitchell et al.*, 1995) and 8q11 (*Caubet et al.*, 1985) respectively, our mapping results are in agreement with comparative mapping data (*Goureau et al.*, 1996). Two pig genes were mapped by means of *in situ* hybridization using this DAB/peroxidase method. These genes are of interest in the field of carcinogenesis. Cyclin-dependent kinase 4 (CDK4) in complexes with D-type cyclins is involved in the control of cell proliferation during the G1 phase of the cell cycle. Mutations of CDK4 result in the production of a tumor-specific antigen and presumably contribute to malignant transformation of melanomas. c-MOS is a member of a group of proto-oncogenes coding for protein serin/threonin kinases. Although detection of these genes in this manner is laborious due to the requirement for repeated layering, it gives results within one day. It does not require autoradiography which not only requires several weeks but also decreases spatial resolution of mapping. Another advantage of the current method is that the signals are stable. This allows accurate counting and statistical evaluation of the dots, thus increasing the sensitivity of the method. We demonstrated that non-radioactive, non-fluorescent *in situ* hybridization used here is a rapid, safe, sensitive, and accurate method of choice for pig gene mapping.

Supported by the Grant Agency of the Czech Republic (grant No. 523/97/P151) and the Ministry of Agriculture (grant No. EP0960996093).

Fig. 1.: **Localization of CDK4 gene. Histogram showing the dot distribution on pig chromosomes. A highly significant number of dots was found in chromosome region 5p12.**

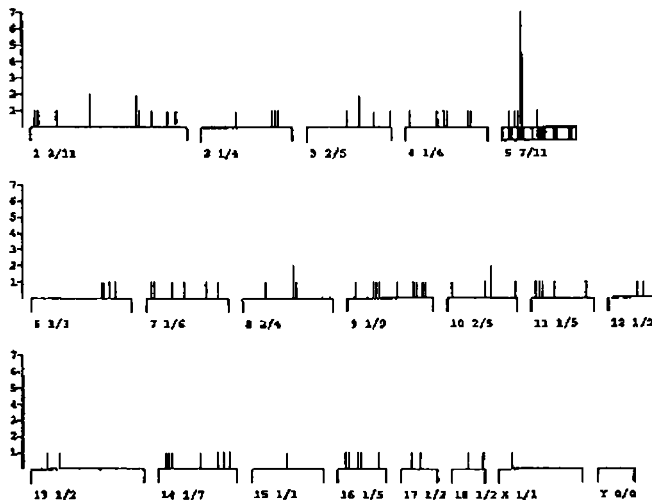
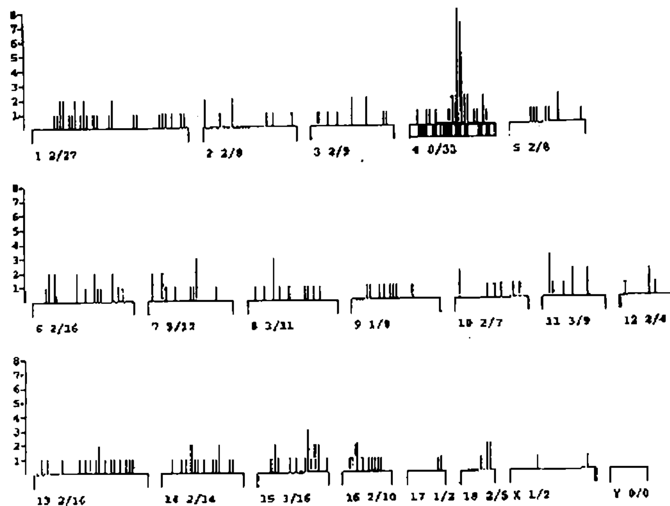


Fig. 2.: **Localization of c-MOS gene. Histogram showing the dot distribution on pig chromosomes. A highly significant number of dots was found in chromosome region 4q14–15.**



REFERENCES

Bobrow, M.N. – Litt, G.J. – Shaughnessy, K.J. – Mayer, P.C. – Conlon, J.(1992): The use of catalyzed reporter deposition as a means of signal amplification in variety of formats. J. Immuno.l Methods, 150. 145–149.p.

Caubet, J.F. – Mathieu-Mahul, D. – Bernheim, A. – Larsen, C.J. – Berger, R.(1985): Human protooncogene c-mos maps to 8q11. EMBO J., 4. 2245–2248.p.

Goureau, A. – Yerle, M. – Schmitz, A. – Riquet, J. – Milan, D. – Pinton, P. –Frelat, G. – Gellin, J.(1996): Human and porcine correspondence of chromosome segments using bidirectional chromosome painting. Genomics, 36. 252–262.p.

Gustavsson, I.(1988): Standard karyotype of the domestic pig. Committee for the Standardized Karyotype of the Domestic Pig. Hereditas, 109. 151–157.p.

Kagiyama, N. – Fujita, S. – Momiyama, M. – Kondoh, Y. – Nishiyauchi, M. – Hori, S.H.(1995): A novel fluorogenic substrate for alkaline phosphatase for the use of nucleic acid hybridization histochemistry and cytochemistry. Acta Hystochem. Cytochem., 6. 581–589.p.

Mitchell, E.L.D. – White, G.R.M. – Santibanez-Koref, M.F. – Varley, J.M. – Heighway, J.(1995): Mapping of gene loci in the q13-q15 region of chromosome 12. Chromosome Res., 3. 261–262.p.

Speel, E.J.M. – Ramaekers, F.C.S. – Hopman, A.H.N.(1995): Cytochemical detection systems for *in situ* hybridization, and the combination with immunocytochemistry. Who is still afraid of red, green and blue? Histochem. J., 27, 833–858.p.

Trask, B.J.(1991): DNA sequence localization in metaphase and interphase cells by fluorescence *in situ* hybridization. Methods in Cell Biology, 35. 3–35.p.

Authors' address: Veterinary Research Institute
Hudcova 70, 621 32 Brno, Czech Republic

THE USE OF CHROMOSOMAL MARKERS FOR THE IDENTIFICATION OF QTLS CONTROLLING FATTENING, AND CARCASS AND MEAT QUALITY TRAITS IN PIGS*

KOMISAREK, J. — SZYDLOWSKI, M. — SWITONSKI, M. — KURYL, J.

This study is a part of the Polish Pig Genome Mapping Project, which aimed at the identifcation of QTLs (quantitative trait loci) controlling fattening, and carcass and meat quality traits. A three-generation reference family was established on the basis of crossing between Polish Large White sows and Zlomicka Spotted boars. Forty quantitative traits were analysed in F_2 male offspring. The segregation of genetic markers type I, type II and chromosomal markers was analysed.

In the pig karyotype, size polymorphism of constitutive heterochromatin blocks of acrocentric chromosomes (pairs No. 13–18) and nucleolar organizer regions - NORs (pairs No. 8 and 10) are known as sources of chromosomal markers.

Size polymorphism of constitutive heterochromatin (C-banding) and nucleolar organizer regions (Ag-NOR staining) was analysed in F_1 and F_2 generations. Two size variants: large (++) and small (+) were recognized in pairs 10, 15 and 17. The segregation of the markers was analysed in progeny originating from informative crosses of F_1 generation. A total number of 52, 60 and 60 F_2 offspring were available for marker systems on chromosome pairs 10, 15 and 17, respectively. A general linear model was used to obtain F test values for the signifcance of within full-sibs difference among marker effects in the presence of nongenetic factors. The analysis showed an association between C-band polymorphism on chromosome 15 and weight of Longissimus dorsi. Evidence was also found for the association of Ag-NOR polyznorphism on chromosome 10 with the weight of ham muscles and body weight at 150th day of life. These associations suggest the presence of QTLs, controlling the mentioned production traits, on chromosome 10 and 15.

* Supported by the Committee for Scientifc Research, grant No. 5 PO6D 02808.

Authors' address: *Komisarek, J. – Szydlowski, M. – Switonski, M.*: Agricultural University of Poznan, 60-637 Poznan, Poland
Kuryl, J.: Institute of Genetics and Animal Breeding, Jastrzebiec, Os-551 Mroków, Poland

BOVINE CHROMOSOME FRAGMENT SPECIFIC LIBRARIES —TOOL FOR THE GENERATION OF REGION SPECIFIC HIGH DENSITY MARKER MAPS

GOLDAMMER, T. — BRUNNER, R.M — KÜHN, C. — WEIKARD, R. — SCHWERIN, M.

SUMMARY

A method for the generation and characterization of bovine chromosome fragment specific libraries is described. The method involves the isolation of only a single chromosome band by microdissection identified via G-banding, the amplification of this dissected chromosome fragments by DOP-PCR, plasmid cloning of generated DNA sequences, and the analysis of the species and chromosome specificity of PCR products and the species specificity of the generated libraries. Such libraries are a useful tool for the isolation of chromosome fragment specific DNA markers.

INTRODUCTION

The efficient use of identified quantitative trait loci (QTL) with significant effects in marker assisted selection requires the availability of tightly linked markers, i.e. fine mapping of QTL. In the last few years considerable efforts in the USA, Australia and Europe revealed a more or less complete genetic map in cattle (*Eggen and Fries,* 1995; *Barendse et al.,* 1997) that is useful for raw QTL mapping. Until now mostly these markers were randomly detected. Because of the low marker density their application in breeding programs is restricted. Both the effective use of markers in selection and the identification of genes which are candidates for performances, need high density marker maps. Therefore in recent years methodical strategies were developed for the directed generation of bovine chromosome specific marker maps (*Ponce De León et al.*, 1996) as well as bovine chromosome fragment specific marker maps (*Weikard et al.*, 1997). This study is based on the microdissection of single G-banded bovine chromosome regions (*Goldammer et al.,* 1996) that correlate to quantitative trait loci, their PCR amplification using a degenerate oligonucleotide primer (DOP; *Telenius et al.*, 1992) followed by the construction of chromosome fragment specific libraries via plasmid cloning. The PCR products were characterized by chromosome painting using fluorescence *in situ* hybridization (FISH). The bovine specificity of the generated libraries was proved by dot blot analysis. We present here the generation of chromosome libraries of goat chromosome fragment CHI 1q42-q44, and cattle chromosome fragments BTA 6q12-q21, BTA 6q13-q18, BTA 18q22-q24, BTA 18q24-qter, BTA 23q13-q22, BTA 23q14-q22, and BTA 23q15-q23.

MATERIAL AND METHODS

Chromosome preparation, microdissection, and DOP-PCR: Metaphase chromosomes were prepared from bovine embryonic fibroblasts, G-banded and Giemsa stained. Metaphase spreads were karyotyped according to the ISCNDA 1989. Microdissection of chromosomes was performed under an inverted microscope with glass microcapillaries controlled by motor driven micromanipulators described in detail by *Goldammer et al.* (1996). Dissected chromosome material was firstly pretreated with Topoisomerase I at 37 °C for 30 min, followed by a denaturation step at 96 °C for 10 min and than directly used for PCR. Ten pmol of the DOP-primer 6-MW 5'-CCGACTCGAGNNNNNNATGTGG-3' were used for the oil free PCR reaction. After an initial denaturation step of 5 min at 94 °C for 1 min, eight cycles of Sequenase-PCR with an annealing step at 30 °C for 2 min, and an extension step at 37 °C for 2 min were performed. After this preamplification a PCR reaction according to *Telenius et al.* (1992) was performed in the same tube. Thirty five cycles of DOP-PCR were carried out with denaturation at 94 °C for 1 min, annealing at 56 °C for 1 min and extension at 72 °C for 2 min. Biotinylation of amplified microdissected chromosome material was done as described above using 2 µl of the first PCR mix and 20 µM biotin-16-dUTP. The PCR products were analyzed by agarose gel electrophoresis. All PCR reactions were performed in a DNA Thermal Cycler (Biometra) equipped with a heated lid.

FISH: Hybridization of probes to bovine chromosomes was performed essentially as described by *Pinkel et al.* (1988). A DNA mixture containing 200 ng of the probe, 2 µg bovine Cot-1 DNA and 2 pg salmon sperm DNA was prepared, vacuum dried and then suspended in 2 µl of deionized water and 8 µl of hybridization mixture containing 50% formamide, 10% dextransulphate and 2xSSC for 1 to 2 hours. The DNA-hybridization mixture was denatured at 75 °C for 8 min followed by a preannealing step at 37 °C for 2 hours. Slides with digitized G-banded metaphase spreads were put on a hot plate, overlaid with 100 µl 70% formamide, 2xSSC, covered with cover slips and denatured for 3 min at 80 °C. After this procedure the slides were quickly transferred to a Coplin jar containing ice-cold 70% ethanol and agitated to rinse off the denaturing solution and than dehydrated serially in ethanol (80, 90, 100%). The preblocked DNA-probes were then placed on prewarmed (37 °C) slides and covered with cover slips. The slides were incubated in a moist chamber at 37 °C for 16 to 20 hours to complete the DNA hybridization. After hybridization, the cover slips were removed gently, and the slides were washed three times in 50% formamide, 2xSSC at 45 °C for 3 min and three times in 2xSSC at 45 °C for 2 min each. The slides were then blocked for 30 min in PNM-buffer [0.1 M NaH_2PO_4, 0.1 M Na_2HPO_4 (pH 8.0), 0.1% Nonidet P-40; 5% nonfat dry milk; 0.02% sodium azide] at ambient temperature. In order to visualize the probe slides were overlaid twice with FITC conjugated to avidin in PNM (3:1000) at 37 °C covered with cover slips and incubated at 37°C for 40 min. The signal was amplified using one layer of 100 µl biotinylated antiavidin antibody in PNM (11:1000) at 37 °C for 30 min. Slides were washed three times between incubations in 0.1 M PN-buffer (PNM without nonfat dry milk) for 2 min each at ambient temperature. Chromosome material was counterstained with PI and finally slides were overlaid with DABCO antifade solution. Metaphase spreads were analyzed with a Nikon Microphot FXA microscope equipped with a dual band pass filter for FITC and Texas red (Chroma Technologies). Images were captured using a CCD camera system, and enhanced with MacProbe FISH software (PSI).

Chromosome libraries: Preparation of libraries was performed as described by *Weikard et al.* (1997). DNA fragments in 30 µl of DOP-PCR amplification were alcohol precipitated and end-polished with 2.5 units Pfu DNA Taq Polymerase (Stratagene) at 70 °C for 30 min. The DNA products of the polishing reaction were used for ligation into p-Bluescript SK(+) derivative (pCR-Script, Stratagene) by incubation with 4 units T4 DNA ligase at ambient temperature for 1 h. Aliquots of the ligation reaction were used to transform *E. coli* XL1-Blue MRF' Kan supercompetent cells (Stratagene).

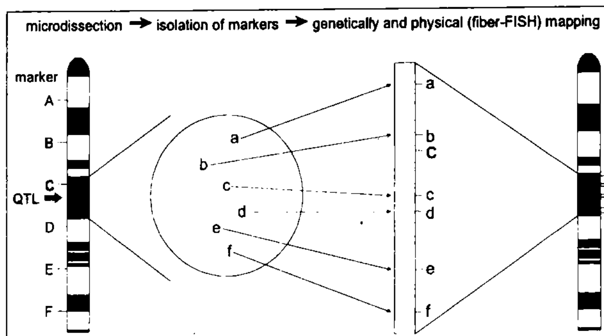
Twenty to 30 chromosome fragment specific clones were randomly picked and used as targed DNA in dot blot analysis. Therefore 200 ng of each clone suspended in 5µl deionized water were spotted onto nitrocellulose filters and hybridized using standard conditions. Briefly, after denaturation the DNA was fixed to the filter by baking it for 1 hour at 80 °C. After prehybridization for 2 hours at 65 °C with 1% bovine serum albumin (BSA), 7% sodium dodecyl sulfate (SDS), 1 mM EDTA. 0.263 M Na_2HPO_4, 25 ng of denatured ^{32}P-labeled DNA-probes (total genomic bovine or human DNA) were added. The filters were hybridized overnight at 65 °C. After hybridization the filters were washed two times in 2x standard saline citrate (SSC) for 15 min at ambient temperature, 30 min in 1xSSC at 65 °C and 30 min at 65 °C in 0.1xSSC. The filters were exposed for 2 hours at –80 °C using an intensifying screen. Autoradiograms were developed on Medical X-ray film XBD using standard conditions.

RESULTS AND CONCLUSIONS

According to the procedure shown in *Fig. 1.* for the generation of chromosome fragment specific markers, described by *Weikard et al.* (1997), a method for the standardized generation and characterization of bovine chromosome fragment specific libraries was described.

The method is based on microdissection of chromosome fragments, their subsequent DOP-PCR and cloning of the generated DNA sequences into a plasmid vector. The scraping of only one chromosome fragment is necessary for the generation of one library. To minimize the risk of contamination with foreign DNA each methodical step requires an accurate handling.

Fig. 1.: **Diagram of the procedure for the generation of high density marker maps**



To prove the species and chromosome fragment specificity the amplified chromosome material was hybridized via FISH to bovine metaphase chromosomes before cloning. For proving the species specificity of the generated chromosome fragment specific libraries randomly picked clones were analyzed using dot biot analysis. Using this method 9 fragment specific libraries were generated from the following chromosome regions of goat (Capra hircus) or cattle (Bos taurus): 2 x CHI 1q42-q44, BTA 6q12-q21, BTA 6q13-q18, BTA 18q22-q24 BTA 18q24-qter, BTA 23 q13-q22, BTA 23 q14-q22, and BTA 23 q15-q22. Chromosome fragments were microdissected as demonstrated in *Fig 2.* e.g. for the isolation of the middle part of chromosme 5. DOP-PCR amplification of isolated DNA material resulted in DNA sequences with molecular weights between 100 and 1000 bp as shown in *Fig. 2.* (right). The characterization of the generated PCR products and chromosome fragment specific libraries is demonstrated for the chromosome fragment BTA 6q16-q21 in *Fig. 3 and 4.*

Fig. 2.: **Microdissection of middle part of BTA 5 (left, middle), and characterization of a DOP-PCR amplified chromosome fragment via gel electrophoresis (right), M = λHind III/pBR Alu I, 1 = size of generated fragment specific DNA-sequences**

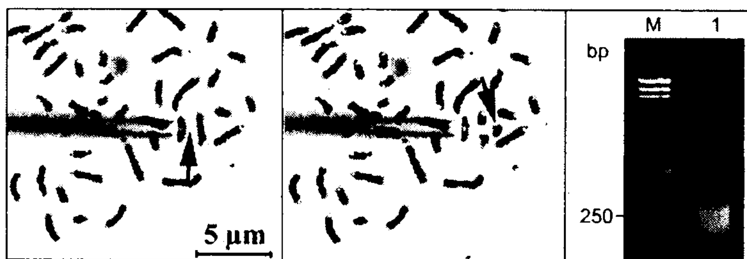


Fig. 3: **Verification of chromosome fragment specificity of DOP-PCR amplified chromosome fragment BTA 6q12-q21. G-bands (left), Double-FISH (middle) of BTA 6q12-q21 together with CIOBT 589 (*Kühn et al.*, 1996), Ideogram (scraped region was moved) and karyogram of BTA 6 (right)**

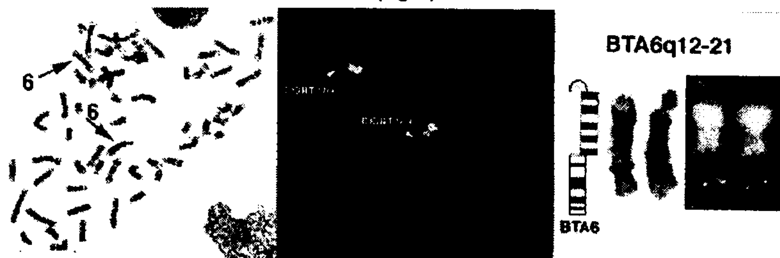
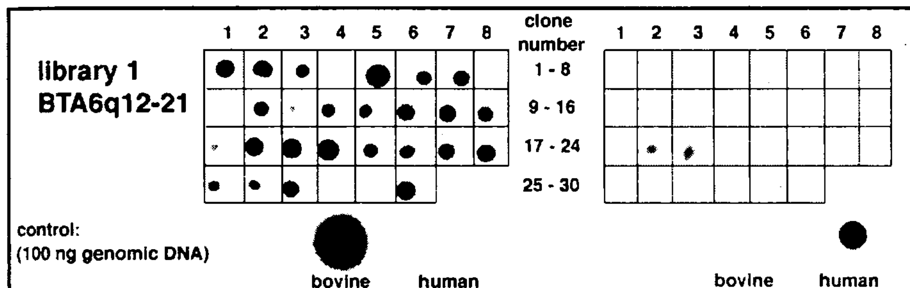


Fig. 4: **Verification of species specificity of chromosome fragment specific libraries. Radioactive filter hybridization of 30 randomly picked clones with P^{32}-dCTP labelled genomic DNA from cattle (left) and human (right). Filters were enhanced together using the software Photoshop 3.05. Picture processing was stopped as soon as no signal could be detected on the bovine control DNA which was hybridized with human genomic DNA**



Microdissection of single G-banded bovine chromosome regions and DOP-PCR allow the generation of bovine chromosome fragment specific DNA-sequences. The generated probes show a high fragment specificity as shown by chromosome painting, and as demonstrated by the complex hybridization patterns the microdissected and amplified probes are representative for the microdissected chromosome region. The microdissected, DOP-PCR amplified and cloned bovine chromosome specific DNA-sequences are a useful prerequisite for the production of targeted type I and II markers to fill in the bovine marker map directly in chromosome regions near to a quantitative trait. Additionally, the chromosome fragment specific sequences can be used for the identification of chromosome fragment specific lambda- cosmide-, P1-, BAC-, and YAC-clones or the identification and characterization of individual bovine chromosomes by chromosome painting.

REFERENCES

Barendse, W. – Vaiman, D. – Kemp, S.J. – Sugimoto, Y. – Armitage, S.M. – Williams, J.L. – Sun, H.S. – Eggen, A. – Agaba, M. – Aleyasin, S.A. – Band, M. – Bishop, M.D. – Buitkamp, J. – Byrne, K. – Collins, F. – Cooper, L. – Coppettiers, W. – Denys, B. – Drinkwater, R.D. – Easterday, K. – Elduque, C. – Ennis, S. – Erhardt, G. – Ferretti, L. – Zaragoza, P. – et al.(1997): A medium-density genetic linkage map of the bovine genome. Mamm. Genome, 8. 21–28.p.

Eggen, A. – Fries, R.(1995): An integrated cytogenetic and meiotic map of the bovine genome. Anim. Genet., 26. 215–236.p.

Goldammer, T. – Weikard, R. – Brunner, R.M. – Schwerin, M.(1996): Generation of chromosome fragment specific bovine DNA sequences by microdissection and DOP-PCR. Mamm. Genome, 7. 291–296.p.

ISCNDA 1989 - International System for Cytogenetic Nomenclature of Domestic Animals. DI Berardino, D. – Hayes, H. – Fries, R. – Long, S.(eds)(1990): Cytogenet. Cell Genet., 53. 65–79.p.

Kühn, C.H. – Weikard, R. – Goldammer, T. – Olsaker, I.(1996): FBN3 (D6S24) – a bovine microsatellite derived from cosmid CIOBT475 at chromosome 6q12–14. Anim. Genet., 27. 57.p.

Pinkel, D. – Landegent, J. – Collins, C. – Fuscoe, J. – Segraves, R. – Lucas, J. – Gray, J.W.(1988): Fluorescence *in situ* hybridization with human chromosome specific libraries: Detection of trisomy 21 and translocations of chromosome 4. Proc. Natl. Acad. Sci., USA, 85. 9138–9142.p.

Ponce De León, F.A. – Ambady, S. – Hawkins, G.A. – Kappes, S.M. – Bishop, M.D. – Robl, J.M. – Beattie, C.W.(1996): Development of a bovine X chromosome linkage group and painting probes to assess cattle, sheep, and goat X chromosome segment homologies. Proc. Natl. Acad. Sci., USA, 93. 3450–3454.p.

Telenius, H. – Carter, N.P. – Bebb, C.E. – Nordenskjöld, M. – Ponder, B.A.J. – Tunnacliffe, A.(1992): Degenerate oligonucleotide-primed PCR: General amplification of target DNA by a single degenerate primer. Genomics, 13. 718–725.p.

Weikard, R. – Goldammer, T. – Kühn, C. – Barendse, W. – Schwerin, M.(1997): Targeted development of microsatellite markers from the defined region of bovine Chromosome 6q21-31. Mamm. Genome, 8. 836–840.p.

Authors' address: Fachbereich Molekularbiologie, Forschungsinstitut für die Biologie landwirtschaftlicher Nutztiere,
D-18196 Dummerstorf, W.-Stahl-Allee 2, Germany

CHROMOSOMAL LOCALIZATION OF THE PRION PROTEIN GENE (PRNP) IN CATTLE, RIVER BUFFALO, SHEEP AND GOAT BY FISH AND R-BANDING

IANNUZZI, L. — PALOMBA, R. — DI MEO, G.P. — PERUCATTI, A. — FERRARA, L.

Prion protein gene (PRNP) mutations can be related to transmissible neurodegenerative diseases in several species, including humans (Creutzfeld-Jacob CJD), cattle (bovine spongiform encephalopathy, BSE) and sheep (Scrapie). PRNP has been localized on HSA2Op12-pter, while in cattle it has been only assigned to syntenic group U11 which maps in cattle chromosome 13 (*Eggen and Fries*, 1995). Phsical localization of this important gene on a specific chromosome region and band is still lacking in domestic bovids. Peripheral blood lymphocyte cultures were treated for late incorporation of both BrdU and Hoechst 33258 (15µg/ml for each one). For probe synthesis, two pairs of specific primers were employed. The probe was denatured at 72 °C for 15 min and hybridized on slides (1 ng/µl) ovemight. After the detection step with FITC-avidin and antiavidin antibody (Vector), the slides were mounted with antiphade/Hoechst 33258 (1 µg/ml). This allowed us to obtain RBH-banding and FITC-signals. The two different images were captured by using a CCD camera (Sensys, Photometrics). To improve banding contrast, the slides were later counterstained with acridine orange to obtain RBA-banded preparations which were also captured by CCD camera. Chromosome identifcation and banding followed the ISCNDA89 (1990) for cattle (BTA), sheep (OAR) and goat (CHI) and the CSKBB (1994) for river buffalo (BBU). Ideograms for both sheep and goat were according to *Iannuzzi et al.* (1995, 1996). In thirty preparations we examined for each species clear hybridization signals were detected on BTA/OAR/CHI 13 and BBU14. These chromosomes are homoeologous among the four species. Our data confirm the assignment *of PRNP to* bovine U11 (*Ryan and Womack*, 1993) which maps in BTA13. This is the first molecular marker assignment to CHI13 and BBU14. It allowed us to indirectly assign, for the first time, all bovine (expressed genes only) U11 (ADA, AR VP, HCK, IL2R, *ITPA,* OXT; SOD1L1, *VIM, Eggen and Fries*, 1995) to these chromosome species.

ACKNOWLEDGMENTS

We thank Mr. D. Incarnato for technical assistance and microscope i image processing. This study was partly been supported by "INC Biologia e Produzione Agaria del CNR, PS Biologia e Produzione Agaria per un a Agricoltura sostenibile" and partly by "Progetto strategico del CNR Encefalopatia Spongiforme Bovina".

Authors' address: National Research Council (CNR), I.A.B.B.A.M., Naples, Italy

CHARACTERIZATION OF THE PRION GENE (PRNP) LOCUS IN CATTLE, SHEEP AND MAN BY PHYSICAL MAPPING

CASTIGLIONI, B. — COMINCINI, S. — DRISALDI, B. —
HILLS, D. — WILLIAMS, J.L. — FERRETTI, L.

Prions are infectious particles causing transmissile neurodegenerative diseases in mammais, such as Scrapie of sheep, Bovine Spongiform Encephalopathy (BSE) of cattle, Creutzfeldt-Jakob disease (CJD) and Fatal Familial Insomnia (FFI) of man. Prions are the product of a single gene, which is highly conserved in mammais.

We performed the direct localization of the PrP gene in cattle, sheep and man by means of fluorescence *in situ* hybridization (FISH) with PCR-generated probes. The bovine and ovine PrP locus was mapped on the chromosomes of the two species either with single or with combined probes in a dual-color FISH experiment. Direct incorporation of label in PCR-generated probes was preferred to standard nick-translation protocols since the intensity of the hybridization signal was much greater. The chromosomal assignment for the PrP gene was 13q17 in cattle and 13q17/q18 in sheep. The ovine and bovine hybridization spots on the same metaphases overlapped, as anticipated from the high conservation of the locus in the two species. FISH with the human probe showed that PRNP gene maps to chromosome 20p12/p13. In addition, the protein coding region of the human prion gene (738 bp) was used as a hybridization probe on sheep and cattle chromosomes, showing specific spots on at least one of the two chromosomes 13, on the same bands as with homologous probes.

Clones from a bovineYAC library isolated with a PrP probe are being ordered into a contig spanning the PrP region with the aim of establishing a detailed physical map and to isolate additional markers.

Authors' address: *Castiglioni, B. – Comincini, S. – Drisaldi, B. – Ferretti, L.*: I.D..V.G.A. – C.N.R., via F.lli Cervi 93, 20090 Segrate (MI), Italy
Drisaldi, B.: Dipartimento di Genetica e Microbiologia, via Abbiategrasso 207, 27100 Pavia, Italy
Hills, D. – Williams, J.L.: Roslin Istitute (Edinburgh), Roslin, Midlothian, EH25 9PS, Scotland, UK

COMPARATIVE PHYSICAL MAPPING OF PROC, EN1, ALPI, TNP1 AND IL1B GENES IN CATTLE AND SHEEP

LOPEZ-CORRALES, N.L. — SONSTEGARD, T.S — SMITH, T.P.L

SUMMARY

The cytogenetic locations of Protein C (PRO C), Transition Protein 1 (TNP1), Intestinal Alkaline Phosphatase *(ALPI,* Engrailed *(EN)* and Human Protointerleukin beta *(IL1B)* genes have been compared between cattle and sheep. Bovine YAC and cosmid clones were used as FISH probes to determine the order (centromere-telomere orientation) of four of these genes on sheep chromosome 2 (OAR2q and chromosome 3p (OAR3p, IL1B). In cattle *IL1B* and *EN1* were assigned to BTA11 and BTA2 respectively. The alignment between ovine, bovine and human physical maps using this data shown that segments of conserved synteny and chromosomal rearrangements detected between cattle and human, are also found in sheep where the cattle order is conserved.

INTRODUCTION

Comparative mapping allows the use of gene mapping information gathered in one species to be applied to all species for which the framework of conserved genome organization is known (*Andersson et al.*, 1996). An overview of segments of conserved synteny which exist between distantly related mammalian species can be revealed by ZOO-FISH methodologies (*Solinas-Toldo et al.*, 1995; *Hayes et al.*, 1995; *Radsepp et al.*, 1996; *Rettenberger et al.*, 1995; *Chowdhary et al.*, 1996). The comparative approach is especially important to facilitate the development of livestock gene maps. The *success* of this type of analysis is dependent on the development of comparative maps between humans, mice and livestock species that identify breakpoints in the conservation of synteny and changes in gene order within the conserved syntenic groups. Comparative mapping of the bovine homologues of genes found on HSA2q *(Homo sapiens)* (*Fisher et al.*, 1997; *Smith et al.*, 1997; and *Sonstegard et al.*, 1997) has defined a segment of conserved synteny between human and cattle chromosome 2. These comparisons were useful in predicting the location of the "double-muscling" locus (mh) (*Smith et ai.*, 1997). ZOO-FISH and linkage data (*Hayes*, 1995; *Solinas-Toldo et al.*, 1995; *Chowdary et al.*, 1996; *Fisher et al.*, 1997; *Sonstegard et al.*, 1997) indicates that most of cattle chromosome 2 *(Bos taurus* BTA2q12-q42) corresponds to HSA2q. Within this conserved synteny, a rearrangement of gene order between the BTA2q12-q44 and HSA2q14-q37 regions was detected by positioning *COL3A1* gene (HSA2q31-q32.3) in the pericentromeric region of BTA2 (*Fisher et al.*, 1997; *Sonstegard et al.*, 1997) and linkage data placing interleukin-1 receptor alpha (IL1RA; HSA2q12) on BTAII (*Yoo et al.*, 1994), indicated that two specific rearrangements occurred on HSA2q with respect to BTA2. In contrast, one segment containing GCG (glucagon) and NEB (nebulin) genes shows a larger region of gene order conservation of four human genes on BTA2 (*Sonstegard et al.*, 1997; *Smith*, 1997) which has also been reported in goat and sheep (*Lopez-Corrales et al.*, 1997). The objective of this study was to apply a comparative mapping strategy to more narrowly refine the position of evolutionary chromosomal changes and determine if the rearrangements and breakages in the conserved synteny observed between HSA2q and BTA2 have also been conserved in sheep *(Ovis aries)*. We used five probes of bovine type I loci *(PROC, TNP1*, ALPI, *IL1B and EN1)* to detect these rearrangements in sheep. Three probes *(PROC, TNP1, ALPI)* were previously assigned in humans and cattle by FISH (*Sonstegard et al.*, 1997). The data should provide two new physical assignments of Type I loci to the cattle map *(IL1B* and *EN1*), and extend the physical map coverage in sheep with five new cytogenetic assignments including *TNP1* which was previously located on sheep chromosome 2 *(Ovis* aries, OAR2) (*Pitei et al.*, 1994).

MATERIALS AND METHODS

Sequences of the human versions of EN1 and IL1B genes were obtained from Gen-Bank Cosmid probes corresponding to each loci were prepared from a bovine cosmid library in pWE15 (Stratagene, La Jolla, Calif.). Isolation and characterization of YAC and cosmid probes for TNP1,

PROC and ALPI were described previously (*Sonstegard et al.*, 1997). Chromosome preparation and FISH analyses were performed according to *Lemieux et al*, (1992); *Lichter et al*, (1990) and *Ponce de León et al.*, (1996). Digital images were obtained using a Zeiss Axioscope epifluorescence microscope coupled to a cooled CCD camera. Hybridization signals were assigned according to the standard R-banding pattems (*ISCNDA*, 1990; *Iannuzzi et al.*, 1995).

RESULTS

Probes for FISH analysis were chosen on the basis of loci which were known or predicted to reveal chromosomal rearrangements between cattle and humans. In sheep IL1B gene was shown to hybridize to OAR3p (3p25-p26). The assignments for *PROC, TNP1, EN1* and *ALPI* were observed on OAR2q (*Fig. 1.*: 2q12, 2q33-34, 2q28-q210, and 2q35). These genes should maintain the same order and centromere-telomere orientation in sheep, cattle, and human. Furthermore, the location *of EN1* and *IL1B* in sheep at OAR2q28-q210 and OAR3p25-p26, respectively (*Fig. 1.*) and in cattle at BTA2q32-q33 and BTA11q22.1-q22.3, respectively, (*Fig. 2.*) show that breakage in the conserved synteny between cattle and humans is conserved in sheep. The breakpoint lies in the narrow interval between *IL1B* and *EN1 as* defined by positioning of these loci on the transcript human map (http://www.ncbi.nlm.nih. gov/cgibin/SCIENCE96/chr?2).

Fig 1: **FISH analysis of five probes in sheep chromosome 2q (OAR2q) and cromosome 3p (OAR3p): PROC (a), TNP1(b), ALPI (c), EN1 (d) and IL1B (e)**

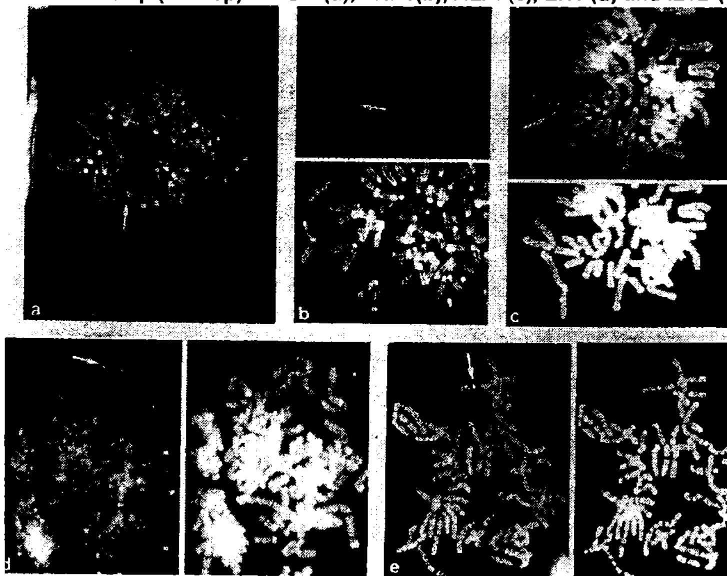


Fig 2: **Cytogenetic location of Engrailed (EN1) and Interleukin 1B in cattle: a) EN1 at BTA2q31-q33 and b) IL1B located at BTA11q22.1-22.3**

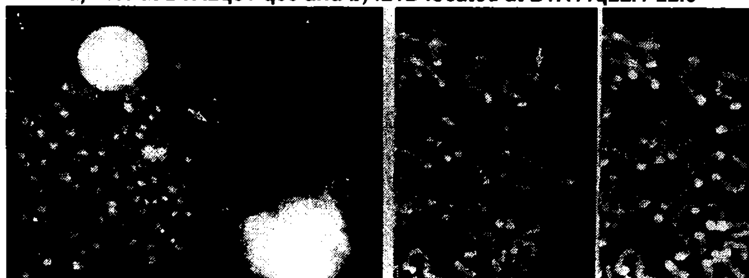
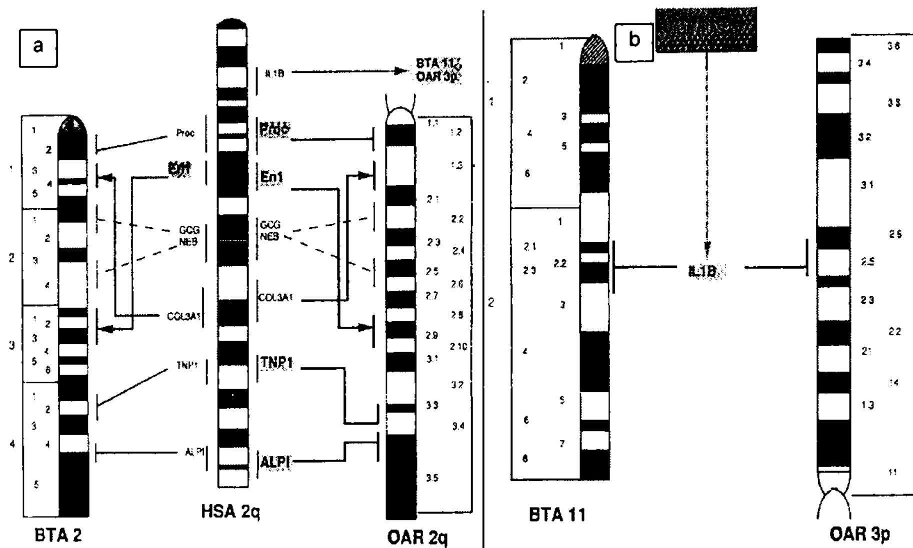


Fig. 3.: **a) Comparison of the cytogenetic location of five probes between human (HSA2q), cattle (BTA2) and ovine (OAR2q) chromosome 2. The new rearnangement concerning EN1 has been indicated and b) Physical location of IL1B on BTA11 and OAR3p**



DISCUSSION

A marked conservation of synteny between HSA2q *(Homo* sapiens), MMA2 *(Mus* musculus) and BTA2 *(Bos taurus)* has been already indicated by *Pitel et al.* (1994). Likewise, a comparative study between HSA2q and BTA2 showed PROC, *TNP* ,and *AIPI* genes maintain the same chromosomal centromere-telomere orientation but are not maintened within a single segment of conserved synteny (*Sonstegard et al.*, 1997). Additionally *Lopez-Coerales et al.* (1997) described a conserved relative physical locations for Glucagon and Nebulin in humans (HSA2q, cattle (BTA2), sheep (OAR 2q and goat chromosome 2 (CHI2). Here the assignments of EN1 and ILI B respectively support this previous study by further refining the breakpoints in the seemingly large segment of conserved synteny between humans and bovids. One of the disruptions in gene order conservation appears to be located within a portion of genome corresponding to HSA2q22. Likewise, in cattle and sheep the loci order *is PROC-GCG-NEB-EN1-TNP1 ALPI as* compared *to PROC-EN1-GCG-NEB-TNP1-ALPI on* HSA2q. These results demonstrate that chromosomal homology between mammals on a gross scale is not always indicative of conservation of gene order. The presence of interruptions in conserved synteny and gene order rearrangements underscore the need to develop dense comparative maps using either genetic linkage data, physical assignments or radiation hybrid maps. The conservation of synteny between HSA2q, mouse chromosome 2 and BTA2 indicated by *Pitel et al.* (1994) is also found in sheep. This physical mapping data supports previous comparative physical (INHBB, *Goldammer et al.*, 1995, COL3A1, *Broad et al.*, 1995; *Sonstegard et al.*, 1997; GCG and NEB, *Lopez-Corrales et al.*, 1997) and linkage mapping studies (*De Gortari et al.*, 1998) which suggested a relatively high degree of loci order conservation between sheep and cattle, especially between BTA2 and OAR2. Our findings also show that the breakpoint defined by the location of IL1B on BTA11 is conserved in sheep with the assignment of this loci to OAR3p. Although, no differences have been detected in gene order or synteny between BTA2 and OAR2q, subchromosomal changes between related species exist (*Iannuzzi et al.*, 1995; *Ponce De León et al.*, 1996). A more refined comparative map between closely related karyotypes should clarify the relationship between chromosomal banding homology and conserved synteny.

REFERENCES

Andersson, L.(1996): Comparative Genome Organization: First International Workshop. Mamm. Genome, 7. 7171–734.p.

Broad, T.E. – Lewis, P.E. – Burkin, D.J. – Gleeson, A.J. – Carpenter, M.A. – Jones, C. – Pearce, P.D. – Maher, D.W. – Ansari, H.A.(1995): Thirteen loci physically assigned to sheep chromosome 2 by cell hybrid analysis and *in situ* hybridisation. Mamm. Genome, 6. 862–866.p.

Chowdhary, B.P. – Frönicke, L. – Gustavsson, I. – Sherthan, H.(1996): Comparative analysis of the cattle and human genomes: detection of ZOO-FISH and gene mapping-based chromosomal homologies. Mamm. Genome, 7. 297–302.p.

De Gortari, M. – Freking, B. – Cuthbertson, R.P. – Kappes, S.M. – Keele, J.W. – Stone, R.T. – Leymasta, K.A. – Dodds, K.G. – Crawford, A.M. – Beattie, C.W.(1998): A second-generation linkage map of the sheep genome. Mammalian Genome 9, 204-209.

DiBerardino, D. – Iannuzzi, L.(1989): Detailed description of R-banded bovine chromosomes. J. Hered., 73. 434–438.p.

Fisher, S.R. – Beever, J.E. – Lewing, H.A.(1997): Genetic mapping of COL3A1 to bovine chromosome 2. Mamm. Genome, 8. 76–77.p.

Goldammer, T. – Brunner, R.M. – Hiendleder, S. – Schwerin, M.(1995): Comparative mapping of sheep inhibin subunit B to chromosome 2 in sheep and cattle by fluorescence *in situ* hybridization. Anim. Genetics, 26. 199–200.p.

Hayes, H. – Petit, E. – Dutrillaux, B.(1991): Comparison of RBG-banded karyotypes of cattle, sheep and goats. Cytogenet. Cell Genet., 57. 51–55.p.

Hayes, H.(1995): Chromosome painting with human chromosome-specific DNA libraries reveals the extent and distribution of conserved segments in bovine chromosomes. Cytogenet. Cell Genet., 71. 168–174.p.

Iannuzzi, L. –Di Meo, G.(1995): Chromosomal evolution in bovids: a comparison of cattle, sheep and goat G- and R-banded chromosomes and cytogenetic divergences among cattle, goat and river buffalo sex chromosomes. Chromosome Research, 3. 291–299.p.

Iannuzzi, L. – Di Meo, G.P. – Perucatti, A. – Ferrara, L.(1995): G- and R- Banding comparison of sheep (Ovis aries L.) chromosomes. Cytogenet. Cell Genet., 68. 85–90.p.

ISCNDA. International System for Cytogenetic Nomenclature of Domestic Animals,1989.(1990): Cytogenet. Cell Genet., 53. 65–79.p.

Lemieux, N. – Dutrillaux, B. – Viegas-Pequignot E.(1992): A simple method for simultaneous R- or G- banding and fluorescence *in situ* hybridization of small single-copy genes. Cytogenet. Cell Genet., 59. 311–312.p.

Lichter, P. – Tang, C.C. – Call, K. – Hamanson, G. – Evans, G.A. – Housman, D. – Ward, D.C. (1990): High resolution mapping of human chromosome 11 by *in situ* hybridization with cosmid clones. Sci., 237. 64–69.p.

Lopez-Corrales, N.L. – Sonstegard, T.S. – Smith, T.P.L. – Beattie, C.W.(1997): Physical assignment of glucagon and nebulin in cattle, sheep and goat. Mamm. Genome, 8. 428–429.p.

Pitel, F. – Lantier, I. – Tabet-Aoul, K. – Saidi-Mehtar, N. – Elsen J-M. Lantier – Gellin, J.(1994):. TNPI a sheep polymorphic marker for a conseved region among mammals. Mamm. Genome, 5. 390–392.p.

Ponce De Leôn, A. – Ambady, A. – Hawkins, G.A. – Kappes, S.M. – Bishop, M.D. – Robl, J.M. – Beattie, C.W.(1996): Development of a bovine x chromosome linkage group and painting probes to asses cattle, sheep and goat X chromosome segment homologies. Proc. Natl. Acad. Sci., USA, 93. 3450–3454.p.

Raudsepp, T. – Fronicke, L. – Scherthan, H. – Gustavsson, I. – Chowdhary, B.(1996): ZOO-FISH delineates conserved chromosomal segments in horse and man. Chromosome Research, 4. 218–225.p.

Rettenberger, G. – Kunz, J. – Vogel, W. – Hameista, H.(1995).Visualization of the conservation of synteny between humans and pigs by heterologous chromosomal painting. Genomics, 26. 372–378.p.

Smith, T.P.L. – Lopez-Corrales, N.L. – Kappes, S.M. – Sonstegard, T.S.(1997): Myostatin maps the interval containing the bovine mh locus. Mamm. Genome, 8. 742–744.p.

Solinas-Toldo, S. – Lengauer, C. – Fries, R.(1995): Comparative genome map of human and cattle. Genomics, 27. 489–496.p.

Sonstegard, T.S – Lopez-Corrales, N.L. – Kappes, S.M. – Beattie, C.W. – Smith, T.P.L.(1997): Comparative mapping of bovine and human chromosome 2 identifies segments of conserved synteny containing the bovine mh locus. Mamm. Genome, 8. 750–754.p.

Yoo, J. – Stone, R.T. – Kappes, S.M. – Beattie, C.W.(1994): Linkage analysis of bovine interleukin receptor types I and II (IL-1R I, II). Mamm. Genome, 5. 820–821.p.

Authors' address: *Lopez-Corrales, N.L.:* Roslin Institute, (Edinburgh), Roslin, Midlothian, EH25 9PS, UK
Sonstegard, T.S.: ARS-USDA Beltsville Agricultural Research, Bdlg, 200BARC-East, Beltsville, MD 20705, USA
Smith, T.P.L.: ARS-USDA Roman L Hruska, Meat Animal Research Center, Clay Center 18 D Spur, PO Box 166, NE, 68933, USA

SHEEP GENE MAPPING USING SOMATIC CELL HYBRIDS: CHARACTERIZATION OF OVINE CHROMOSOMES 4, 5 AND 6

TABET-AOUL, K. — SAIDI-MEHTAR, N. — LANTIER, I. — VAIMAN, D. — LANTIER, F.

A panel of 24 hamster-sheep somatic cell hybrids has been previously produced by fusing sheep cells, lymphocytes or fibroblasts, with immortalized, mutated hamster cells (1). This panel has already been used for gene mapping. Because the cytogenetic characterization was not possible, the frst step in the characterization of this hybrid panel was to identify their ovine chromosome contents. Analysis of 90 ovine or bovine microsatellites distributed along the 26 autosomes resulted in the first characterization of our panel with association of every syntenic group to ovine chromosome (2). To improve this characterization and to determine whether the ovine chromosomes are entirely or partially present in the hamster-sheep hybrids we have choosen, in this study, new microsatellites, localized either on sheep, cattle or, goat chromosomes. All microsatellites were analyzed by Polymerase Chain Reaction (PCR). Annealing temperatures were sometimes adjusted for a better sheep DNA amplifcation. Amplif ed fragments were separated by agarose electrophoresis and visualized by ethidium bromide staining. Ovine and hamster genomic controls were systematically included in the analysis of each microsatellite. Correlation between new markers and those previously localized on sheep chromosomes were determined according to the rules proposed by Chevalet and Corpet (3) With 24 hybrids, assignment to a syntenic group was assumed for a correlation coeffcient greater than, or equal to, 0.74. To ascertain our results, the typing of discordant hybrids from the same syntenic group have been duplicated.

As an example, we will present the status of sheep chromosome 4, 5 and 6 in the 24 hamster-sheep hybrid cells. For each chromosome we have data about the hybrids possessing the entire chromosome and those which lost a part of it.

This work results in the frst chromosome characterization for OAR4, 5 and 6 in our hamster-sheep hybrid panel. The characterization of the entire ovine chromosomes is in progress and allows the use of this panel for regional mapping in sheep. This panel may also serve as a tool to produce microsatellites in a region of interest, such as sites of putative QTL in sheep.

REFERENCES

Chevalet, C. – Corpet, F.(1986): Cytogenet. Cell Genet., 43. 132–139.p.
Saidi-Mehtar, N. et al. (1981): Cytogenet. Cell Genet., 30. 193–204.p.
Tabet-Aoul, K. et al. (1997): 10th North American Colloquium on Gene Mapping and Cytogenetics in Human and Domestic Species, Florida, USA

Authors' address: *Tabet-Aoul, K. – Lantier, I. – Lantier, F.*: Laboratoire de Pathologie Infectieuse et Immunologie, INRA, Tours, France
Saidi-Mehtar, N.: Laboratoire de Biologie Moléculaire et Génétique, Université Oran, Es-Sénia, Algeria
Vaiman, D.: Laboratoire de Génétigue Biochimique et Cytogénétigue, INRA, Jouy-en-Josas, France

LOCALIZATION OF 18S+28S RDNA IN THE RABBIT (*ORYCTOLAGUS CUNICULUS*) BY FISH AND AN UPDATE OF THE CYTOGENETIC MAP OF THIS SPECIES

ZIJLSTRA, C. — KORSTANJE, R. — DE HAAN, N.A. —
van LITH, H.A. — van ZUTPHEN, L.F.M. — BOSMA, A.A.

SUMMARY

The chromosomal location of the 18S+28S ribosomal RNA genes in the rabbit was determined by means of fluorescence *in situ* hybridization. Our data map these genes in the secondary constrictions of chromosomes 13, 16 and 20, and in the telomeric region of the q-arm of chromosome 21. These results confirm previous localizations obtained with other methods. In addition, the cytogenetic map of the rabbit genome was updated and shown to include fifty-four mapped loci.

INTRODUCTION

Despite the importance of the rabbit as a laboratory, production and pet animal, the gene maps of this species are only poorly developed. In order to increase the number of loci present on the physical gene map, we recently initiated mapping studies in which fluorescence *in situ* hybridizaton (FISH) is used as mapping tool.

The rabbit has 2n=44 chromosomes, and a standard karyotype was published in 1981 by the Committee for Standardized Karyotype of *Oryctolagus cuniculus*. This standard includes a numbering system and idiograms of GTG-banded chromosomes. On the basis of the position of the centromere, four morphological classes of chromosomes can be distinguished: metacentrics (Chrs.1–6), submetacentrics (Chrs. 7–11, X and Y), subtelocentrics (Chrs. 12–17) and acrocentrics (Chrs. 18–21). Comparison between the R-banded chromosomes of the rabbit and those of different primates revealed a high degree of similarity between the banding patterns of rabbit and human chromosomes (*Dutrillaux et al.*, 1980). Part of the observed homology between the chromosomes of these two species was later on confirmed by means of gene mapping studies.

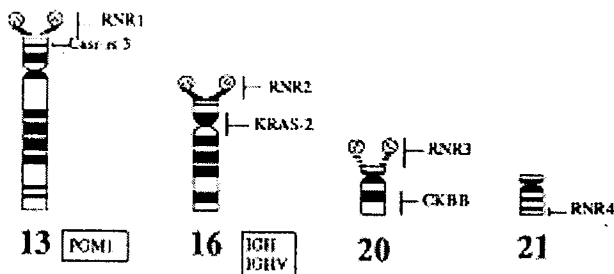
The present paper describes the localization of the 18S+28S ribosomal RNA genes (18S+28S rDNA) in the rabbit by means of FISH and summarizes the present status of the cytogenetic map of this species.

Mapping of 18S+28S rRNA genes

Materials and methods: For FISH, metaphase chromosomes were prepared from blood lymphocytes of a male New Zealander White following standard procedures. Chromosomes were GTG-banded prior to hybridization and well-banded metaphase spreads were photographed. A human rDNA probe (5.2 kb) was biotinylated and hybridized to rabbit chromosomes. Hybridization was carried out at 37 °C for 20h with a final probe concentration of 5 ng/µl. Specific hybridization sites were detected with FITC-conjugated avidin and signals were amplified twice using additional layers of biotinylated goat-anti-avidin and avidin-FITC. After hybridization, previously photographed metaphases were re-examined and chromosomes carrying specific fluorescent signals were identified on the basis of their GTG-banding pattern. Numbering of the chromosomes is in accordance with the recommendations of the Committee for Standardized Karyotype of *Oryctolagus cuniculus* (1981).

Results and discussion: After FISH, eight fluorescent spots were found in all metaphases examined, corresponding to four chromosome pairs. These pairs were identified as Chrs. 13, 16, 20 and 21. Clear fluorescent spots were found on both chromosomes of pair 21 in the telomeric region of the q-arm, band q16. In the other three chromosome pairs, prominent signals were present at the secondary constriction sites (*Fig. 1*). These data demonstrate that in the rabbit, as in many other mammalian species, the 18S+28S rRNA genes are located on several chromosomes. The regional localization of these genes as described here is in full agreement with previous localizations accomplished by selective silver staining of chromosomes for nucleolar organizer regions (Ag-NORs) (*Martin-DeLeon et al.*, 1978) and *in situ* hybridization with a radioactively labelled RNA probe on unbanded chromosomes (*Martin-DeLeon*, 1980).

Fig. 1.: **Idiograms of the chromosomes to which the 18S+28S rRNA genes (RNR1, 2, 3 and 4) were mapped, illustrating the position of these genes and of other loci mapped to the respective chromosomes**



STATUS OF THE RABBIT CYTOGENETIC MAP

In order to prepare an updated version of the physical gene map of the rabbit, an inventory of all published gene localizations was made. This inventory showed that up till now fifty-four genes have been mapped to individual rabbit chromosomes. Twenty-seven of these genes were regionally mapped by means of *in situ* hybridization, the others were assigned to individual chromosomes by means of analysis of somatic cell hybrids. In addition, this inventory showed that Chr. 1 is the chromosome to which most genes were mapped (in total three loci mapped regionally and four loci assigned) and that there are five rabbit chromosomes without any mapped locus (Chrs. 2, 7, 10, 11 and 18). In order to develop the cytogenetic map further, we will concentrate on these gene-poor chromosomes and will make use of the information provided by comparative painting and by the gene-rich maps of human and mouse.

REFERENCES

Committee for Standardized Karyotype of Oryctolagus cunicuius(1981): Standard karyotype of the laboratory rabbit, *Oryctolagus cunicuius*. Cytogenet. Cell Genet., 31. 240–248.p.

Dutrillaux, B. – Viegas-Pequignot, E. – Couturier, J.(1980): Tres grande analogie de marquage chromosomique entre le lapin (*Oryctolagus cuniculus*) et les primates, dont l'homme. Ann. Génét., 23. 22–25.p.

Martin-DeLeon, P.A.(1980): Location of the 18S and 28S rRNA cistrons in the genome of the domestic rabbit (*Oryctolagus cuniculus* L.). Cytogenet. Cell Genet., 28. 34–40.p.

Martin-DeLeon, P.A. – Petrosky, D.L. – Fleming, M.E.(1978): Nucleolar organizer regions in the rabbit (*Oryctolagus cuniculus*) as shown by silver staining. Can. J. Genet. Cytol., 20. 377–382.p.

Authors' address: *Zijlstra, C. – de Haan, N.A. – Bosma, A.A.*: Department of Cell Biology and Histology, Faculty of Veterinary Medicine, Utrecht University, P.O. Box 80.157, 3508 TD Utrecht, The Netherlands

van Lith, H.A. – van Zutphen, L.F.M. – Korstanje, R.: Department of Laboratory Animal Science, Faculty of Veterinary Medicine, Utrecht University P.O.Box 80.166, 3508 TD Utrecht, The Netherlands

A RADIATION HYBRID MAP OF PIG CHROMOSOME 4

LOPEZ-CORRALES, N.L. — MUNGALL, C. —MCCARTHY, L. —
MCDOWALL, S. — GOODFELLON, P.N. — ARCHIBALD, A.L.

The linkage maps of the pig genome being developed in Europe and the US cover 90% of the genome at a resolution of 10–20 cM and include about 1500, mainly microsatellite, markers. The linkage maps are being used to map quantitative trait loci (QTL) to 20–30 cM intervals. The subsequent isolation of the corresponding trait genes is likely to follow a (comparative) positional candidate gene approach exploiting the conservation of synteny amongst higher vertebrates. It is critical to the transfer of mapping information from humans and mice to livestock species such as pigs that we know the limits of conservation. A high resolution comparative gene map is essential for this purpose and radiation hybrids (*Walter et al.* 1994) provide one of the most effective ways of developing such a map. Thus our objective is to produce a radiation hybrid map of the entire pig genome.

Pig donor cells (primary fibroblasts established at Roslin from an F1 large White x Meishan male) were irradiated and fused to hamster A23 (TK^-) recipient cells. A radiation dose of about 3 Krads was used to generate fragments of the appropriate size range for a map of 1 Mbp resolution. Selection in HAT medium yielded radiation hybrid clones which have retained random fragments of the pig genome in addition to the porcine TK locus on chromosome 12. Three hundred hybrid clones were established and small quantities of DNA prepared for analysis. One hundred and eighty hybrids were evaluated for their retention of 27 pig genetic (microsatellite) markers. The average pig marker retention frequency of 25% compares favourably with successful radiation hybrid mapping panels.

Hybrids selected for high marker retention were expanded and DNA prepared. The DNA from these hybrids constitutes the T43 pig radiation hybrid panel. The T43 pig radiation hybrid panel consists of 92 hybrids chosen for high retention of pig genetic markers.

The distribution of pig chromosomes and chromosomal fragments has been assessed by fluorescence *in situ* hybridization (FISH) using total pig genomic DNA as the probe. This FISH analysis revealed pig chromosomal fragments both integrated into hamster chromosomes and as free rearranged pig chromosomes.

Forty microsatellite markers selected from the published USDA-MARC and PiGMaP linkage maps of chromosome 4 have been optimised for PCR analysis of the radiation hybrid panel. To date eighteen of these markers have been genotyped in the panel. Analysis of these data with RHMAPPER has allowed us to establish linkage groups for chromosome 4. We anticipate completeing the radiation hybrid map of chromosome 4 within the next two months.

This pig radiation hybrid mapping panel represents a valuable resource for pig *gene* mapping and allows integration of physical and genetic maps.

Authors' address: *Lopez-Corrales, N.L. – Mungall, C.. – Archibald, A.L.:*
Roslin Institute (Edinburgh), Roslin, Midlothian, EH25 9PS Scotland, UK
McCarthy, L. – McDowall, S.– Goodfellon, P.N.:
Department of Genetics, University of Cambridge, Cambridge

SPONTANEOUS FREQUENCIES OF SISTER CHROMATID EXCHANGES IN THE HORSE

MURAMATSU, M. — MIYAZAKI, H. — MURAMATSU, S. — YOSHIZAWA, M. — FUKUI, E.

INTRODUCTION

The sister chromatid exchanges(SCEs) are useful as one of the excellent indicators for the studies estimating the effects of environmental mutagens because of their high sensitivity to serve chemical and physical agents (*Kato,* 1977; *Latt,* 1978). The SCEs in mitotic chromosomes of domestic animals are studied on various species, such as cattle, water buffalo, goat, sheep, pig and so on (*DiBerardino and Shoffner,* 1979; *McFee and Sherill,* 1979; *Hanada and Muramatsu,* 1982; *Nirasawa et al.,* 1983; *Rubs,* 1987; *Catalon et al.,* 1994; *DiBerardino et al.,* 1997), and reported the results on the various factors affecting the incidence of the spontaneous and inducing SCEs in cells. Studies on SCEs in the horse are very scanty until now. Thus, we are continuing the analysis on the spontaneous rates of the SCEs in mitotic chromosomes of the horse in order to collect the fundamental data for the mutagenecity tests of several mutagens.

The present paper deals with our preliminary results on the equine chromosomes.

MATERIALS AND METHODS

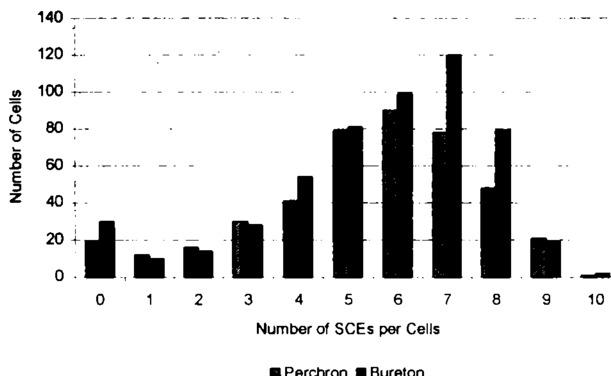
Peripheral blood samples were obtained from healthy horses of the Percheron (2 males and 3 females) breeds and the Breton(1 male and 4 females). Each sample was cultured by usual method described previously (*Hanada and Muramatsu,* 1982). Final concentration of BudR was 5.0 µg/ml. Air-dried preparations ware stained by the BudR-Giemsa techniques of *Kato and Sandberg* (1977).

SCEs ware counted on 70 or more metaphases in second division selected randomly for each animal.

RESULTS AND DISCUSSION

The data obtained from ten animals of two breeds are shown in *Table 1* of and *Figure 1*. The number of SCEs in each cell distributed in the range among zero to ten.

Fig. 1.: **Distribution of cells accompanied with SCEs**



In Percheron, the individual mean rates and standard errors per cell are ranged from 4.58±2.10 to 6.04±2.73, with an average of 5.43±2.24. In Breton, the individual mean rates and standard errors per cell are ranged from 5.25±2.26 to 5.78±2.56, with an average of 5.62±2.45. In comparison with the results shown in *Table 1.*, there are no significant differences on the spontaneous rates of SCEs per cell between both breeds.

Table 1.

Frequency of SCEs per cells

Breed	Sex	No.	Total number of cells	Total number of SCEs	SCEs per cells
Percheron	♂	1	78	425	5.45±2.02
		3	85	544	6.04±2.73
	♀	5	83	380	4.58±1.99
		6	84	385	4.58±2.10
		8	96	578	5.81±2.59
Total			426	2312	5.43±2.24
Breton	♂	2	83	436	5.25±2.26
	♀	4	77	445	5.78±2.56
		7	101	584	5.78±2.56
		9	134	738	5.51±2.34
		11	134	772	5.76±2.43
Total		10	529	2975	5.62±2.45

Kato (1997) reported that the mean frequancy of SCEs per cell in the horse estimated as 5.65±0.53 in the cultured cells in vitro. The average values of SCEs in the present study are very close to those reponded by *Kato* (1977).

A series of experiments is in progress to resolve the effects of culture condition and BudR-dose response relationship.

CONCLUSION

Based on the present results, the spontaneous frequencies of SCEs per cell in two breeds are estimated as 5.43±2.25 in the Percheron and 5.62±2.45 in the Breton.

REFERENCES

Catalon,J. – Moreno, C. – Arruga, M.V.(1994): Distribution and sources of variability of sister chromatid exchange frequencies in cattle. Genet. Sel. Evol., 26. 3–14.p.

DiBerardino, D. – Ramunno, L. – Jovino, V. – Pacelli,C. – Lioi, M.B. – Scarfi, M.R. – Burguete, I. (1997): Spontaneous rate of sister chromatid exchanges (SCEs) in mitotic chromosomes of sheep(*Ovis aries L.*) and comparison with cattle (*Bos taurus L.*), goat (*Capra hircus L.*) and river buffalo (*Bubalus bubalis L.*). Hereditas, 127. 231–238.p.

DiBerardino, D. – Shoffner, R.N.(1979): Sister chromatid exchanges in chromosomes of cattle (Bos taurus l.) J. Dairy Sci., 62. 627–632.p.

Hanada, H. – Muramatsu, S.(1982): The optimum culture time for detecting the chromosome damage induced in bovine lymphocytes. Bull. Nat. Inst. Anim. Ind., (Japan) 39. 107–110.p.

Kato, H.(1977): Spontaneous and induced sister chromatid exchanges as revealed by the BUdR-labeling method. Int. Rev. Cytol., 49. 55–97.p.

Kato, H. – Sandberg, A.A.(1977): Effects of herpes simplex virus on sister chromatid exchange and chromosome abnormalities in human diploid fibrobflasts. Exp. Cell Res.,109. 423–427.p.

Latt, A.V. – Thompson, L.H. – Lincle, P.A. – Minkler, J.L.(1978): Sister chromatid exchanges as an indicator of mutagenesis. Nature, 271. 551–553.p.

McFee, A.F. – Sherill, H.N.(1979): Species variation in BrdUrd-induced sister chromatid exchanges. Mutation Res., 62. 131–138.p

Nirasawa, K. – Muramatsu, S. – Hanada, H.(1983): Division cycle analysis of pig lymphocytes in vitro(in Japanese with English summary). Bull. Nat. Inst. Anim. Ind., (Japan) 40. 11–15.p.

Rubes, J.(1987): Chromosomal aberrations and sister chromatid exchanges in swine. Mutation Res., 191.105–109.p.

Authors' address: Faculty of Agriculture, Utsunomiya University,
350 Minemachi, Utsunomiya, Tochigi 321–8505, Japan

CHROMOSOME BANDING STUDIES OF RACCOON DOG (*NYCTEREUTES PROCYONOIDES PROCYONOIDES*)

PIENKOWSKA, A. — SWITONSKI, M.

INTRODUCTION

The karyotype of Euro-Asiatic subspecies of raccoon dog *(Nyctereutes procyonoides procyonoides)* consists of 54 A chromosomes and variable number (0–4) of B chromosomes. In the karyotype of the Japanese subspecies of raccoon dog *(Nyctereutes procyonoides viverrinus)* the number of A chromosome set is 38 and the number of B chromosomes varies from 0 to 6. In both subspecies Nucleolar Organizer Regions (NORs) are located on three autosome pairs and on the Y chromosome (*Mäkinen et al.*, 1986; *Ward et al.*, 1987).

There are contradictory opinions on the C-banding patterns of B chromosomes in the Euro-Asiatic subspecies. According to *Ward et al.* (1987) the B chromosomes do not show C-banding

patterns. *Wurster-Hill et al.* (1986) postulated that the Bs have individual C-banding patterns more distinct than those revealed by G-banding. *Mäkinen et al.* (1986) reported that C-banded Bs were stained positively along their whole length.

The aim of the present study was to characterize cytogenetically a group of Euro-Asiatic raccoon dogs froma commercial fur animal farm in Poland.

MATERIAL AND METHODS

Althogether 40 animals, from a farm in Jeziory near Poznań, were examined cytogenetically (*Sumner*, 1972).

The following tehniques were applied: Giemsa staining, CBG-banding and silver staining (Ag-I) (*Goodpasture and Bloom,* 1975).

RESULTS

1. Distribution of B chromosomes: Distribution of B chromosomes was analysed in animals for which 8 to 10 good chromosome spreadscould be analysed. Extensive intraindividual variability, in the range from 0 to 4, was found (*Table 1*). Usually two B chromosomes in a spread (36.4%) were observed. Spreads with one or three B chromosomes were found with similar frequency: 24.4% for one B chromosome and 23.1% for three Bs.

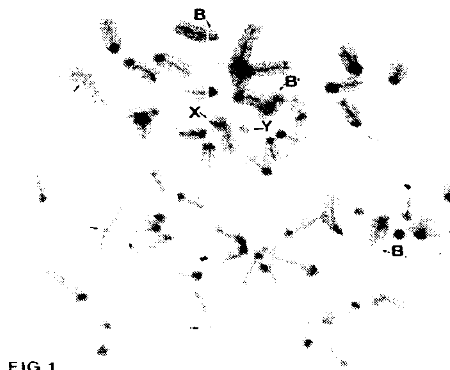
Table 1.

Variability of the number of B chromosomes in 23 raccoon dogs

Sex	No.	No. of analysed spreads	B chromosomes (number of spreads)				
			0	1	2	3	4
	1	10	—	—	5	5	
F	2	10	1	—	2	4	3
	3	10	—	—	3	7	—
E	4	10	—	1	7	2	—
	5	9	1	4	4		—
M	6	10	4	1	2	3	—
	7	10	2	1	3	4	—
A	8	10	1	6	2	1	—
	9	10	2	3	3	2	—
L	10	10	1	4	5	—	—
	11	10	—	6	4	—	—
E	12	10	—	2	2	6	—
	13	9	1	2	2	2	2
S	14	10	2	3	5	—	—
	15	10	—	6	4	—	—
	16	10	1	2	2	3	2
Σ		158	16	41	55	39	7
%			10.1%	25.9%	35.4%	24.7%	4.4%
	17	10	—	1	4	5	—
M	18	10	—	1	2	7	—
A	19	10	3	3	4	—	—
L	20	9	—	3	6	—	—
E	21	8	—	—	6	1	1
S	22	10	4	4	2	—	—
	23	10	5	2	3	—	—
Σ		67	12	14	27	13	1
%			17.9%	20.9%	40.3%	19.4%	1.5%
Together		225	28	55	82	52	8
%			12.4%	24.4%	36.4%	23.1%	3.6%

2. *Distribution of C-bands:* CBG banding revealed constitutive heterochromatin in centrimeric regions of all A autosomes, except of two large biarmed chromosomes. The B chromosomes appeared to be negative after CBG-banding (*Fig. 1.*).

Fig. 1.: **CBG-banding. B chromosomes and two pairs of bi-armed A autosomes without distinct centromeric C-band are indicated by arrows**



3. *Distribution of silver stained NORs:* NORs were found on three autosome pairs and on the Y chromosome (*Fig. 2.*). Mean number of silver stained NORs in a group of 40 animals was 4.9 and the value was higher in males (5.2) than in females (4.8). The modal number was the same for both sexes (*Table 2.*).

Table 2.

Activity of Nucleolar Organizer Regions (Ag-NOR) in raccoon dogs

Sex	Number of animals	Number of metaphase spreads	Number of active NORs	
			mean	modal number
Females	27	213	4.8	5
Males	13	100	5.2*	5*
Together	40	313	4.9	5

* active NOR on Y chromosome was found in 31% of metaphase spreads

Fig. 2.: **Silver staining. Silver deposits are indicated by arrows**

CONCLUSIONS

1. Number of B chromosomes demonstrates extensive intranidividual variability. The number varied from 0 to 4 and most frequently two Bs were found.

2. CBG-banding revealed lack of constitutive heterochromatin on B chromosomes. All autosomes of the A set, exept of two biarmed ones, showed distinct centromeric constitutive heterochromatin.

3. Three autosome pairs and Y chromosome bear Nucleolar Organizer Regions. Mean number of active (silver stained) NORs was 4.8 in females and 5.2 in males.

REFERENCES

Goodpasture, C. – Bloom, S.E.(1975): Vizualization of Nuclear Organizer Regions in mammalian chromosomes using silver staining. Chromosoma, 55. 37–50.p.

Mäkinen, A. – Kuokkanen, M.T. – Valtonen, M.(1986): A chromosome banding study in the Finnish and Japanese raccoon dog. Hereditas, 105. 97–105.p.

Sumner, A.T.(1972): A simple technique,for demonstrating centromeric heterochromatin. Exp. Cell. Res., 75. 304–306.p.

Ward, O.G. – Wurster-Hill, D.H. – Ratty, F.J. – Song, Y.(1987), Comparative cytogenetics of Chinese and Japanese raccoon dogs, *Nyctereutes procyonoides*. Cytogenet. Cell Genet., 45. 177–186.p.

Wurster-Hill, D.H. – Ward, O.G. – Kada, H. – Whittemore, S.(1986), Banded chromosome studies and B chromosomes in wild- caught raccoon dogs, *Nyctereutes procyonoides viverrivus*. Cytogenet. Cell Genet., 42. 85–93.p.

Authors' address: Department of Genetics and Animal Breeding, Agricultural University of Poznan, Wołynska 33, 60-637 Poznan, Poland

INHERITANCE OF TWO MORPHOLOGICAL FORMS OF CHROMOSOME 4 IN ZATORSKA GEESE

RABSZTYN, A. — JASZCZAK, K. — JASZCZAK, J. — KAPKOWSKA, E.

SUMMARY

The investigations were carried out in the population of Zatorska breed of geese, which was developed 40 years ago on basis of several indigenous varieties. The basal varieties included both *Anser anser* and *Anser cygnoides* . The two species differ in morphology of the fourth autosome (submetacentric vs. metacentric). Earlier investigations revealed that, after many years of closed flock selection program for egg number and body weight, both forms of chromosome four were segregating in the population of Zatorska geese, suggesting possible relationship with embryonic mortality and hatchability.

Males with metacentric chromosome 4 were mated with both metacentric and submetacentric females and karyotypic observations were made in early embryos and in blood lymphocytes of the progeny. The frequency of the two morphological forms of chromosome 4 depended on the type of mating but the observed proportion of embryos carrying metacentric chromosome four was smaller than theoretically expected in both types of mating (53.5% vs. 75% and 32.1% vs.50%, respectively in MxM and MxS types of mating). The proportion of female embryos with metacentric chromosome 4 was slightly smaller than metacentric male embryos. The putative difference in frequency of metacentric chromosome 4 observed between early goose embryos and goslings could suggest a relationship with hatchability.

INTRODUCTION

The breed of Zatorska geese was created forty years ago on basis of several Polish varieties of geese (*Anser anser*) and the hunchbeak White Chinese geese *(Anser cygnoides)* maintained in Poland since many, many years (*Bączkowska et al.*,1967). The latter is characterised by different morphology of the fourth autosome (metacentric instead of submetacentric) as compared to *A. anser* (*Silversides et al.*, 1988).

After many years of intrapopulation selection on egg performance and body weight chromosomal polymorphism in autosome 4 was found in the population (*Jaszczak et al.*,1996). Some efforts are being made on observations whether or not there is an influence of parental karyotype on hatchability and embryonic mortality (*Rabsztyn et al.*,1997) which was also suggested in geese by *Liptói and Hidas* (1997). The objective of the study was to investigate whether the inheritance of the two morphological forms of chromosome 4 segregating in the population was in accordance with theoretical principle, after the parents were purposely mated according to their karyotype instead of performance.

MATERIAL AND METHODS

Four males with metacentric chromosome 4, in heterozygous condition, were pedigree mated with 24 females, half of them with metacentric chromosome 4 in heterozygous state (MxM mating), and half of (submetacentric karyotype (MxS mating).

Karyotypic studies were made in early embryos and in blood lymphocytes in the progeny of the pedigree mated individuals. The eggs were collected daily and stored up to 15 days. They were incubated for 18 hours and then the blastodiscs from fertile eggs were cultured for 2 hours. Further preparation followed the method described by *Zartman and Jaszczak* (1979).

Peripheral blood lymphocytes were collected from 11-week and 7-month old progeny and metaphase chromosomes (five largest pairs and sex chromosomes) were analysed.

The chi-square goodness of fit test was used to test data on sex ratio in embryos (*Steel and Torrie*, 1980).

RESULTS AND DISCUSSION

In two reproductive seasons, 1997 and 1998, a total of 202 fertile eggs were examined. 40.6% of embryos were males and 59.4% were females (*Table 1.*). In male embryos 52.4% were found to have submetacentric autosome 4, and 47.6% were metacentric. In female embryos, however, 60.0% were observed to be submetacentric and only 40.0% had metacentric chromosome 4.

Frequencies of embryos carrying two different morphological forms of autosome 4 in respect to the type of mating are presented in *Table 2.* There were 53.5% of embryos with metacentric chromosome 4 resulting from MxM type of mating and 32.1% resulting from MxS type of mating. Assuming normal segregation, the observed numbers of embryos with metacentric chromosome 4 were less than expected. The deviation from theoretically expected proportions could be related to some impairment of metacentric karyotype during spermatogenesis or oogenesis, fertilization and early embryonic development. *Crawford* (1990) refers to *Gray's* (1958) indication in his check-list of bird hybrids that F_1 progeny of *A. anser* x *A. cygnoides* have normal fertility but hatchability in backcrosses is very low.

Table 1.

Number of embryos with submetacentric and metacentric autosome 4 in eggs from experimental mating.

Fertile eggs				
202	82** (40.6%) Chromosome 4		120** (59.4%) Chromosome 4	
	Submetacentric	metacentric	Submetacentric	metacentric
	43 (52.4%)	39 (47.6%)	72 (60.0%)	48 (40.0%)

** significant deviation from 1:1 ratio, P<0.01

Preliminary karyotypic observations in peripheral blood lymphocytes in F_1 progeny have shown apparently smaller proportion of individuals with metacentric than submetacentric chromosome 4 (respectively, 20% and 80% in female progeny and 15% and 85% in male progeny). The frequency of metacentric chromosome 4 in the selection population of Zatorska geese based on blood examinations was found by *Rabsztyn* (1997) to be 8.57%. This needs further investigation. There was a significant deviation from the expected 1:1 sex ratio in embryos (*Table 1.*) in data pooled over two years, although the deviation was observed only in the first year of investigation and was not seen in the second year.

Table 2.

Number of goose embryos with metacentric or submetacentric chromosome 4 resulting from MxM or MxS type of mating (pooled over 2 seasons)

Type of mating	MxM	MxS
Number of embryos	114	84
Chromosome 4		
- metacentric	61 *(53.5%)*	27 *(32.1%)*
- submetacentric	53 *(46.5%)*	57 *(67.9%)*

MxM – *metacentric* x *metacentric;* MxS – *metacentric* x *submetacentric*

CONCLUSIONS

There was slightly smaller proportion of female embryos with metacentric chromosome 4 as compared to male embryos. The frequency of the two morphological forms of autosome 4 was related to the type of mating. The observed proportion of embryos carrying metacentric chromo-

some 4 was smaller than could be expected on theoretical basis. The putative difference in frequency of metacentric chromosome 4 between early goose embryos and goslings could suggest a relation with hatchability.

REFERENCES

Bączkowska, H. – Freundlich, A. – Rychlicka, J. – Bieliński, K.(1967): Nowa odmiana gesi w wyniku krzyżowania krajowych grup użytkowych (in Polish, with English summary: A new breed of geese as a result of crossing of home-bred utility groups). Acta Agr. Silv., VII. 1. 3–18.p.

Crawford, R.D.(1990): Origin and history of poultry species. In: Poultry Breeding and Genetics. Ed.: Crawford, R.D., Elsevier, Amsterdam, 1–41.p.

Jaszczak, K. – Rabsztyn, A. – Jaszczak, J.(1996): Cytogenetic study of embryos in geese of preservable stock. Arch. Zootec., 45. 229–231.p.

Liptói, K. – Hidas, A.(1997): Investigation of chromosomal abnormalities in early dead goose embryos. Zeszyty Naukowe Przeglądu Hodowlanego, 31. 205–206.p.

Rabsztyn A.(1997): Wpływ czynników genetycznych na niektóre cechy reprodukcyjne u gesi (in Polish) , State Committee for Scientific Research, Project nr 5 P06D, Kraków

Rabsztyn, A. – Niedziółka, J. – Jaszczak, K. – Kapkowska, E.(1997): Preliminary studies on the effect of parental karyotype on embryonic mortality in Zatorska geese. Proc. 12th International Symposium on Current Problems in Avian Genetics "Aviagen" Pruhonice, Sept 1–3. 167–168.p.

Silversides, F.G. – Crawford, R.D. – Wang, H.C.(1988): The cytogenetics of domestic of geese. J. Hered., 79. 6–8.p.

Steel, R.G.D. –Torrie, J.H.(1980): Principles and Procedures of Statistics. McGraw-Hill Book Company, New York, 469.p.

Zartman, D.L. – Jaszczak, K.(1979): A procedure for chromosome preparations from early chick embryos. Pr. Mater. Zoot., 23. 115–118.p.

Authors' address: *Rabsztyn, A. – Kapkowska E.*: Agricultural University, Poultry Science Department, 24/28 Mickiewicza St., 30-059 Kraków, Poland

Jaszczak, K. – Jaszczak, J.: Institute of Genetics and Animal Breeding, Department of Molecular Cytogenetics, Polish Academy of Sciences, Jastrzebiec, 05–551 Mroków, Poland

NEW MARKERS FOR THE INTEGRATION OF CHICKEN MICROCHROMOSOME CYTOGENETIC AND GENETIC MAPS

FILLON, V. — PITEL, F. — MORISSON, M. — POUZADOUX, A. — BERGÉ, R. — ZOOROB, R. — AUFFRAY, C. — GELLIN, J. — VIGNAL, A.

SUMMARY

The chicken karyotype, composed of 2n=78 chromosomes, is separated into two classes: the macrochromosomes and the microchromosomes. The standard karyotype for the 8 large macrochromosome pairs and the sex chromosomes, ZW (female) or ZZ (male), has been established after chromosome banding studies but the thirty microchromosome pairs remain indistinguishable, visible only as dots on metaphase preparations.

The chicken genetic map is composed of large linkage groups usually assigned to macrochromosomes, and a greater number of small linkage groups which probably correspond to microchromosomes, but with no specific chromosomal assignment.

To integrate the chicken genetic and cytogenetic maps, Bacterial Artificial Chromosome (BAC) and P1-derived Artificial Chromosome (PAC) clones were localised by Fluorescence *In situ* Hybridisation (FISH) on chromosomes and by genetic mapping on the East Lansing and Compton reference families by developing either microsatellite or Single Strand Conformational Polymorphism (SSCP) markers.

These new genetic markers will increase the number of those already available but their particular value is that they are both physical and genetic markers and thus were used to assign linkage groups on chicken microchromosomes. Altogether, 11 markers were generated and enabled the assignment of linkage groups to 8 different microchromosomes.

INTRODUCTION

The chicken karyotype is composed of 2n=78 chromosomes, including the ZW (female) or ZZ (male) sexual chromosomes. The standard karyotype for the 8 large macrochromosomes, as well as the sexual ones, has been established after chromosome banding studies, but the 30 microchromosomes remain indistinguishable and are ordered arbitrarily by approximate decreasing size. The chicken genetic map is actually composed of a few large linkage groups usually assigned to the macrochromosomes, and a greater number of small linkage groups often containing few markers, corresponding either to microchromosomes or to specific regions of macrochromosomes. The aim of the present study is to provide markers useful for both the genetic and cytogenetic maps, especially directed on microchromosomes.

In a first approach, BAC and PAC clones containing microsatellites were identified and then microsatellites were subcloned and characterized by sequencing. Those revealing polymorphism were mapped on the international Compton and East Lansing chicken reference back-cross mapping populations and regionally assigned to chicken chromosomes by FISH.

Our second strategy was especially developed to complete the under-developed genetic map of microchromosomes. Random BAC and PAC clones localized on chicken microchromosomes by FISH were subcloned and a SSC polymorphism was developed in order to integrate the cytogenetic and genetic maps.

MATERIALS AND METHODS

Clones: Large insert-containing clones were selected to generate strong FISH signal. They were randon PAC or BAC clones (*Zoorob et al.*, 1996), some of which pre-screened for presence of a (CA)n dinucleotide repeat.

FISH mapping: Metaphase spreads were from embryo fibroblast cultures. Two-colour FISH was performed by labelling probes with digoxigenin (digoxigenin-11-dUTP, Boehringer Mannheim) or biotin (biotin-16-dUTP, Boehringer Mannheim) by random priming. Probes were hybridised to the

metaphases for 24 hours. Biotin labelled probes were detected with avidin-Texas red (Vector) and digoxigenin labelled ones with FITC antibodies (Boehringer Mannheim).

Genetic mapping: Subcloning (CA)n microsatellite-containing BAC or PAC fragments was performed by *Sau*3AI or *Alu*I digestion, followed by ligation into pTZ19R plasmid vector. BAC clones not containing a microsatellite were digested with *Hae*III and cloned into pUC18 at the *Sma*I site, using the Sureclone ligation kit (Pharmacia). Sequencing was performed on an automatic sequencing machine (Applied Biosystems). Genotyping of the clones was performed on the mapping populations either by PCR and denaturing poly-acrylamide gel electrophoresis, for the (CA)n microsatellites, or by PCR and SSCP analysis. When possible, segregation analyses were performed both in the Compton (*Bumstead and Palyga*, 1992) and East Lansing (*Crittenden et al.* 1993) chicken reference back-cross mapping populations. Genetio localisation on the maps was calculated with the Map-Manager software (*Manly*, 1993).

Mapping markers on both genetic and cytogenetic maps

Microsatellite markers: 9 PAC and BAC clones containing microsatellite and previously localised on microchromosomes were subcloned in pTZ19R. The positive subclones were sequenced and primers were designed for all of them but we obtained cytogenetic and genetic map locations for only 2 clones called P3C6 and P10G12 (*Table 1.*).

SSCP markers: Single Stranded Conformational Polymorphisms (SSCP) were developed as an alternative approach to generate markers. First, the microsatellite markers that could not be mapped in the reference families, were tested by migration on non-denaturing poly-acrylamide gels and one (P1A6) could be mapped as an SSCP.

Then, 13 PAC or BAC clones, all located by FISH on microchromosomes, were subjected to a simple subcloning and sequencing of the largest fragments obtained. A panel of enzymes was used for each marker, to break the primary PCR products into fragments of sizes around 200 bp. For the marker P1-8, a PCR-RFLP was detected directly when testing the restriction enzymes, with different enzymes in the two families. Four markers could be mapped directly as SSCPs after PCR and 3 others by SSCP after restriction digestion (*Table 1.*).

Table 1.

Cytogenetic and genetic mapping of clones

Genetic marker	Clone name	Polymorphism	Cytogenetic mapping	Genetic mapping	
			Chromosome[1]	East Lansing	Compton[3]
				Linkage groups	
GCT0014	P3C6	microsat	MC (13–16)	E18	NP
GCT0016	P1A6	SSCP	MC (9)	E36[2]	NP
GCT0022	P10G12	microsat	MC (25–27)	New	C35
GCT0901	B6E8	SSCP-rest	MC (11–13)(A)	E48	NP
GCT0902	P10B1	SSCP	MC (23–29)(C)	NP	C34
GCT0903	P1-8	PCR-RFLP	MC (12–14)(B)	E35	C18
GCT0904	P2-3	SSCP	MC (23–29)(C)	E53	C34[4]
GCT0905	P2-4	SSCP	MC (18–22)	E49	NP
GCT0906	P2-7	SSCP-rest	MC (19–23)	E46	NP
GCT0907	P3-1	SSCP	MC (11–13)(A)	NP	C28
GCT0908	P6V11	SSCP-rest	MC (12–14)(B)	NP	C37

NP: Non Polymorphic; UN: Unlinked; New: new linkage group, to be named; MC: microchromosome. [1]Only the estimated size range of microchromosomes, for example MC (11–13), could be given after cytogenetic mapping. Identical microchromosomes are indicated by identical additional letters (A, B, C). [2]GCT0016 was not polymorphic as a microsatellite in the reference crosses, but showed two alleles in the East Lansing population through Single Strand Conformational analysis. [3]The Compton linkage group numbers are from the new maps of January 1998 (N. Bumstead, personal communication). [4]This is a new linkage group, markers from the ancient C34 linkage group are now in C18 (N. Bumstead, personal communication and Chicken Genome Database (http://www.ri.bbsrc.ac.uk/chickmap/)):

SSCP: markers mapped directly as SSCPs after PCR .

SSCP-rest: markers mapped by SSCP after restriction digestion.

PCR-RFLP: detected directly when testing the restriction enzymes on PCR product.

DISCUSSION AND CONCLUSION

As a first approach, microsatellite markers were developed for genetic mapping but the SSCP technique has been more effective for developing polymorphic integration markers from large insert clones. Altogether, 11 markers linking the microchromosome cytogenetic and genetic maps were generated and enabled the assignment of linkage groups to 8 different microchromosomes.

Our genetic mapping data have shown that P10B1 and P2-3 are close together on the same linkage group (5.4 cM on the Compton map) (*Table 1.*). The localisation of these two markers on the same microchromosome was confirmed by a two-colour FISH experiment.

Another interesting result was obtained from the genetic marker GCT0903, derived from the PAC clone P1-8 located on one of microchromosomes 12, 13 or 14, that enabled us to link the Compton linkage groups C18 and C34 together. The resulting linkage group, now named C18, has a genetic size of 37.5 cM, but a much larger linkage group is now expected for this chromosome, as the genetic marker GCT0908, derived from the PAC clone P6V11 located on the same microchromosome by a two colour FISH experiment, was not linked to any other marker on the current genetic map. Together with the data on the two genetically independent *B@* and *Rfp-Y@* Major Histocompatibility Complexes, which are both located on microchromosome 16 (*Fillon et al.,* 1996), this is the second time a high recombination frequency has been associated with a chicken microchromosome but it is too early to establish a reliable ratio between the physical and genetic size of the microchromosomes: the chicken genetic map is far from being saturated for this fraction of the genome.

ACKNOWLEDGEMENTS

We would like to thank N. Bumstead for providing DNA samples of the Compton reference family and L.B. Crittenden and H. Cheng for providing DNA samples of the East Lansing chicken reference family.

This work is part of the European ChickMap project (contract BIO4 CT95-0287).

V. Fillon is supported by a Ministére de l'Enseignement Supérieur et de la Recherche grant.

REFERENCES

Bumstead, N. – Palyga, J.(1992) A preliminary linkage map of the chicken genome. Genomics, 13. 690–697.p.

Crittenden, L.B. – Provencher, L. – Santangelo, L. – Levin, I. – Abplanalp, H. – Briles, R.W. – Briles, W.E. – Dodgson, J.B.(1993) Characterization of a Red Jungle Fowl by White Leghorn backcross reference population for molecular mapping of the chicken genome. Poultry Sci., 72. 334–348.p.

Fillon, V. – Zoorob, R. – Yerle, M. – Auffray, C. – Vignal, A.(1996) Mapping of the genetically independent chicken major histocompatibility complexes B@ and Rfp-Y@ to the same microchromosome by two-color fluorescent *in situ* hybridization (FISH). Cytogenetics and Cell Genetics, 75. 7–9.p.

Manly, K.F.(1993) A Macintosh program for storage and analysis of experimental genetic mapping data. Mammalian Genome, 4. 303–313.p.

Zoorob, R. – Billault, A. – Severac, V. – Fillon, V. – Vignal, A. – Auffray, C.(1996) Two chicken genomic libraries in the PAC and BAC cloning systems: Organization and characterization. Animal Genetics, 27. (suppl. 2), 69.p.

Authors' address: *Fillon, V. – Pitel, F. – Morisson, M. – Pouzadoux, A. – Bergé, R. – Gellin, J. – Vignal, A.:* Laboratoire de Génétique Cellulaire, INRA, BP27
31326 Castanet-Tolosan Cedex, France
Zoorob, R. – Auffray, C.: Génétique Moléculaire et Biologie du Développement, CNRS UP420, Villejuif, France

MOLECULAR CYTOGENETIC STUDIES IN DOMESTIC GOOSE

HIDAS, A.

SUMMARY

European domestic goose breeds are originated from the Anser anser, while the Asian and African ones were bred from the Anser cygnoides. Following conventional Giemsa staining, the karyotypic difference is obvious on the 4th chromosome pair, which is submetacentric in case of European breeds, while metacentric in the others. CBG staining reveals an intercalaric and centromeric heterochromatic segments in the A. cygnoides type chromosome 4 as a further difference. Satellite DNA comparison was made by GISH (Genomic In Situ Hybridization). Satellite DNA (occupying the heterochromatic regions) sequences tend to hybridize much more quickly and effectively than unique and moderately repeated sequences, therefore their target sequences can be well detected on the chromosomes. The A. cygnoides satellite DNA hybridized well to apparently all of the heterochromatic regions of the A. anser chromosomes showing high sequence homology. The A. anser satellite DNA also hibridized to the heterochromatic segments of the A. cygnoides karyotype (including centromeric heterochromatin of chromosome 4, which is missing in the A. anser) except for the intercalaric heterochromatin of the chromosome 4. The centromeric heterochromatin of A. cygnoides type chromosome 4 seems to contain sequences similar to some or all other heterochromatic regions. The intercalaric heterochromatin satellite DNA represents a completely unique satellite family with sequences obviously different from the other heterochromatic regions and missing from the A. anser genome (or might be present, but in single or few copy number).

INTRODUCTION

The two goose species which from most of the goose breeds were domesticated are the greylag goose (Anser anser) and the Chinese swan goose (*Anser cygnoides*). These closely related species and breeds (their hybrids are fertile) show karyotypic differences. The chromosome 4 is submetacentric in the A. anser karyotype (*Hammar*, 1966), while it is metacentric in the A. cygnoides (*Bhatnagar*, 1968).

Early observations supposed a pericentric inversion in the chromosome structure change (*Silversides et al.*, 1988). Our studies with C-banding and restriction endonuclease banding revealed other differences as well concerning the heterochromatin distribution on the chromosome 4 (*Hidas*, 1993). While the submetacentric A. anser chromosome does not carry heterochromatin, the metacentric A. cygnoides type chromosome 4 shows both centromeric and intercalaric heterochromatin.

The genomic *in situ* hybridization (GISH) was applied between the different karyotypes to get some more information on the heterochromatin distribution differences. GISH is generally suitable for chromosome identification in hybrids and especially effective for those chromosomal regions which contains satellite DNA (heterochromatin) since these sequences hybridize more intensively (*Houseal et al.*, 1995; *Shi et al.*, 1996). GISH is also capable for detection of amplification events (frequently observed in tumor cells), since the extreme increase in the copy number of a sequence of the probe genome gives a stronger signal at the affected site on the normal chromosome (*Thompson and Gray*, 1993; *Schrock et al.*, 1994; *Fischer et al.*, 1994; *Houldsworth et al.*, 1994).

MATERIAL AND METHODS

Goose embryos for chromosome and DNA preparations were obtained from the "Kuban" breed stock of the Goose Research Station of the Gödöllő University of Agricultural Sciences and from the Institute for Small Animal Research.

Genomic DNA was prepared from the liver of 6-7 day old goose embryos of different karyotypes (MM=both chromosome 4 is metacentric and SS=both chromosome 4 is submetacentric). The isolated DNA was nick translation labelled with fluorescein-dUTP (Boehringer).

Chromosome preparations were made from goose embryonic fibroblast cultures and the slides were stored for 1–2 weeks before *in situ* hybridization.

In situ hybridization were initiated with simultaneous denaturation of probe and chromosomes at 70 °C for 1–2 minutes. Following denaturation the slide was cooled on ice and hybridization was carried out at 37 °C for 24–48 hours. Hybridization was terminated by 10 min washing in 37 °C 2xSSC and preparations were examined directly or counterstained with propidium iodide.

RESULTS AND DISCUSSION

The results of *in situ* hibridization experiments are shown in the *Table 1.*

The MM karyotype DNA hybridized to the same type chromosomes revealed the distribution of all satellite DNA present in the genome. The intercalaric heterochromatin is also occupied by a huge amount of satellite DNA.

Table 1.

Satellite DNA hybridization signals on the chromosome 4 in different combinations of metaphase chromosome preparation and probe karyotype

Karyotype of chromosome preparation	Karyotype of probe DNA	Centromeric hetero-chromatin	Intercalaric hetero-chromatin
MM ZW	MM ZZ	+	+
MM ZZ	SS ZZ	+	—
MM ZZ	SS ZW	+	—
SS ZZ	MM ZZ	—	—

The MM probe hybridized to the SS karyotype did not give any outstanding labelling on the submetacentric chromosome 4 as it was expected if the intercalaric heterochromatin would be a result of local amplification and subsequent heterochromatinization (This event might remain hidden if the amplified unit is a very short sequence).

The probe produced from the genomic DNA of SS karyotype (without the intercalaric heterochromatin) hybridized to the metacentric chromosome showed the cross-hybridization signal on the centromeric heterochromatin. Sequences of this region therefore look similar to those present on the other or some of the other chromosomes. These might be originated from other chromosomes or evolved with them (by nonhomologous exchanges during the evolution, which is responsible for maintenance of sequence similarity between different heterochromatic regions of nonhomologous chromosomes). Although we do not know the direction of evolutionary progress, loss of this heterochromatin seems to be more likely (M→S) than gaining (S→M). The intercalaric heterochromatin was completely free of heavy labelling which was observed at the MM-MM hybridization. It suggests the lack of these sequences in the SS karyotype and also the difference from the satellite DNA of other heterochromatic regions.

ACKNOWLEDGEMENTS

Thanks for the kind provision of the samples by Dr Kozák, J.; Dr Bódi, L.; and Mrs Karsainé Kovács, M. from the Goose Research Station of the Gödöllő University of Agricultural Sciences. This work was supported by the Hungarian Research Fund (F 016664)

REFERENCES

Bhatnagar, M.K.(1968): Mitotic chromosomes of white Chinese geese. J. Hered., 59. 191–195.p.

Fischer, U. – Wullich, B. – Sattler, H.P. – Gottert, E. – Zang, K.D. – Meese, E.(1994): DNA amplifications on chromosomes 7, 9, and 12 in glioblastoma detected by reverse chromosome painting. Eur. J. Cancer, 30A. 1124–1127p.

Hammar, B.(1966): The karyotypes of nine birds. Hereditas, 55. 367–385.p.

Hidas, A.(1993): Cytogenetic studies on a species hybrid goose breed. Proc. 8th North American Colloqium on Domestic Animal Cytogenetics and Gene Mapping, Guelph, Canada, 153–155.p.

Houldsworth, J. – Chaganti, R.S.K.(1994): Comparative genomic hybridization: an overview. American Journal of Pathology, 145. 1253–1260.p.

Houseal, T.W. – Cook, J.A. – Modi, W.S. – Hale, D.W.(1995): Identification of highly conserved loci by genome painting. Chromosome Research, 3. 175–181.p.

Jacobsen, E. – Dejong, J.H. – Kamstra, S.A. – Vandenberg, P.M.M.M. – Ramanna, M.S.(1995): Genomic *in situ* hybridization (GISH) and RFLP analysis for the identification of alien chromosomes in the backcross progeny of potato(+)tomato fusion hybrids. Heredity, 74. 250–257.p.

Le, H.T. – Armstrong, K.C. – Miki, B.(1989): Detection of rye DNA in wheat-rye hibrids and wheat translocation stocks using total genomic DNA as a probe. Plant Molec. Bioi. Rep., 7 .150–158.p.

Schrock, E. – Thiel, G. – Lozanova, T. – Dumanoir, S. – Meffert, M.C. – Jauch, A. – Speicher, M.R. – Nurnberg, P. – Vogel, S. – Janisch, W. – Doniskeller, H. – Ried, T. – Witkowski, R. – Cremer, T. (1994): Comparative genomic hybridization of human malignant gliomas reveals multiple amplification sites and nonrandom chromosomal gains and losses. American Journal of Pathology, 144. 1203–1218.p.

Shi, L. – Zhu, T. – Morgante, M. – Rafalski, J.A. – Keim, P.(1996): Soybean chromosome painting: A strategy for somatic cytogenetics. J. Hered., 87. 308–313.p.

Silversides, F.G. – Crawford, R.D. – Wang, H.C.(1988): The cytogenetics of domestic geese. J. Hered., 79. 6–8.p.

Thompson, C.T. – Gray, J.W.(1993): Cytogenetic profiling using fluorescence insitu hybridization (FISH) and comparative genomic hybridization (CGH). Journal of Cellular Biochemistry, 139–143.p.

Wenham, P.R.(1992): DNA-based techniques in clinical biochemistry: a beginner's guide to theory and practice. Ann. Clin. Biochem., 29. 598–624.p.

Authors' address: Institute for Small Animal Research
H-2103 Gödöllő, P.O.B. 417., Hungary

RECURRENT TRISOMIES IN CHICKEN EMBRYOS

HIDAS, A. — VÁRKONYI, E. — LIPTÓI, K. —
SAYAHZADEH, H. — LENNERT, L. — SZALAY, I.

SUMMARY

Chromosomal abnormalities are frequent causes of early embryonic wastage during the incubation (1–15%). These abnormalities are generally the numerical changes of the chromosomes (haploidy, triploidy, polyploidy and aneuploidy). The proportion of chromosomally aberrant embryos seems to be genetically predisposed. Mostly the hens are kept to be responsible for these abnormalities (meiotic, fertilization errors). That is why selection of breeding stocks is possible through a large scale investigation of the hens' embryos. Layer breeding lines were investigated for the occurrence of embryonic chromosomal abnormalities for this purpose.

Aneuploidies (monosomies, trisomies) are relatively less frequent causes of embryonic death. Therefore, recurrent occurrence from a hen is quite unlikely without any predisposition. It was the case with two hens. Surprisingly not only the aneuploidy was repeated, but the involved chromosomes were the same. The other hens fertilized by the same cocks did not show aneuploidy, therefore predisposition for meiotic nondisjunction is suspected at the hens.

INTRODUCTION

Chromosomal abnormalities are frequent (1–15%) causes of early embryonic wastage during the incubation (*Fechheimer,* 1990). These abnormalities are generally numerical changes of the chromosomes (haploidy, triploidy, polyploidy and aneuploidy). The proportion of chromosomally aberrant embryos seems to be genetically predisposed. Mostly the hens are kept to be responsible for these abnormalities (meiotic, fertilization errors). That is why selection of breeding stocks is possible through a large scale investigation of the hens' embryos (*Szalay,* 1989). Layer breeding lines were investigated for the occurrence of embryonic chromosomal abnormalities for this purpose.

MATERIAL AND METHOD

Early dead embryos from registered eggs (TETRA SL layer hybrid lines, Bábolna, Hungary) were selected at the candling on the 5th day of incubation. Following classification and 1–2 hours of colchicine treatment they were hypotonized and fixed for slide preparation according to *Bloom and Hsu* (1975).

RESULTS AND DISCUSSION

Aneuploidy (monosomy, trisomy) is relatively a rare cytogenetic cause of the embryonic death (observed in 7% of the chromosomal abnormal embryos in the investigated line). Therefore, recurrent occurrence from a hen is quite unlikely without any predisposition. It was the case with two hens.

The hen No. 227B produced two karyotypically abnormal dead embryos, both showing trisomy for chromosome number 1. Higher embryonic mortality was observed at the hen 370B with 4 chromosomal aberration including two trisomies for chromosome 2 and both 2 and 3. In the latter case haploidies were still found in two of the other dead embryos. Surprisingly not only the aneuploidy was repeated, but the involved chromosomes were the same (*Table 1.*). The other hens fertilized by the same cocks did not show aneuploidy, therefore predisposition for meiotic nondisjunction is suspected at the hens.

Table 1.

Incubation, embryological and karyological data of the two hens showing repeated trisomies

Hen No.	Eggs	Fertility%	Dead embryo	Dead %	Chromosomal abnormalities	Aneu-ploidy
227Q	39	90.0	4	9	2	2
370B	39	82.0	8	18	4	2

Accumulation of aneuploidy in full and halfsib families was also reported (Fechheimer 1981) so genetic establishment is very likely.

The mechanism which responsible for higher incidence of this kind of chromosomal abnormalities is not clear, errors in female meiosis are probable (*Fechheimer 1981*). Chromosomal nondisjunction in meiosis (leading to aneuploidies) is suspected to be related to the lack or high level of recombination which might has influence on normal pairing and segregation of the homologous chromosomes (*Abruzzo and Hassold,* 1995; *Buraczynska et al.,* 1989; *Chakravarti et al.,* 1989; *Hassold et al.,* 1991; *Sherman et al.,* 1991).

REFERENCES

Abruzzo, M.A. – Hassold, T.J.(1995): Environmental and Molecular Mutagenesis, 25. 38.p.

Bloom, S.E. – Hsu, T.C.(1975): Chromosoma, 51. 261.p.

Buraczynska, M. et al.(1989): In: Molecular and Cytogenetic Studies of Non-Disjunction, 101.p.

Chakravarti, A. et al. (1989): In: Molecular and Cytogenetic Studies of Non-Disjunction, 45.p.

Fechheimer, N.S.(1981): Poultry Science, 60. 1365.p.
Fechheimer, N.S.(1990): Chromosomes of chickens. In: Domestic Animal Cytogenetics. Ed.: McFeely, Adv. in Vet. Sci. and Comp. Med., 34. 170.p.
Hassold, T.J. et al.(1991): Am. J. Hum. Genet., 49. 253.p.
Thorne, M.H., et al.(1987): Cytogenet. Cell Genet., 45. 21.p.
Sherman, S.L., et al.(1991): Am. J. Hum. Genet., 49. 608.p.
Szalay, I.(1989) Ph.D. Thesis, Gödöllő

Authors' address: Institute for Small Animal Research
H2103 Gödöllő, P.O.B. 417., Hungary

INVESTIGATION OF CHROMOSOMAL AND EMBRYONIC ABNORMALITIES IN EARLY DEAD GOOSE EMBRYOS

LIPTÓI, K. — HIDAS, A. — SZALAY, I.

SUMMARY

Three goose lines (No 4, 7 and 9) were investigated in Artigueres (France, INRA). The aim was to determine the types of embryonic mortality, karyotype of dead embryos and determination of the ratios of embryonic and chromosomal abnormalities of the lines in different periods of the laying cycle. For this purpose, samples were collected at the beginning (in February), in the middle (in April) and at the end (in June) of laying. Incubated eggs discarded by candling on the 5th day were processed for phenotypic classification of embryos and karyotype analysis.

The rate of chromosomally abnormal embryos was found 7.14%, 16.83% and 12.73% in lines 4, 7 and 9 respectively. All abnormalities proved to be numerical alterations. Haploid, haploid/diploid, triploid, triploid/diploid, diploid/polyploid karyotypes were identified.

Summarized data of embryonic abnormalities however showed a significantly higher ratio in the first three weeks than in the middle period of egg collection. It was 10.49% at the beginning, 6.18% in the middle and 7.16% at the end of the laying cycle, calculated as percentages of fertile eggs.

On the basis of individual records of abnormalities, a complete analysis of full sister and half sister families was also accomplished. There were some families in every line showing significant difference from the population average for chromosome, embryonic or both abnormalities (3 families in line 4, 4 in line 7 and 2 in line 9).

INTRODUCTION

Goose is a poultry species with a relatively poor reproductive performance. Hatchability — being one of the most important traits in the poultry breeding — is about 60% of goose eggs compared to 80–90% in chicken. During the first seven days of incubation many eggs are lost because of infertility and early death of embryos. The latter is often due to genetic abnormalities, including chromosome aberrations. Because of the possible genetic background of some types of chromosome abnormalities, the application of cytogenetic methods may be promising in improving reproduction traits of the goose, a species with a very limited data in this respect.

Three goose lines were investigated in Artigueres (France, INRA). The aim was to determine the types of the embryonic mortality, the investigation of karyotypes in the dead embryos, examination of the ratio of embryonic and chromosomal abnormalities in the different periods.

MATERIAL AND METHODS

The samples were collected at the beginning (in February), in the middle (in April) and at the end (in June) of the laying period.

The eggs collected from trapnest were marked with the number of the line, pen and the layer. Four layers and one gander were in each pen. Those eggs, which seemed "empty" or "bloody" at candling on the 5th day of incubation were opened and early dead embryos were injected with vinblastine for mitotic arrest and incubated for one hour. Dead embryos were phenotypically classified according to *Szalay* (1989) and membranes were processed for cytogenetic analysis by hypotonic treatment and fixation. Those eggs, which seemed to be "empty," sometimes contained very early dead embryonic tissues. They cannot be determined by candling just after braking. Air-dried slides were prepared from the collected samples and stained with Giemsa.

RESULTS

In the line 4 the fertility was very low (*Table .1*). The highest was in the line 7 and the ratio of chromosomal abnormality in the abnormal embryos too. The ratio of chromosomal abnormality in the fertile eggs was the highest in the line 9. (*Fig. 1., Fig. 2.*).

Table 1.

Line	Number of the layers	All eggs	Fertile eggs in all eggs %	Embryonic abnormality in fertile eggs%	Chromosomal abnormality in dead embryos%	Chromosomal abnormality in fertile eggs%
4	70	1437	40.36	5.48	7.14	0.69
7	119	2906	75.46	12.29	16.83	0.78
9	89	2412	58.46	4.56	12.73	0.99

All of the chromosomal abnormalities were numerical alterations. Haploid, haploid/diploid, triploid, triploid/diploid, diploid/polyploid karyotypes were identified.

The ratio of the embryonic abnormalities was investigated in the lines at different periods (*Table 2*). Only if we observed just the first three weeks of egg collection and taken together the lines, the ratio of the embryonic abnormalities was significantly higher at this time than in the middle period. It was 10.49%, 6.18% and 7.16% in the fertile eggs.

All 3 lines have some layers, which produced outstanding embryonic mortality (*Table 1.*). In the line 4 the No. 4501 (4 malformed embryos, 100% of the fertile eggs), No. 5804 (4 malformed embryos, 22.22% of the fertile eggs), No. 4701 (5, 35.71% of the fertile eggs). These three layers gave 23.4% of the all abnormalities of this line. In the line 7 there was only one layer, the No. 3103 (5 malformed embryos, 31.25% of the fertile eggs). In the line 9 there were two females, the No. 8804 (4, 22.22%) and the No: 8403 (5, 38.46%).

Some layers was selected showing embryonic mortality over the population average and producing chromosomal abnormality are shown in the *Table 3.*

Full and half sister families were analysed on the basis of embryological and karyotypical observations. There were some families in every line, which showed significant difference from the population average for chromosomal, embryonic, or both abnormalities (*Table 4.*).

Table 2.

The ratio of embryonic and chromosomal abnormality in the different periods

Line	Period	Embryonic abnormality in fertile eggs %	chromosomal abnormality in PD+bloody %	chromosomal abnormality in fertile eggs %
4	13/02-27/02	16.28	0	0
	3/04-17/04	8.20	12.00	0.98
	21/05-5/06	10.34	4.17	0.24
7	13/02-27/02	5.94	13.33	0.79
	3/04-17/04	3.60	13.33	0.48
	21/05-5/06	5.64	20.59	1.67
9	13/02-27/02	10.96	17.50	1.92
	3/04-17/04	7.03	9.30	0.65
	21/05-5/06	6.24	11.11	0.69

Fig. 1.

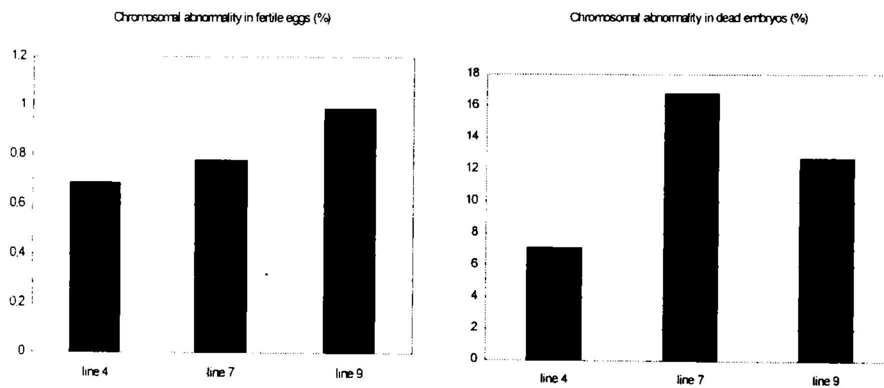


Fig. 2.

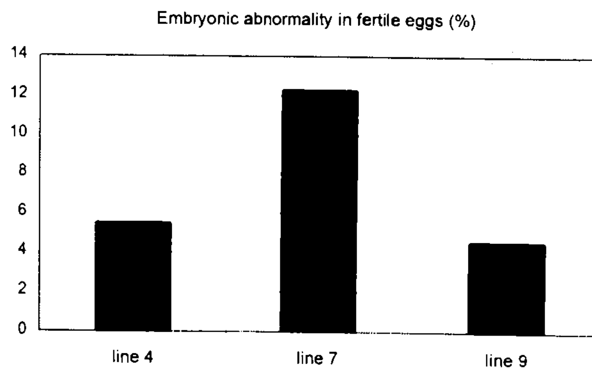


Table 3.

Selected layers on the basis of their abnormal embryos

Line	N° of the layers	Ertile eggs	Dead embryos in the fertile eggs %	Phenotype of the abnormal embryos	Chromosomal abnormality (N)
4	pop. average	8	10		
	4402	17	18	bwe, bwe, d2, pd	
	4501	4	100	bwe, bwe, bwe, bwe	1
	4701	14	29	bwe, bwe, d3, d5, pd	
	5104	8	25	bwe, bwe, bwe	1
	5203	12	17	bwe, bwe, bwe	
	5804	18	22	bwe, bwe, d2, ae	
7	pop. average	16	5		
	801	18	22	d3, d4, pd, pd	
	1902	8	38	bwe, bwe, pd	2
	3103	16	31	bwe, bwe, bwe, ae, ae	
	802	11	27	pd, pd, pd	
	804	14	14	d4, pd	
	2002	28	7	bwe, ae,	2
	3102	20	15	bwe, bwe, bwe	1
	603	20	15	bwe, ae, ae,	
	701	23	13	pd, ae, ae	
	2903	17	18	bwe, bwe, pd	1
9	pop. average	14	7		
	8804	18	22	bwe, bwe, d2, ae	2
	8503	8	38	pd, bwe, bwe	3
	8403	13	38	bwe, bwe, bwe, bwe, bwe	1
	7804	21	14	pd, ae, ae	
	7803	8	25	d5, d5, pd	1
	7503	23	13	pd, pd, pd	
	6902	12	17	ae, ae	1

bwe: blastoderm without embryo; pd: positive development; ae: abnormal embryo;
d1- 5: embryonic death on the 1- 5 day of the incubation

Table 4.

Comparison of full and half sister families with population average which showed significant difference in embryonic abnormalites and/or chromosomal abnormalities

		Dead embryos in fertile eggs (%)	Abnormal karyotype in fertile eggs (%)	Abnormal karyot. in dead embryos (%)
Line 4				
Population average		9.66	0.69	7.14
Full sister families	(a1)	20.00	5.00	25.00
	(a2)	28.57	9.52	33.33
Half sister families	(b)	22.73	13.64	60.00
Line 7				
Population average		4.97	0.78	15.60
Full sister families	(a)	19.23	7.69	40.00
Half sister families	(b1)	20.69	6.90	33.33
	(b2)	12.96	0.00	0.00
	(b3)	5.88	5.88	100.00
Line 9				
Population average		7.80	0.99	12.73
Half sister families	(b1)	23.53	11.76	50.00
	(b2)	20.00	10.00	50.00

We would like to study the inheritance of the chromosomal and embryological abnormalities with the selected animals.

This work was supported by "BALATON" project (F-35/96).

REFERENCES

Szalay I.(1989): Cytogenetic aspects of early embryonic development in meat type poultry. Ph.D. Thesis, Gödöllö

Authors' address: Institute for Small Animal Research
H-2100 Gödöllö, POB 417., Hungary

GENETIC DIFFERENTIATION IN *ALECTORIS RUFA* AND *A. GRAECA* FROM SPAIN

SAZ, J. — ARRUGA, M.V. — TEJEDOR, M.T. — VILLARROEL, M. — SAVVA, D.

SUMMARY

The native Spanish red-legged partridge (*Alectoris rufa*) has been genetically polluted by unregulated breeding with the non-native rock partridge (*A. graeca*). In order to establish methodologies allowing an investigation of the extent of hybridisation between the two species as well as their genetic characterisation, we have analysed pure stocks of both species cytogenetically and by protein electrophoresis, DNA fingerprinting and genotyping by the polymerase chain reaction (PCR). Here we report that we have used the PCR and specific DNA microsatellite markers to develop a method which enables us to distinguish between the red-legged and the rock partridge; this method makes it possible to identify with certainty pure-bred (non-hybrid) individuals and to establish the current amount of interbreeding between the two species in the wild.

INTRODUCTION

The native Spanish red-legged partridge (*Alectoris rufa*) has been genetically polluted by unregulated breeding with the non-native rock partridge (*Alectoris graeca*). In order to establish methodologies allowing an investigation of the extent of hybridization between the two species as well as their genetic characterisation, we have analysed pure stocks of both species cytogenetically and by protein electrophoresis, DNA fingerprinting and genotyping by the polymerase chain reaction (PCR).

MATERIAL AND METHODS

We have analyzed 151 wild-living birds coming from different Spanish regions: Zaragoza (n=27), Ciudad Real (n=8), Badajoz (n=16), Santander (n=5), La Rioja (n=18), Toledo (n=26), Albacete (n=20), Murcia (n=19), Andalucia (n=7) and Castilla-León (n=5). Two small samples of rock partridge were also studied (Rock 1 , n=6 and Rock 2, n=7).

Cytogenetic studies were performed from white blood cells by usual techniques of macro and micro culture.

Individual liver extracts were prepared. Horizontal cellulose acetate electrophoresis was used to resolve the following enzimatic loci:

— *Phosphogluconate dehydrogenase* (*PGD*, EC 1.1.1.44)
— *Glucose phosphate isomerase* (*GPI*, EC 5.3.1.9)
— *Glutamate oxaloacetate transaminase 1* (*GOT1*, EC 2.6.1.1)
— *Isocitrate dehydrogenase 1* (*IDH1*, EC 1.1.1.42)
— *Malic enzyme 1* (*ME1*, EC 1.1.1.40)
— *Mannose phosphate isomerase* (*MPI*, EC 5.3.1.8).

Buffers, electrophoretical dynamics and staining recipes were adapted from *Meera Khan* (1971), *Harris and Hopkinson* (1973), *Van Someren et al.* (1974) and *Womack and Moll* (1986).

Comparisons between allele frequencies for pairs of populations were performed by means of Nei's identity (*Nei*, 1972).

DNA studies were carried out from blood or individual liver samples. DNA fingerprints were obtained using *Alu* I and pV47 probe, according to Longmire *et al.* (1990). The labelling of the probe was made using biotin (*Feinberg and Vogelstein*, 1983), modified by *Hodgson and Fisk*, 1987). Statistical analysis of the bands from fingerprints were made according to *Jeffreys et al.* (1985) and *Georges et al.* (1988). Calculations became easier using DNA POP Program (*Pena and Nunes*, 1990). The parameter considered in this work was the proportion of shared bands.

PCR amplification was performed in a DNA thermal cycler. Primer pairs proven informative for other galliformes were assayed in the polymerase chain reaction (PCR) to amplify red-legged partridge and rock-partridge genomic DNA loci. We selected three primers pairs that amplified species specific alleles and assayed them on 120 individuals not related from various geographical locations.

RESULTS

I. Cytogenetic studies: Spanish red-legged partridges have 2n= 18 macrochromosomes and 30 pairs of microchromosomes (*Fig.* .).

II. Enzyme studies: Allele frequencies were estimated for each locus and population. Nei's identity values were calculated for all loci as a whole (*Table 1.*). and for each particular locus from every possible pair of considered populations. Many conspecific bird population are little differentiated and is difficult to find local populations with low identity values (*Barrowclough*, 1983).

III. DNA fingerprinting: *Table 2.* shows proportion of shared bands between individual pairs.

IV Microsatellites: Amplification of three primer pairs loci were very informative. The three of the 10 microsatellite loci fulfil basic requirements to make them attractive for differentiation of partridge species. *Fig. 2.* shows the image of microsatellites obtained with silver staining.

Fig. 1. **Karytype of Spanish red-legged partridge (Alectoris rufa)**

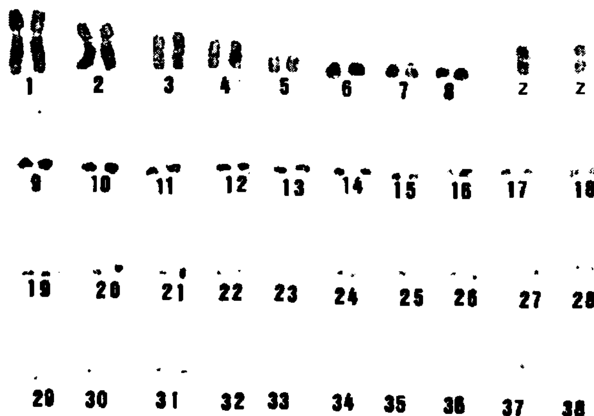


Table 1.

Values of Nei's similarity index for all studied loci. Values <0.80 are shown in bold

	Zar.	C. Real	Bad.	Sant.	Rioja	Tol.	Alb.	Mur.	And.	C.León	Rock 1	Rock 2
Zar.	0.971	0.995	0.993	0.947	0.990	0.910	0.980	**0.763**	0.943	0.859	0.941	
C.Real		0.975	0.954	0.910	0.988	0.960	0.985	0.828	0.956	**0.798**	0.895	
Bad.			0.995	0.953	0.987	0.903	0.977	**0.738**	0.927	0.844	0.943	
Sant.					0.956	0.975	0.874	0.961	**0.699**	0.909	0.851	0.949
Rioja						0.931	0.845	0.928	**0.704**	0.857	0.877	0.970
Tol.						.	0.954	0.994	0.823	0.964	0.837	0.912
Alb.								0.960	0.905	0.954	**0.733**	**0.790**
Mur.								0.842	0.963	0.817	0.899	
And.									0.915	**0.624**	**0.655**	
C.León										**0.757**	0.837	
Rock 1											0.915	
Rock 2												

Fig. 2.: **Image of microsatellites obtained with silver staining**

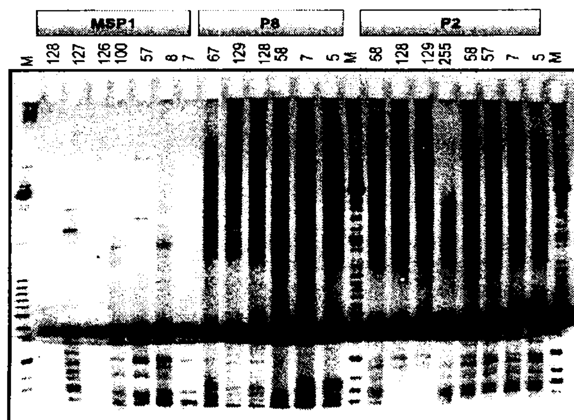


Table 2.

Proportion of shared bands for every pair of studied individuals

	Rock1	Rock2	Rock3	Red1	Red2	Red3	Red4	Red5	Red6	Red7	Red8	Red9	Red10	Red11	Red12
Rock1	1.00	0.53	0.56	**0.00**	**0.11**	**0.09**	**0.00**	**0.00**	**0.08**	**0.21**	**0.00**	**0.13**	**0.11**	**0.14**	**0.19**
Rock2		1.00	0.62	**0.00**	**0.14**	**0.00**	**0.00**	**0.00**	**0.00**	**0.29**	**0.00**	**0.07**	**0.00**	**0.17**	**0.00**
Rock3			1.00	**0.00**	**0.12**	**0.00**	**0.12**	**0.11**	**0.09**	**0.24**	**0.00**	**0.13**	**0.13**	**0.22**	**0.20**
Red1				1.00	0.27	0.21	0.13	0.25	0.10	0.13	0.43	0.14	0.29	0.32	0.07
Red2					1.00	0.09	0.22	0.21	0.17	0.33	0.35	0.19	0.24	0.29	0.13
Red3						1.00	0.36	0.17	0.30	0.18	0.19	0.34	0.10	0.13	0.23
Red4							1.00	0.21	0.26	0.33	0.24	0.26	0.35	0.14	0.39
Red5								1.00	0.58	0.21	0.11	0.31	0.22	0.14	0.19
Red6									1.00	0.09	0.13	0.27	0.27	0.30	0.11
Red7										1.00	0.35	0.19	0.12	0.21	0.19
Red8											1.00	0.13	0.25	0.15	0.20
Red9												1.00	0.33	0.29	0.23
Red10													1.00	0.37	0.27
Red11														1.00	0.15
Red12															1.00

Note: In bold, proportion of shared bands between rock and red-legged partridges

CONCLUSIONS

I. Cytogenetic studies: The number of microchromosomes does not appear to be a reliable indicator of species type.

II. Enzyme studies:

1) Considering the six enzyme loci as a whole, high identity values occurred in the studied wild populations for allele frequencies. Only Andalucia population showed some identity values below 0.80. In respect to relationships among wild-living populations and Rock partridges samples, Rock 1 usually had lesser identity values than Rock 2.

2) For particular loci, only IDH1 and ME1 showed interesting differences between populations, even when Rock partridges were involved in the comparisons.

3) Andalusian population showed small identity values for most other populations. The small size and heterogeneity of this sample might be the cause of this situation. More research is needed for accurate characterization of this interesting population.

III. DNA fingerprints:

1) Significant differences ($p<0.05$) were found in shared bands for rock and red-legged partridge pairs between three Red-legged partridges (Red 7,11 & 12) and the rest of them. So, these three birds, previously supposed to be pure-breed *A. rufa*, might be considered as hybrids *A. rufa* x *A. graeca*.

2) This result pointed out the possibility of using DNA fingerprinting in the identification of this kind of hybrids. More research is necessary to confirm this preliminary conclusion.

IV Microsatellites: The results point to a real possibility of differenciate both species on the basis of microsatellite studies.

REFERENCES

Barrowclough, G.F.(1983): Perspectives in ornithology. Cambridge Univ. Press, 223–261.p.

Feinberg, A.P. – Vogelstein, B.(1983): Analytical Biochemistry, 132. 6–13.p.

Georges, M. – Lequarre, A.S. – Castelli, M. – Hanset, R. – Vassart, G.(1988): Cytogenet. Cell Genet., 47. 127.p.

Harris, H. – Hopkinson, D.A.(1976): Handbook of enzyme electrophoresis in human genetics. North Holland Publishing Co.

Hodgson, C.P. – Fisk, R. Z.(1987): Nucleic Acids Research, 15. 6295.p.

Jeffreys, A. – Wilson,V. – Thein,S.L.(1985): Nature, 316. 76.p.

Longmire, J.L. – Kraemer, P.M. – Brown, N.C. – Hardekopf, L.C. – Deaven, L.L.(1990): Nucleic Acids Research, 18. 6. 1658.p.

Meera Khan, P.(1971): Arch. Biochem. Biophys., 145. 470–483.p.

Nei, M.(1972): Am. Nat., 106. 283–292.p.

Pena, S.D.J. – Nunes, A.C.(1990): Fingerprint News, 2. 3. 7–8.p.

Van Someren,H. – Van Henegouwen, H.B. – Los, W. – Wurzer-Figurelli, E. – Dopper,B. – Vervloet, M. – Meera Khan, P.(1974): Humangenetik, 25. 189–201.p.

Womack, J.E. – Molly, D.(1986): J. Hered, 77. 2–7.p.

Authors' address: *Saz, J. – Arruga, M.V. – Tejedor, M.T. – Villarroel, M.:*
Laboratory of Cytogenetics and Molecular Genetics.
Faculty of Veterinary. Miguel Servet
177. 50013 Zaragoza (Spain)
Savva, D.: School of Animal and Microbial Sciences.
The University of Reading Whiteknights
P.O. Box 228. Reading RG 6AJ (United Kingdom)

INVESTIGATION OF THE KARYOTYPE OF *VIPERA URSINII RAKOSIENSIS*

LIPTÓI, K. — ÚJVÁRI, B. — KORSÓS, Z. — HIDAS, A.

SUMMARY

In 1995 a research project began for the investigation of the *Vipera ursinii rakosiensis* population in Hungary. One part of this work was the chromosome analysis of this species. The aim was to define the karyotype and to compare the samples from different areas. The individuals were collected in Dabas and in Bugac in the middle part of Hungary.

16 macro and 20 microchromosomes were identified. Seven pairs from the macrochromosomes were submetacentric, one pair was acrocentric, and the both sex chromosomes were acrocentric macrochromosomes. One female from Dabas showed 16 microchromosomes instead of 20.

INTRODUCTION

The first article on the chromosomal constitution of snakes was published in 1922 by *Thatcher* about the spermatogenesis of *Tamnophis butleri*.

The chromosome number of the snakes is between 32 and 48 with microchromosomes among them. Similarly to birds, the sex karyotype is ZZ for the male and ZW for the female (*Becak* 1969).

Kobel (1967) described the karyotype of Vipera ursinii. He found 16 macrochromosomes and 20 microchromosomes.

In 1995 a research project began for the investigation of a rare snake, Vipera ursinii rakosiensis population in Hungary. One part of this work was the chromosome analysis of this species. The aim was to define the karyotype and to compare the samples from different areas.

MATERIAL AND METHODS

The snakes were collected in Dabas and in Bugac in the middle part of Hungary. Metaphase chromosomes were obtained from lymphocyte cultures, and from fibroblast cultures of dead animals.

Fibroblast culture: According to *Shoffner et al.* (1967) and *Ansari* (1986): A piece of muscle tissue was put in the culture flask after disinfection with alcohol. It was incubated for 6-8 weeks on 30 °C in TC-199 medium and 15% FCS supplemented with L-Glutamin. The cells were treated by colchicine and were removed by trypsine. After hypotonic (0.56% KCl) treatment and fixation (1:3 acid acetic:ethanol), cell suspension was spread on slides and stained with 5% Giemsa.

A female was captured in Bugac which had 7 malformed progenies, three of them had abnormal skull. We could use them for tissue culture.

Lymphocyte culture: According to *Au et al.* (1975) and *Thorne et al.* (1987): Heparinized blood was taken from tail vein (0.05–0.15 ml) into TC-199 medium and 15% FCS containing 0.1% Phytohemagglutinin and incubated for 4–5 days.

C-banding: According to *Sumner* (1994): The slides were treated with 0.2 M HCl for 1 h and 5% barium hidroxyde and SSC at 50 °C. They were stained with 5% Giemsa.

RESULTS

16 macro and 20 microchromosomes were identified (*Fig. 1.*). Seven pairs from the macrochromosomes were submetacentric, one pair was acrocentric and both sex chromosomes were submetacentric (*Fig. 2.*). In a fibroblast culture we found tetraploid divisions (*Fig. 3.*). It was impossible to determine whether it was originated from the malformed snake or it was an artifact of the long time culture. One female from Dabas showed 16 microchromosomes instead of 20. This individual had normal phenotype, so the missing 4 chromosomes are unlikely to contain essential genes. The microchromosomes of this species are heterochromatic, similarly to birds.

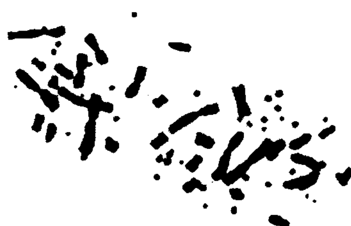
Despite of the observed alteration, the number of the examinable samples were not enough to give a correct answer whether these populations contain any chromosomal differences.

This work was supported by Hungarian Research Fund (OTKA-T16608).

Fig. 1.: **Diploid metaphase**

Fig 2.. **C-band staining of adder metaphase chromosomes**

Fig. 3.: **Tetraploid metaphase**



REFERENCES

Ansari, H.A. – Takagi, N. – Sasaki, M.(1986): Interordinal conservatism of chromosome banding patterns in *Gallus domesticus* (Galliformes) and *Melopsittacus undulatus* (Psittaciformes). Cytogenet. Cell Genet., 43. 6–9.p.

Au, W. – Fechheimer, N.S. – Soukop, S.(1975): Indentification of the sex chromosomes in the bald-eagle. Can. J. Genet. Cytol., 17. 187–191.p.

Becak, W.(1969): Karyotypes, sex chromosomes and chromosomal evolution in snakes. In Venomous animals and their venoms. 53–95.p.

Kobel, H.R.(1967): Morphometrische Karyotypanalyse einiger Schlangen-Arten, Genetica, 38. 1–31.

Shoffner, R.N. – Krishan, A. – Haiden, G.J. – Bammi, R.K. – Otis, J.S.(1967): Avian chromosome methodology. Poult. Sci., 46. 333–344.p.

Sumner, A.T.(1994): Absorption Chromosome Banding. in Gosden, R.D.: Chromosome Analysis Protocols 1994, Vol. 29. 64–79.p.

Thatcher, L.E.(1922): Science, 56. 372.p.

Thome, M.L. – Collins, R.K. – Sheldon, B.L.(1987): Live haploid-diploid and other unusual mosaic chickens(Gallus domesticus). Cytogenet. Cell Genet., 45. 21–25.p.

Authors' address: *Liptói, K.– Hidas, A.*: Institute for Small Animal Research
H-2100 Gödöllő, POB 417., Hungary
Újvári, B. – Korsós, Z.: Hungarian Natural History Museum
H-1088 Budapest, Baross u. 13., Hungary

SPONTANEOUS TRIPLOIDY IN THE AFRICAN CATFISH *CLARIAS GARIEPINUS* (PISCES: CLARIIDAE)

RÁCZKEVI-RADICS, J.— RADICS, F.

A karyological analysis on the basis of Giemsa stained chromosomes was performed in two strains of the African catfsh *Clarias gariepinus* maintained in Szarvas, Hungary. The chromosomes of 23 juvenile individuals studied were obtained from cephalic kidney by direct method. Macroscopic sex determination was confrmed using aceto-carmine squashing of gonads. 22 of the specimens investigated were characterized with 2n=56 chromosome number, one satellite-bearing chromosome pair, and a heteromorphic pair in females. They appear karyologically corresponding to the three strains from different geographical localities reported formerly (namely in males: 8m+24sm+24a; NF=88; while in females: 8m+25sm+23a, NF=89). Based on the heteromorphic pair of females found in all populations the species was considered to belong to sex determination of ZZ-ZW type, but the results of induced gynogenesis and molecular genetic study performed recently revealed XX-XY system.

One specimen proved to be spontaneous triploid, as possessed 84 chromosomes representing 28 triplets instead of 28 pairs. In external morphology this individual was indistinguishable from the diploid ones and was determined as a male with developing testes. The triplet corresponded to the heteromorphic pair of females consists of two acrocentric and one large submetacentric elements. The most likely mechanism that gave rise to spontaneous triploidy in populations studied is the fusion of haploid spermatozoon with an unreduced oocyte. Spontaneous retention of second polar body due to oocyte ageing was described in other species as well as was conf rmed recently by us in *Clarias gariepinus*.

Authors' address: *Ráczkevi-Radics, J.:* Fish Culture Research Institute
Szarvas, P.O.Box.47. Hungary
Radics, F.: Szarvas Fish Ltd. Szarvas, I.kk.57. Hungary

CONNECTION BETWEEN OVERMATURATION OF THE EGGS AND CHROMOSOMAL ABNORMALITIES IN *SILURUS GLANIS* L.

VÁRKONYI, E. — HORVÁTH, L. — OZOUF-COSTAZ, C. — BILLARD, R.

SUMMARY

The effects of aging oocytes for 6 hours before fertilization in the European catfish *Silurus glanis* are compared with the same experiment under normal conditions (stripping and fertilization immediately after ovulation). Fertilization rate and hatching rate are lower in the aged groups, while the percentages of malformed larvae and chromosomal abnormalities are significantly higher. Several kinds of abnormalities are described in detail.

INTRODUCTION

During the development of eggs in hatcheries or on natural spawning grounds, abnormal embryos with deformed bodies have been reported (*Garman and Denton*, 1886; *Yamazaki et al.*,1989). *Linhart and Billard* (1995) showed that when fertilizing intraovarian aged oocytes of the European catfish, *Silurus glanis*, the percentage of malformed larvae among the hatchlings increased up to 50% after 6 h compared with fertilization under normal conditions. It was hypothesised that the aging phenomenon may result in an error in the chromosome distribution during fertilization (*Saito et al.*, 1993), so that morphological abnormalities are due to epigenetic and developmental effects.

MATERIAL AND METHODS

Aging

Four females and 2 males were selected for artifical spawning after external examination. Fish were transferred to indoor tanks, weighed, individually tagged by colour filaments and injected intramuscularly with lyophilised carp pituitary, according to routine procedures of the fish farm. Females were checked by evaluation for ovulation two and one hour before the expected time. Only about half of the estimated number of eggs of each fish was stripped initially. The rest remained in the fish and were stripped 6 h later. Body weight (at first hypophysation), egg weight at first (T_0) and second (T_6) stripping and the pseudo-gonadosomatic index (PGSI) (*Richter et al.*, 1985) were obtained.

PGSI=weight of stripped eggs/(weight of fish before injection — weight of stripped eggs).

Two males (M_1 and M_2) were killed and their testes minced into 3–5 mm pieces. Sperm was rapidly removed from the testicular tissue and was ready to use for the first (T_0) fertilisation. The stored part of the sperm was kept at 5 °C and used for fertilization at T_6. Motility was checked each time. A mixture of the sperm from both males (0.3–0.4 ml/100 g eggs) was used for the first and second batch of eggs.

Oocytes were fertilized within 10 min after stripping and transferred into Zuger jars for incubation (*Horváthné-Tamás et al.*, 1982). Fertilization rates were determined from the samples of 3 x 200–300 eggs 22 h after fertilization and at hatching.

Chromosome preparation

Chromosome analysis of parental broodfish: Fish-fins of four females and two males (A,B,C,D,M_1,M_2) were made fibroblast cell culture.The fibroblast cells were used for chromosome analysis. Five slides from each individual were prepared and at least 100 metaphase spreads per individual examined for chromosome abnormalities.

Chromosome analysis of larvae from the aged (T_6) and the control (T_0) groups: A direct method was used for chromosome preparation from larvae. From well aerated freshwater with a mitostatic agent (0.05% colchicine) following 3–3.5 h of incubation, larvae were put into hypotonic solution (distilled water) for 25 to 35 min and the hypotonic solution replaced by fresh fixative for 20 min. The fixative was changed three times.

The cells were dispersed with a micropipette in a few drops of 50% acetic acid, dropped onto a prewarmed slide (heating-plate at 50 °C) and then removed very slowly by a pipette (modified after *Baksi et al.*, 1988). The slides were dried and stained. The samples for chromosome analysis were taken separately from each larvae (morphologically normal and abnormal), 30 larvae per female at each time. Two slides were prepared from each individual and at least 50 metaphase spreads per individual were examined for chromosome abnormalities.

Statistical analysis

Statistical significance between the control (T_0) and the aged (T_6) groups was assessed using one-way analysis of variance, followed by Fisher's least significant difference test (LSD). Significance values of less than 0.05 were considered as significant. The relationship between the morphological and chromosomal aberrations were proved by the χ^2 test.

RESULTS

Aged eggs: Fertilization, hatching rate and malformed embryos

The fertilization and hatching rates were not significantly different in the groups T_0 and T_6 (*Fig. 1., 2.*). Abnormal larvae were found in all T_6 groups and none in the T_0 control groups. The frequency of abnormality strongly increased up to 15% in female C.

The overall frequency of malformed larvae was significantly lower (P<0.001) in the control (0%) than in the aged groups (2–15%). The fertility and hatching rates were 95–98%. The good environmental conditions (level of O_2, water flow, etc.) and the genetic background of parental-lines may explain the absence of malformed larvae in the control groups (*Fig. 3.*). Three types of abnormalities were described in the aged egg groups: strong curvature of the spinal column, deformed tail and pericardial oedema. The frequency of chromosomal abnormalities was significantly higher in the group of malformed embryos than among the phenotipically normal embryos (P<0.01).

Fig. 1.: **Mean frequency of fertilization (±SD) for females at stripping time (T_0), and 6 h later (T_6)**

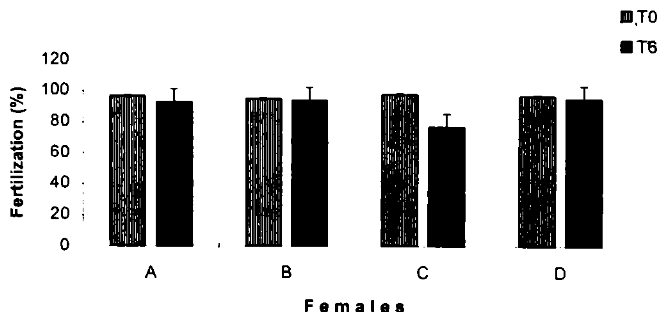


Fig. 2.: **Mean frequency of hatching (±SD) for females at stripping time (T_0), and 6 h later (T_6)**

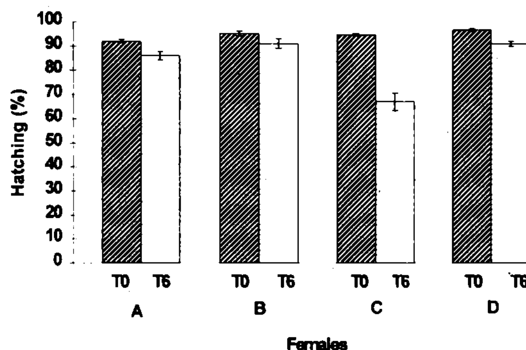
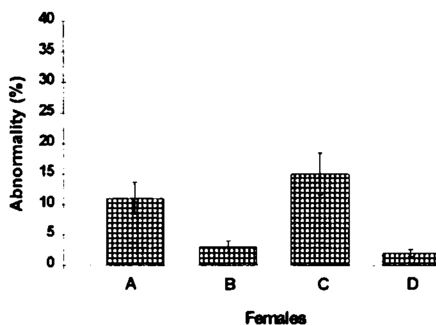


Fig. 3.: **Mean frequency of abnormal larvae (±SD) for females in the aged group (T_6)**



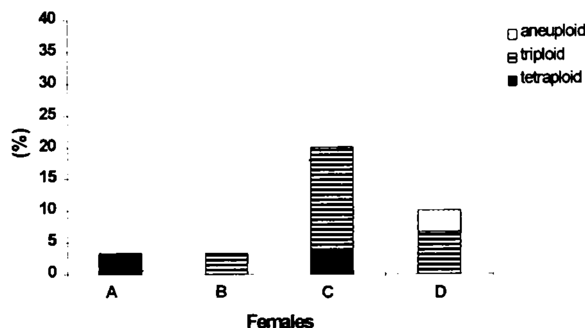
Chromosome analysis of parental broodfish

The four females (A, B, C, D) and both males (M_1, M_2) were karyologically normal (*Ráb et al.*, 1991) and had 2n=60 chromosome number.

Chromosome analysis of larvae from the aged (T_6) and control (T_0) groups

There was no chromosomal abnormality in the control groups (AT_0, BT_0, CT_0, DT_0). One tetraploid (4n=120) was found in the AT_6 group (*Fig.4.*) which was phenotypically abnormal with oedema of the pericardium. One triploid (3n=90) was found in the BT_6 group (*Fig. 4.*) which was phenotypically normal. The CT_6 group was characterised by an especially high frequency of chromosomal abnormalities: one tetraploid with a normal phenotype, three triploids also with a normal appearance and two triploids with malformations (*Fig. 4.*).One phenotypically normal triploid, one triploid and one aneuploid (2n=64) (*Fig. 4.*) with a strong curvature of the spinal column were found in the DT_6 group. Thus the frequency of karyologically abnormal larvae was 3.33–20% after fertilization of aged oocytes, which is very high compared with the control group.

Fig. 4.: **Type and frequency of chromosomal abnormalities in larvae karyologically analysed for each female in the aged group. (T_6)**



DISCUSSION

The rate of abnormal embryonic development significantly increases with the retention time of the eggs in the ovarian cavity following ovulation (*Sakai et.al.*, 1975; *Bromage,* 1995; *Linhart and Billard,* 1995). *Yamazaki and Arai* (1982) found no significant increase in abnormal salmon embryos from eggs fertilized with sperm obtained 5 to 28 days after spermiation in masu salmon which suggests that the source of the aberrations is in the female gamete. *Yamazaki et al.* (1989) showed chromosomal aberrations following oocyte aging in salmonids, but the exact time of ovulation was not determined. *Ueda* (1996) described triploidy in embryos after oocyte aging in rainbow trout. True tetraploids were found in the .present experiment among embryos produced from eggs retained in the ovarian cavity, which may be due to cell membrane alteration during the process of oocyte aging. In induced tetraploidy, inhibition of the plasma membrane formation between the daughter cells at mitotic divisions takes place so that the whole genome is duplicated (*Chourrout,* 1984). The high frequency of triploids detected in the treated groups, may have arisen from suppression of the second polar body followed by nondisjunction (*Nagy et al.,* 1978). The aneuploid larva with 64 chromosomes is a type of chromosome abnormality not yet reported in *Silurus glanis*. There were more morphological abnormalities than chromosomal ones; the relationship between the morphological and chromosomal aberrations proved to be statistically significant ($P<0.01$). The chromosomal abnormalities are described here from hatched larvae, but there might be even more in embryos, as suggested by *Yamazaki et al.* (1989) and *Ueda* (1996). Some other types of chromosomal abnormalities could have been detected using banding techniques, but such techniques are difficult to perform on preparations obtained by direct methods especially from larvae. These results indicate, that in hatchery conditions the complete procedure of gamete collection and artifical insemination should be carried out as soon as possible after ovulation. Moreover, both the present study and previous data (Billard, unpublished data.) show that some females are more sensitive to oocyte aging than the others. For example, in female C differed from the others in fertilization and hatching rates and number of malformed embryos (*Fig. 1., .2, 3.*).

In nature, *Silurus glanis* has a very stable karyotype and in the numerous populations studied all over Europe (Rábova, unpublished data.) no case of viable chromosomal aberration could be detected. The present work clearly demonstrates that at least three cases of viable (up to the larval stage) chromosomal abnormalities (triploidy, tetraploidy and aneuploidy) may occur. Polyploids are viable, not always sterile and do not exhibit any morphological abnormalities.

REFERENCES

Baksi, S.M. – Means, J.C.(1988): Preparation of chromosomes from early stages of fish for cytogenetic analysis. Journal of Fish Biology, 32. 321–325.p.

Bromage, N.R.(1995): Broodstock management and seed quality. In N.R. Bromage and R.J. Roberts Broodstock Management and egg and larvai quality. Blackwell, 1–24.p.

Chourrout, D.(1984): Pressure-induced retention of second polar body and suppression of first cleavage in rainbow trout: production of all-triploids, all-tetraploids, and heterozygous and homozygous diploid gynogenetics. Aquaculture, 36. 111–126.p.

Garman, S. – Denton, F.(1886): Abnormal embryos of trout and salmon. Sci. Observer, 49. 1–7.p.

Horváthné-Tamás, G. – Horváth, L. – Tölg, L.(1982): Fortschritte in der Teichwirtschaft, Spezielle Methoden. P. Parey-Akadémiai Kiadó, 38.p.

Linhart, O. – Billard, R.(1995): Survival of ovulated oocytes of the European catfish (*Silurus glanis*) after in vivo and in vitro storage or exposure to saline solutions and urine. Aquatic Living Resources, 8. 317–322.p.

Nagy, A. – Rajki, K. – Horváth, L. – Csányi, V.(1978): Investigation on carp, *Cyprinus carpio* L. gynogenesis. Journal of Fish Biology, 13. 215–224.p.

Ráb, P. – Mayr, B. – Roth, P.(1991): Chromosome banding study of European catfish, *Silurus glanis* (Pisces, Siluridae). Genetica, 83. 153–157.p.

Richter, C.J.J. – Eding, E.H. – Roem, A.J.(1985): 17α-hydroxyprogesterone induced breeding of the African catfish, Clarias gariepinus (Burchell), without priming with gonadotropin. Aquaculture, 44. 285–293.p.

Saito, H. – Koike, K. – Saito, T. – Nohara, M. – Kawagoe, S. – Hiroi, M.(1993): Aging changes in the alignment of chromosomes after human chorionic gonadotropin stimulation may be a possible cause of decreased fertility in mice. Hormone Research, 39. 28–31.p.

Sakai, K. – Nomura, M. – Takashima, F. – Oto, H.(1975): The over-ripening phenomena of rainbow trout-II. Changes in the percentage of eyed eggs, hatching rate and incidence of abnormal alevins during the process of over-ripening. Bulletin of the Japanese. Society of Science and Fisheries, 41. 855–860.p.

Ueda, T.(1996): Chromosome aberrations in salmonid fish embryos using prolonged stored eggs in coelomic fluid. Chromosome Information Service, 61. 13–15.p.

Yamazaki, F. – Arai, K.(1982): Developmental genetics concerning abnormal ontogeny in salmonids. Kasen Gata Kenkyu Group Report, Hokkaido Salmon Hatchery, 173–178.p.

Yamazaki, F. – Goodier, J. – Yamano, K.(1989): Chromosomal aberrations caused by ageing and hybridization in charr, masu salmon and related salmonids. Physiology and Ecology of Japan, Spec., 1. 529–542.p.

Authors' address: *Várkonyi, E. – Ozouf-Costaz, C. – Billard, R.*: Lab. d'Ichtyologie,
Muséum National d'Histoire Naturelle, Paris, France
Várkonyi, E.: Institute for Small Animal Research
H-2100 Gödöllő, POB 417., Hungary
Horváth, L: Gödöllő Univ. of Agricultural Sciences
H-2103 Gödöllő, Páter K. u. 1., Hungary

CHROMOSOME RESEARCHES ON SILVER CRUCIAN CARP (*CARASSIUS AURATUS GIBELIO*)

VÁRADI, L. — TÓTH, B.

SUMMARY

The number of individuals of silver crucian carp has been increasing dramatically in the last 10 years. This process leads to the disappearance of the biodiversity in natural waters and causes extraordinary demages in fish farms. This huge population growth can be explained by the ecological simplicity and the special reproductive mechanism. There are unisex populations containing only triploid (3n) females. These fish reproduce by gynogenesis in their long spawning period (from April, to August). The females produce triploid eggs, which are induced by males of other species (usually carp), or even diploid crucian carp males. The genetic staff of the sperm doesn't play roles in developing offspring so all female populations have clonal lines. Literature on the subject shows that there are diploid crucian carp populations too, which reproduce tradicionally. These populations have both female and male individuals. In our study we tried to separate the diploid and triploid individuals in a closed channel (Hajta stream in Hungary).. Morpho-meristic estimations, nucleoli numbers, erythrocyte diameters and chromosome numbers were studied. Chromosome preparations were made from fibroblast cultures of fins.

All males were found to be diploid with a chromosome number of 100. Females were triploid (150 chromosomes could be counted) or diploid (with 100 chromosomes). We also found a few males showing mosaicity with different chromosome numbers in different cells. Other study methods, as nucleoli numbers, erythrocyte diameters and morpho-meristic data led to the same result.

INTRODUCTION

Silver crucian carp (*Carassius auratus gibelio* BLOCH) is not inhabited in our natural waters. Settlements into farms were carried out in 1954. From the Danube river János Tóth detected this species for the first time in 1975. The individual number of the silver crucian carp has been increasing dramatically in the last 10–15 years. Before that time only triploid unisex female populations were recorded, but a few years ago male individuals were also detected in some natural waters. Those males are supposed to be members of wild goldfish (*Carassius auratus auratus)* populations, that morphologically cannot be separated from silver crucian carp. It presumes that there are two forms of the silver crucian carp in Hungary: the diploid type with the chromosome number of 100 and the other form which has 150 chromosomes, and is thought to be triploid. The diploid form spawns traditionally and has an ability to create hybrids with common carp (*Pintér,* 1992). The polyploids reproduce by gynogenesis. In this process the duplication of the chromosomes can be observed, but the first meiotic division doesn't occur. During the second meiotic division the second polar body eliminates, which results triploid eggs. Development of gynogenetic eggs induced by the sperm of other fish species (carp, or other cyprinids), but the genetic staff of the sperm doesn't play any role in forming offsprings.(*Horváth and Orbán,* 1995). This way the offspring have to be clones of their mother. Inbreeding studies were done in Japan in related species called ginbuna (*Carassius auratus langsdorfii*), that spawns in the same way. This study resulted that the 32 individulas made up 7 clones. (*Dong, et al.* 1996). Similar result were found by *Dong et al.* 1997, showing that 248 individuals could be devided into 18 clonal lines.

The literature on the subject shows that there are three kind of crucian carps in Japan. The first is a bisexual diploid (2n=100), the second is a unisexual triploid (150 chromosomes) and the third is a unisexual tetraploid (200 chromosomes). All of the unisexual populations reproduce by gynogenesis. The crucian carp in northeast China with a chromosome number of 150 was considered to be triploid, but after chromosome preparations Fan. Z, and Shen. J. in 1990 realized that chromosomes could be paired into homolouges (21 pairs of metacentric, 37 pairs of submetacentric, and 20 pairs of subtelocentric chromosomes).

In our study we tried to separate the diploid and the triploid individuals in a closed channel (Hajta stream in Hungary). Morpho-meristic estimations, nucleoli numbers, erythrocyte diameters and chromosome numbers were studied. Chromosome preparations were made from fibroblast cultures of fins.

MATERIALS AND METHODS

Fish were taken from a totally closed channel called Hajta channel. After the faunistic examinations, silver crucian carp individuals were transfered into the laboratory in 10 litre tanks.

In the laboratory these individuals were kept in 50 litre tanks (5–6 individuals in one tank) at 28 °C for 1 month. Morpho-meristic data were detected using a simple calliper and weight was measured by scale. The results were estimated according to *Váradi* (1996). Nucleoli numbers were studied from suspended and fixed fin cells (we suspended the cells in 50% acetic acid sollution. fixative: 3:1 methanol: acetic acid), the slides were made from fixed cells and stained with silver stain ($AgNO_3$). The nucleoli counting was carried out with ZEISS Fluovert microscope. The results were appraised according to *Cherfas* (1993). The erythrocyte diameters were detected by using Buerker-chamber. The result was appraised according to *Katsutoshi et al.* (1990).

Chromosome preparations were made from fin fibroblast cultures according to *Várkonyi et al.* (1998). After cutting the fins, we put them into 70% ethanol for sterilization, and then we kept them in a steril trypsine-PBS solution for 7-10 min (0.25% trypsine, SIGMA, T 4799). 2X2 mm tissues were cut from fins, and then were put into culture flasks. 2 hours later SIGMA, F 7524, SIGMA,. H 0763, and SIGMA, M 5017 were added to each flask. Flasks were cultivated at 28 °C for 4 weeks. 0.05% Colhicine solution (SIGMA, C 9754) was added to each flask, and 2 hours later suspension was made from cell cultures in centrifuge tubes using 0.025% trypsine solution at 28 °C. We centrifuged the solution for 7 min at 1500 rpm. After pouring off the supernatant, 5 ml hypotonic solution was added (0.35% Potassium-chloride). 5 min later it was centrifuged again and then fixative was added drop by drop (3:1 methanol:acetic acid). After 20 minutes the fixation was repeated two times. Slides were made from the suspension, and stained with Giemsa (2.4%) for 7 min., and were examined by using LEITZ DIAPLAN microscope.

RESULTS

Comparing the different sexes we have found that female's body weights are higher than male's. The differences between the data of body lenght/head show that females are probably more successful in competition, living in unfavourable ecological conditions, than males.

Nucleoli counting resulted that nucleoli number of the males are different from the females, and showed similarity to the nucleoli number of goldfish.

The amount of genetic staff was found to be different between females and males. The average erythrocyte diameter in males is 6.2 μm, while it is 8.45 μm in case of females. We have determined the dispersion as well as the percentage of dispersion, that showed another difference between the two sexes. Coefficient of variation of the females was 19.32, while males came up with 57.28, which meant that the genetic staff of the males could be different in one individual.

Chromosome preparations showed that there were females with 100 chromosomes, the diploid ones, and with 150 chromosomes, the triploid ones. Males were found to be diploid, but some of them showed mosaicity with chromosome numbers of 100, 125, 132, 156, 174 and186, usually between 100 and 190. Mosaicity was found in Crucian carp (*Carassius carassius)* by *Lingenfelser et al.*(1997) in Ukraine near Chernobil.

Taking into consideration the morphological results, we have found that some of the diploid individuals have morphological defaults. Till this time, this kind of defaults were detected only in goldfish .

DISCUSSION

It is clear that silver crucian carp has an ability to survive extraordinary ecological conditions. We have been trying to find the reasons for the wide tolerance interval of this species, and we would also like to find a method, for regulating the growth of their population. We have done an experiment, that showed that the diploid and triploid populations of the silver crucian carp live

together in a closed channel. Some of the diploid individuals have morphological defaults, but the two forms cannot be separated simply by measuring morphological parameters. Genetic measurements showed that there were differences between the amount of the genetic staff in the males. We have found a phenomena of mosaicitiy.

This study seems to prove the hypothesis, that assumes that the diploid form can be wild goldfish, and adds a new question: Where the mosaic males are come from? According to one hypotheses they can be hybrids of the two forms. This idea supposes that the diploid males spawn with the triploid females, and the sperm's genetic staff stays in the eggs after inducing the development of the offspring. Later some of these „strange chromosomes" eliminate. A related study was done by *Taniguchi and Dong* (1997), that proved, that a heat shock (40 °C) during fertilization gengoroubuna (*Carassius auratus cuvieri*) eggs were treated with common carp (*Cyprinus carpio*) sperm led to the apperance of the carp's fragments in the offsprings' genome.

There are a few more questions that we are planning to examine in the near future. Are there any differences in the hatching ratio, and the resistance for various deseases between the two forms, and we would also like to know if there is an ecological variance depending on the ploidy level.

REFERENCES

Cherfas, N.B.(1993): Induced diploid gynogenesis and polyploidy in ornamental (koi) carp, *Cyprinus carpio* L. 2 Timing of heat shock during the first cleavage. Aquaculture, 111. 281–290.p.

Dong, S. – Ohara, K. – Taniguchi, N.(1997): Introduction of sperma of common carp cyprinus carpio into eggs of ginbuna *carassius auratus langsdorfii* by heat shock treatment and its confirmation by DNA markers. Nippon Suisan Gakkaishi. 63. 2. 201–206.p.

Dong, S. – Taniguchi, N. – Tsui, S.(1996): Identification of clones of ginbuna *Carassius auratus langsdorfii* by DNA fingerprinting and isozym pattern. Nippon Suisan Gakkaishi, 62. 5. 747–753.p.

Dong, S. – Taniguchi, N.(1996): Clonal nature of offsprings of ginbuna *Carassius auratus langsdorfii* by RAPD-PCR and isozym patterns. Nippon Suisan Gakkaishi, 62. 6. 891–896.p.

Fan, Z. – Shen, J.(1990): Studies on the evolution of bisexual reproduction in crucian carp (*Carassius auratus gibelio* BLOCH). Aquaculture, 84. 235–244.p.

Horváth, L. – Orbán, L.(1995): Genome and gene manipulation in the common carp. Aquaculture, 129. 157–181.p.

Katsutoshi, A. – Kumi, M. – Ryo, S.(1991): Karyotype and erythrocyte size of spontaneous tetraploidy and triploidy in the loach Misgurnus anguillicaudatus. Nippon Suisan Gakkaishi, 57. 12. 2167–2172.p.

Lingenfelser, S.K – Dallas, C.E – Jagoe, C.H – Chesser, R.K – Smith, M.H – Lomakin, M.(1997): Variation in blood cell DNA in *Carassius carassius* from ponds near Chernobyl, Ukraine. Ecotoxicology, IV. 187–203.p.

Pintér., K.(1992):The fishes of Hungary (in Hungarian). Mezőgazdasági Kiadó, Budapest

Tóth, J.(1975): A brief account on the presence of the silver crucian carp(*Carassius auratus gibelio* BLOCH 1873) in the Hungarian section of the Danube. Annales Univ: Sci. Budapestiensis Section Biologica, 17.p.

Váradi., L.(1996) Measurement of genetic structures in fish populations and possibilities for manipulations. (PhD-thesis, in Hungarian) Gödöllő, Hungary.

Várkonyi, E. – Bercsényi, M. – Ozouf-Costaz, C. – Billard, R.(1998): Chromosomal and morphological abnormalities caused by oocyte ageing in *Silurus glanis*. Journal of Fish Biology, 52. 899–906.p.

Authors' address: *Váradi, L. – Tóth, B.*: University of Agricultural Sciences
H-2100 Gödöllő, Páter K. u. 1.

MOLECULAR CYTOGENETICS OF FLATFISHES (PLEURONECTIDAE, SCOPHTHALMIDAE, SOLEIDAE)

PARDO, B.G. — BOUZA, C. — CASTRO, P. — MARTÍNEZ, J. — SÁNCHEZ, L.

Recently several flatfshes of commercial interest have began to be cultured in Spain. Cytogenetd'c data in the Pleuronectiformes are very scarce. In the peresent work we made an extensive cytogenetic analysis of different species of three families (Pleuronectidae, Scophthalmidae and Soleidae). heterochromatin was characterized by means of restriction enzyme digestion. The localization of acitve and inactive nucleolar organizer regions (NOR) and their polymorphism were studied by means of A9-, CMA_3 banding and ISH. All these results will be of great interest for future chromosome manipulations in these species.

Authors' address: Dept. Biol. Fundamental, Area Genética,
Universidad de Santiago de Compostela, 27002 Lugo, Spain

APPLICATION OF GENOMIC *IN SITU* HIBRIDIZATION (GISH) IN THE IDENTIFICATION OF FISH HYBRIDS

HIDAS, A. — VÁRKONYI, E.

SUMMARY

In case of hybridization and other parcial genome transfer, the genomic *in situ* hybridization (GISH) proved to be suitable method for identification of various chromosomes from different species in fish. The labelled total DNA of one species was used as probe, and the other unlabelled total genome was used as competitor DNA. In case of hybrids with mixed genome, the chromosomes of one parent-line was detectable with strong fluorescent signal.

The isolated DNA was fluorescein-dUTP labelled with nick translation to provide a possibility for direct detection. The chromosomes of carp light in red colour (rhodamine) and the chromosomes of rosy barb in green colour (fluorescein).

It was determined that from the carp and from the koi carp **50** chromosomes and from the rosy barb **24-25** chromosomes contributed to the genome of both hybrid-groups depending on the individuals.

INTRODUCTION

In case of hybridization and other parcial genome transfer, the genomic *in situ* hybridization (GISH) proved to be suitable method for identification of various chromosomes from different species in fish.The labelled total DNA of one species was used as probe and the other unlabelled total genome was used as kompetitor DNA. In case of hybrids with mixed genome, the chromosomes of one parent-line was detectable with strong fluorescent signal.

MATERIALS AND METHODS

In the first year we produced the fish hybrids-lines using carp (*Cyprinus carpio carpio*) and koi carp (*Cyprinus carpio viridi-violaceus*) mother-lines and Rosy barb (*Barbus conchonius*) father-line. The hybrids were made with artifical insemination. The fertilization rate in blastula stage was 80–85%.

When the population was growing up to 4–5 cm size, the cytogenetic analysis was started. From fish-fins were started tissue cultures (*Alvarez et al.*, 1991). After four weeks when the fibroblast monolayer was almost confluent in the flasks, the tissue culture using one drop of 0.05% colchicine (Sigma C 9754) was stopped. The cell suspension was spread on slides, dried at room temperature, and stained with 5% fresh Giemsa (in phosphate buffer pH 7.0) for 7–8 min (*Earley*, 1975). We determined the number of chromosomes from the 15 individuals of two experimental population.

The isolated DNA (Wenham, 1992) was fluorescein-dUTP and rhodamine-dUTP labelled with nick translation to provide a possibility for direct detection. The chromosomes of carp light in red colour (rhodamine) and the chromosomes of rosy barb in green colour (fluorescein).

The samples were incubated at 16 °C at two hours, after this procedure the DNase enzyme was inactivated at 68 °C at 15 minutes. After cooling the labelled genomic probe was precipitated with alcohol, dissolved in 25 μi formamide and put into the hybridization mixture.

Hybridization mixture:

5 μl probe-competitor DNA (dissolved in formamide),
5 μi dextrane-sulfate (500 μl 50% dextrane-sulfate, dissolved in 250 μl 20xSSC),
0.7 μl 0.2 N HCl.

The probe and the target DNA (chromosome preparations) were denaturated together. After dropping the probe DNA, covering and insulating with rubber cement, the denaturation happened at 70 °C (40–80 sec.). Following the cooling procedure with ice, the hybridization occured at 37 °C and 24–48 hours in vapour chamber.

After the hybridization the washing was 5 minutes in 2xSSC at 37 °C. Staining with propidium-jodid: 0.015 μg/μl, 10 minutes.

After washing, the preparations were examinated using antifading solution (Vectashield) with microscope (LEITZ DIAPLAN).

RESULTS AND DISCUSSION

We established, that the two hybrid-groups has intermediate diploid chromosome number with 74 chromosomes.

Table 1.

	Females		Hybrids		Male Rosy barb
	Carp	Koi carp	Carp x Barbus	Koi carp x Barbus	
No. of chromosomes	101.8	101.8	74.4	74.2	48.1

It was determined that from the carp and from the koi carp 50 chromosomes and from the rosy barb 24–25 chromosomes contributed to the genome of both hybrid-groups depending on the individuals.

This work was supported by the Hungarian Research Found (F 016415)

REFERENCES

Alvarez, M.C. – Otis, J. – Amores, A. – Guise, K.(1991): Short-term cell culture technique for obtaining chromosomes in marine and freshwater fish. J. Fish. Biol., 39. 817–824.p.
Earley, E.M.(1975): Chromosome preparations from monolayer cell cultures. Tissue Cult. Assoc. Man., 2. 31–35.p.
Wenham, P.R.(1992): DNA-based techniques in clinical biochemistry: a beginner's guide to theory and practice. Ann. Clin. Biochem., 29. 598–624.p.

Authors' address: *Hidas, A. – Várkonyi, E.*: Institute for Small Animal Research
H-2100 Gödöllő, P.O.B. 417., Hungary

PHYSICAL MAPPING OF MICROSATELLITE MARKERS ON THE DOG (*CANIS FAMILIARIS*) CHROMOSOMES

SWITONSKI, M. — ŁADON, D. — SCHELLING, C. —
SCHLAPFER, J. — STRANZINGER, G. — DOLF, G.

Nine microsatellite markers. designated CanBern1, CanBern6, ZuBeCa1, ZuBeCa2, ZuBeCa3, ZuBeCa4 and ZuBeCa6, ZuBeCa6 and ZuBeCa7 were physically localized with FISH technique on the canine chromosomes. The cosmid probes were labelled with the random priming method with biotin-16dUTP and then applied on chromosome slides with preidentyfed QFQ banded metaphases.

At present, only a partial standard karyotype of the dog is available, comprising 21 biggest autosome pairs and the sex chromosomes Seven microsatellites were assigned to specifc canine chromosomes (*Switonski et al.*, 1996) while two of them were localized on small autosomes not yet included in the standard:

CanBern 1	13q21
CanBern6	small autosome
ZuBeCa 1	10q22-q24
ZuBeCa2	1q210-q211
ZuBeCa3	9q21-q22
ZuBeCa4	3q15-ql8
ZuBeCaS	19q7
ZuBeCa6	Sq2-q3
ZuBeCa7	small autosome

REFERENCE

Switonski et al. (1996): Chromosome Research 4. 306–309.p.

Authors' address: *Switonski M. – Ładon, D.*: Agricultural University of Poznan
60-637 Poznan, Poland
Schelling, C. – Stranzinger, G.: Swiss Federal Institute of Technology
8092 Zürich, Switzerland
Schlapfer, J., – Dolf, G.: University of Berne, 3012 Switzerland

STANDARDIZATION OF CHROMOSOMES NOS. 22–38 OF THE DOG *(CANIS FAMILIARIS)* WITH THE USE OF CANINE PAINTING PROBES

COMMITTEE FOR THE STANDARDIZED KARYOTYPE OF THE DOG *(CANIS FAMILIARIS)*

BREEN, M. — REIMANN, N. — BOSMA, A.A. — ŁADON, D. —
ZIJLSTRA, C. —BARTNITZKE, S. — SWITONSKI, M. — LONG, S.E. —
DE HAAN, N.A. — BINNS, M.M. — BULLERDIEK, J. — LANGFORD, C.F.

The karyotype of the domestic dog *(Canis familiaris)* has provided a considerable challenge to detailed karyotype analyses. This is not only due to the high diploid chromosome number (2n=78), but also to the acrocentric morphology of all 38 pairs of autosomes which show only a gradual decrease in size.

During the 11th European Colloquium on Cytogenetics of Domestic Animals, held in August 1994 in Copenhagen (Denmark), the Committee for the Standardized Karyotype of the Dog *(Canis familiaris)* was established. In 1996, the Committee published a standard for chromosomes 1 to 21 plus X and Y (*Switonski et al.*, 1996) based on the arrangement made by *Selden et al.* (1975). It includes a numbering system for autosomes nos. 1–21, and ideograms, based on G-banding patterns, with numbered regions and bands. The Committee concluded that it is not possible to reliably identify the smaller autosomes (nos. 22–38) from their banding patterns only.

In collaboration with the Animal Health Trust, the Sanger Centre has produced painting probes from flow-sorted canine chromosomes (*Langford et al.*, 1996). Paints hybridizing to the smaller autosomes have been applied to chromosomes in GTG- and DAPI-banded metaphase spreads from healthy dogs of various breeds in four of our laboratories. During a meeting of the Committee in May 1997 in Utrecht (The Netherlands), the results thus obtained were compared. A numbering system was developed for autosomes nos. 22–38, by following basically the arrangement of *Selden et al.* (1975), and considering the minor modifications of Selden's arrangement introduced by *Reimann et al.* (1996). However, in a large number of cases, agreement with respect to the identity of a particular chromosome could only be reached on the basis of its painting properties.

One of the problems encountered with was the fact that not all paints hybridize to one pair of chromosomes only (paints S, V, W, Z, and cc hybridize to two chromosome pairs) . In two of our laboratories (at the universities of Utrecht and Poznan), differences in fluorescence intensity were observed when applying paint S, a paint hybridizing to chromosome pairs nos. 21 and 23 (the latter pair was found to be painted more brightly).

Identification of individual canine metaphase chromosomes is important in gene mapping studies, in the cytogenetic analysis of dogs with congenital malformations, and in studies on karyotypic abnormalities of tumor cells. A full publication with the Committee's recommendations is in preparation. These recommendations include a numbering system for chromosomes nos. 22–38 that is based on both GTG- and DAPI-banding patterns and painting properties. Full agreement on all details of numbering of regions and bands in these chromosomes could not be reached. In this respect, authors may refer to the extended ideogram of the canine karyotype presented by *Reimann et al.* (1996).

Chromosomes nos. 22–38 hybridize with the following paints (marked by *Langford et al.*, 1996):

Chromosome no.	Paint	Chromosome no.	Paint	Chromosome no.	Paint
22	N	28	V	34	Z
23	S	29	W	35	ff
24	V	30	bb	36	cc
25	T	31	Z	37	dd
26	aa	32	W	38	ee
27	U	33	cc		

REFERENCES

Langford, C.F. – Fischer, P.E. – Binns, M.M. – Holmes, N.G. – Carter, N.P.(1996): Chromosome-specific paints from a high-resolution flow karyotype of the dog. Chrom. Res., 4. 115–123.p.

Reimann, N. – Bartnitzke, S. – Bullerdiek, J. – Schmitz, U. – Rogalla, P. – Nolte, I. – Ronne, M. (1996): An extended nomenclature of the canine karyotype. Cytogenet. Cell Genet., 73. 140–144.p.

Selden, J.R. – Moorhead, P.S. – Oehlert, M.L. – Patterson, D.F.(1975): The Giemsa banding pattern of the canine karyotype. Cytogenet. Cell Genet., 15. 380–387.p.

Switonski, M. – Reimann, N. – Bosma, A.A. – Long, S. – Bartnitzke, S. – Pienkowska, A. – Moreno-Milan, M.M. – Fischer, P.(1996): Report on the progress of standardization of the G-banded canine *(Canis familiaris)* karyotype. Chrom. Res., 4. 306–309.p.

Authors' address: *Breen, M. – Binns, M.M.*: Centre for Preventive Medicine, Animal Health Trust
Lanwades Park, Kentford, Suffolk, CB8 7UU, U.K.

Reimann, N. – Bartnitzke, S. – Bullerdiek, J.: Center for Human Genetics and Genetic Counselling, University of Bremen
Leobener Strasse ZHG, 28359 Bremen, Germany

Bosma, A.A. – Zijlstra, C. – de Haan N.A.: Department of Cell Biology and Histology, Faculty of Veterinary Medicine, Utrecht University
P.O. Box 80.157, 3508 TD Utrecht, The Netherlands

Ładon, D. – Switonski, M.: Department of Genetics and Animal Breeding, Agricultural University of Poznan
Wolynska 33, 60-637 Poznan, Poland

Long, S.E.: Department of Clinical Veterinary Science, University of Bristol
Bristol, BS18 7DU, U.K.

Langford, C.F.: The Sanger Centre, Wellcome Trust Genome Campus, Hinxton
Cambridgeshire, CB10 1SA, U.K.

PHYSICAL LOCALIZATION OF THE CANINE MICROSATELLITE MARKERS ON THE SILVER FOX *(VULPES FULVUS DESM.)* CHROMOSOMES*

ŁADON, D. — SWITONSKI, M. — SCHELLING, C. — SCHLAPFER, J. — DOLF, G.

Progress of the canine marker genome map brings an opportunity to develop the maps of other Canidae species. Until now, thirty two markers were mapped on the silver fox chromosomes with the use of somatic cell hybridization approach (Genetic Maps, 1993).

In this paper we report on localization of fve microsatellite markers, named CanBernl, CanBern6, ZuBeCa1, ZuBeCa3, ZuBeCa4 on the silver fox chromosomes. These markers were previously assigned to the canine chromosomes. The cosmid probes were labelled by the random priming method with biotin-16dUTP and then applied in FISH experiments. Chromosomes were identifed by QFQ banding prior to FISH, and nomenclature for G-banded karyotype of silver fox was followed (*Mäkinen et al.*, 1985).

The markers were localized on the following silver fox chromosomes:

CanBern1	5pter
CanBern6	5pter
ZuBeCa1	16q2
ZuBeCa3	2p2
ZuBeCa4	14q1

* This work was supported by the Committee for Scientifc Research, grant no. 5 P06 D 00 413.

REFERENCES

O'Brien, S.J.(eds.)(1993): Genetic Maps. Cold Spring Harbor Laboratory Press
Makinen et ai. (1985): Hereditas, 103, 171–176.p.

Authors' address: *Ładon, D – Switonski,* M.: Agricultural University of Poznan
60-637 Poznan, Poland
Schelling, C.: Swiss Federal Institute of Technology
8092 Zürich, Switzerland
Schlapfer, J. – Dolf, G.:University of Beme
3012 Berne, Switzerland

A SEQUENCE CONTAINING A JUNCTION BETWEEN SHEEP SATELLITE I AND II DNA MEDIATES ISOCHROMOSOME AND MICROCHROMOSOME FORMATION IN HAMSTER CELLS

CICCARESE, S. — D'AIUTO, L. — CSERPÁN, I. — VONGHIA, G. — BARSANTI, P.

The nature of the DNA sequences necessary to provide in higher organisms full centromere function is still not well understood notwithstanding its importance for the development of stable artificial chromosome vectors. While recent experiments (*Harrington et al.*, 1997) attribute a fundamental role to alpha-satellite for centromeric activity, other evidence (*Choo*, 1997) indicates that alphoid DNA is not necessary for centromeric function or, alternatively, it can be replaced by other sequences with similar properties. The centromeric heterochromatin of domestic sheep *(Ovis aries)* is basically composed of two different repetitive DNA sequences, satellite I and satellite II (*Buckland et al.*, 1983, 1985). Recently, we demonstrated that satellite I and satellite II form discrete and contiguous blocks in the centromeric regions of most sheep chromosomes (*D'Aiuto et al.*, 1997). Further analysis of phage clones containing the junction between satellites I and II has pointed out an unexpected complexity of the regions of contiguity between blocks of different sheep centromeric highly repeated sequences.

To evaluate the possibility that sequences from such regions could induce de *novo* centromere formation in transfected cells, we cotransfected in hamster K20 cells the junction-containing phage 17/3pc together with the pCEPUR132 vector, which carries the puromycine and hygromicine resistance markers. Cotransfection was by calcium phosphate DNA precipitation. To allow for selection of trasformants integrating sheep centromeric sequences, transfected cells were placed under 15 mg/ml puromycine selection for 14 days in F-12 medium. Ten drug-resistant colonies were isolated, expanded and analyzed by Southern blot analysis, using as probe 17/3pc phage DNA. 17/3pc sequences resulted present in multiple copies in the K17/3 clone.

Chromosome preparations from this clone were hybridized *in situ* using as a probe 17/3pc phage DNA biotinylated by nick-translation as described by *D'Aiuto et al.* Chromosome visualization was obtained by DAPI staining. Digital images were obtained using a computer-controlled microscope equipped with a cooled CCD camera. FITC and DAPI fluorescence were recorded as grey scale images and then pseudocolored and computer merged. FISH experiments pointed out the presence of at least four types of 17/3pc DNA-containing chromosomal structures. In the first type, 17/3pc is integrated into the long arm of an endogenous hamster chromosome. In other cells, the signal is located at the extremity of a smaller chromosome that appears to correspond to q ter of the first structure type. The third type of observed structure is a metacentric chromosome on which the hybridization signal is located on the primary constriction; most probably this represents a duplication of the apparently telocentric chromosome above described. In accord with this hypothesis metaphases were also observed where the signal was located on both telocentric and metacentric chromosomes. Finally, in several metaphases the transfected 17/3pc clone was present in a microchromosome.

These results show that sheep centromeric sequences induce chromosomal breakage in transfected hamster cell line and also suggest that functional centromeres were *de novo* formed. A study of the stability of the induced chromosomal structures and of the significant features of their centromeres is in progress.

ACKNOWLEDGEMENT

Research supported by CNR Target Project on "Biotechnology" and MURST, Italy.

REFERENCES

Buckland, R.A.(1983): Nucleic Acids Res., 11. 1349–1360.p.
Buckland, R.A.(1985):J. Mol. Biol., 186. 25–30.p.

Choo, K.H.(1997): The Centromere Oxford University Press
D'Aiuto, L. et al.(1997): Chromosome Research, 5. 375–381.p
Harrington, J.J. et al.(1997): Nature Genetics, 15. 345–354.p

Authors' address: *Ciccarese, S. –D'Aiuto, L. – Barsanti, P.:* Institute of Genetics, University of Bari, via Amendola 165/A, 70126 Bari, Italy
Cserpán, I.: Institute of Genetics, Biological Research Center Hungarian Academy of Sciences, H-6701 Szeged, P.O. Box 521.
Vonghia, G.: Department of Animal Production, University of Bari via Amendola 165/A, 70126 Bari, Italy.

CYTOGENETIC CAUSE OF STERILITY ON THE HYBRIDS BETWEEN YAK (*BOS GRUNNIENS*) AND CATTLE (*BOS TAURUS*)

TUMENNASAN, Kh. — TUYA, Ts. — TSENDTSESMEE, L. —
HOTTA, Y. — TAKASE, H. — SPEED, R.M. — CHANDLEY, A.C.

Crosses between yak and cattle produce an F1 hybrid, known in Mongolia as a khainag, which is fertile in females but sterile in the male. Because of its strength, the khainag serves as a useful beast of burden for Mongolian farmers and can be seen pulling family carts. The karyotypes of B. taurus (2n=60) and B. grunniens (2n=60) appear to be identical cytologically, each consisting of 58 acrocentric or telocentric autosomes. submetacentric X- and Y-chromosomes. Its therefore of interest that the F1 hybrid male khainag is sterile. It can be likened to other interspecifc hybrids which are sterile in one or both sexes despite inheriting closely similar or even identical chromosome sets from the two parents.

Testis measurements, collection of tissue for somatic chromosome analyses, testicular histology meiotic chromosome analysis by air drying, microspeading have been performed by standard methods. 2 yak, 2 cattle, 2 khainag, 2 ortoom and 2 usanguzee have been sampled.The testes of the two cattle were larger than those of the other animals.

Testicular histology consistent with the large size of their testes, the two cattle were the only animals to show complete spermatogenic development to the stage of spermatozoa. All of the remaining animals appeared to be sexually immature, although in the histological sections each showed the presence of germ cells that had developed to the spermatocyte stage. The two yaks had spermatocytes in 25% and 50% of the tubules, respectively; each khainag and each usanguzee had spermatocytes in about one third of the tubules whilst in the two ortoom, about 50% of the tubules showed maturation to this stage. In all remaining tubules, development had proceeded only to the spermatogonial stage. Overall, the findings indicated that puberty is more advanced in cattle than in the yak or in any of the hybrid or backcross animals. Meiotic analyses of air dried preparations: All stages of germ-cell development, including spermatozoa, were found on the slides prepared from the two cattle. but only Sertoli cells spermatogonia, and early spermatocytes were found in those from the yak, khainag and usanguzee.

DISCUSSION

First, it looked as though the cattle reached puberty earlier than the yak or any of the hybrid or backcross animals. Second, it was possible to conclude to that spermatogenesis in all of this later types of animals was proceeding at least to the primary spermatocyte stage. The development in the khainag testis proceeds only to the spermatogonial stage. It seemed from our histological preparations that the overall numbers of spermatogonia were, in fact, reduced in the khainag. A third aspect of this work was the finding of synaptic anomalies in the khainag at meiotic prophase, despite the fact that the chromosomes of yak and cattle are indistinguishable cytologically. The reason for spermatogenetic breakdown in such situations remains to be explained.

But pairing anomalies seen at meiotic prophase in mammals need not necessarily be the cause of spermatogenetic breakdown; rather they may be a consequence of some prior disturbance to the germ line. In the khainag, for example, it appeared, from the histological preparations that spermatogonia were reduced in number in the testis tubules, a sign that the spermatogenetic disturbance preceded the pairing stages.

It was also apparent that not every prophase spermatocyte in the khaínag that examined by microspreading exhibited pairing anomalies. Likewise, backcross animals which are closer chromosomally to one or the other of the parental types, better pairing (and, hence, perhaps better fertility) can be achieved, as was observed in the ortoom.

The germ — line disturbances in general are more pronounced in males than in females, indicating that spermatogenesis is a much more sensitive process to impairment than oogenesis. The "Haldane rule" seems certainly to be obeyed in the cattle X yak hybrid situation, where, as Haldane predicted, the heterogametic sex would be the more likely to be sterile. Based on our data we suggest that backcrosses Fb1, Fb2 tend to become fertile. A futher study of this natural model would contribute to understanding mechanisms of infertility in hybrids between closely related mammalian species.

Authors' address: *Tumennasan, Kh. –Tuya, Ts. –Tsendtsesmee, L.:* Department of Genetics, Institute of Biology, Mongolian Academy of Sciences, Ulaanbaatar (Mongolia)
Hotta, Y. – Takase, H.: School of Biological Sciences, Nara Institute of Sciences and Technology, Ikoma (Japan)
Speed, R.M. –Chandley, A.C.: MRC Human Genetics Unit, Western General Hospital, Edinburgh, Scotland (UK)

EVALUATION OF REPRODUCTIVE PERFORMANCE ON THE BASIS OF SEMEN ANALYSIS IN THE BULLS CARRYING 60,XX/60,XY CHIMERISM

REJDUCH, B. — JAZDZEWSKI, J. — SLOTA, E. —
KOZUBSKA-SOBOCINSKA, A. — DANIELAK-CZECH, B.

After cytogenetic analysis 28 bulls originated from dizygotic twins were chosen as the carriers of 60,XX/60,XY chimerism to evaluate the infuence of this chromosomal abnormality on reproductive performance.

Five animals had to be excluded from this group because of:

— azoospermy – 2 animals,
— very low growth rate – 1 animal,
— poor sexual behaviour – 2 animals.

The semen analysis included the following parameters: volume of ejaculate (ml), motile spermatozoa (%) and sperm concentration (ml/x10°). The 12 semen samples per animal were taken from the bulls aged 12–15 months.

The results obtained were compared with the results of semen quality from 23 bulls with normal karyotype. The results are presented in *Table 1.*

Table 1.

The results of semen quality analysis in the chimeric bulls

No of the bull	Volume (ml)	Motile sperm. (%)	Sperm. conc./mlx10°
1342	4.03	66.7	1182
1417	4.86	65.0	879
1423	5.03	56.7	724
26254	3.46	66.7	870
1473	5.85	73.3	1505
70924	4.88	68.3	858
35152	2.26	60.8	504
1524	2.64	53.6	991
1523	3.00	45.0	552
36142	3.95	64.0	971
04273	1.15	60.0	713
47915	3.11	55.8	863
17675	3.54	59.2	549
90315	4.77	61.7	832
40053	5.58	55.8	91[illegible]
37947	3.03	45.0	60[illegible]
1639	4.26	46.7	552
1673	2.67	53.3	677
1640	5.21	58.3	647
1681	2.62	48.3	560
1690	2.36	41.7	289
35901c	3.24	53.3	766
35901b	1.30	23.5	398
XX/XY n=23	3.60*	55.8**	757**
controls n=23	4.40	69.2	1021

* significant differences (P>0.05) ** highly signifcant differences (P<0.01)

The results showed significant differences with reference to the volume of ejaculate and highly significant differences with reference to the motility of spermatozoa and sperm concentration. These three parameters were lower in the group of chimerism carrying bulls than in the control group (bulls with normal karyotype). The results obtained suggest that it is advisable to eliminate chimeric bulls from A.I. stations. This work was done within of research project no 5 PO6D 003 10, financed by the State Committee for Scientific Research.

Authors' address: *Rejduch, B. – Slota, E. – Kozubska-Sobocinska, A. – Danielak-Czech, B.:* National Research Institute of Animal Production, Department of Immuno and Cytogenetics, 32-083 Balice/Kraków, Poland
Jazdzewski, J.: Experimental Station of Animal Production, 64-122 Pawlowice, Poland

THE LEVEL OF XX;XY LYMPHOCYTE POPULATION IN CHIMERIC BULLS

SŁOTA, E. — REJDUCH, B. — KWACZYNSKA, A. — KOZUBSKA-SOBOCINSKA, A. — DANIELAK-CZECH, B.

SUMMARY

Twenty-five bulls originating from dizygotic twins were taken under cytogenetic control. Blood samples were collected from month-old animals and then every 10 weeks until fifteen months of age. Chromosome preparations obtained after lymphocyte culture were stained by conventional Giemsa staining. The ratio of two cell lines (60,XX:60,XY) was established on the basis of 100 metaphase plates per animal.

Of 25 bulls, 17 animals showed the tendency to increase the XY cell line level and in 8 bulls the level of their own XY cell line decreased. Also the differences between the first and last observation were greater in the bulls in which the level of XY cell line increased. The mean difference between the results of the first and last examination was 25.6 in the group of bulls with the tendency to eliminate the sister (XX) cell line and 10.3 in the group of bulls with the tendency to decrease the level of their own line.

The results obtained indicate that the tendency to eliminate the XX line (obtained from the sister of the twin) is very individual and can be compared to individual immunological tolerance on skin grafts between dizygotic twins.

INTRODUCTION

The most frequently observed chromosome abnormality in cattle is 60,XX/60,XY chimerism in dizygotic twins. The cell chimerism has been found in different tissues: blood, bone marrow, lymph node, skin fibroblasts, spleen, kidney and testes (*Ohno et al.*, 1962, *Teplitz et al.*, 1967, *Dunn et al.*, 1968, *Plante et al.*, 1992). Cellular chimerism is the result of placental vascular anastomosis, which develops between the two embryos at an early stage of intra-uterine life (*Marcum*, 1974).

This cell chimerism was most frequently detected in the lymphocyte population. The proportion of two lymphocyte lines (60,XX; 60,XY) ranged widely from 0 to 100% (*Dunn et al.*, 1979, *Jaszczak et al.*, 1988). The differences in percentage of XX lymphocytes in one animal tested at time intervals was observed previously by *Dunn et al.* (1979).

The aim of this study was to observe of the differences in the XX:XY ratio in the bulls originated from dizygotic twins.

MATERIALS AND METHODS

Twenty-five bulls originating from dizygotic twins were taken under cytogenetic control. Blood samples were collected from month-old animals and then every 10 weeks until fifteen months of age. Chromosome preparations obtained after lymphocyte culture were stained by conventional Giemsa staining. The ratio of two cell lines was established on the basis of 100 metaphase plates per animal.

RESULTS AND CONCLUSIONS

Vascular anastomosis between cattle fetuses begins by about the 30th day of intra-uterine life, resulting very often (about 95% of cases) in blood cell exchange (*Jost and Prepin,* 1966). All of the investigated bulls (25) showed lymphocyte chimerism. In 23 bulls chimerism was evident from birth to fifteen months of age. Also *Basrur and Stoltz* (1966) have demonstrated in studies on bovine twins, triplets and quintuplets that lymphocyte chimerism may occur from birth to sexual maturity.

The level of XY cell lines ranged widely in the chimeric bulls' population from 4 to 100%. Similar results obtained by *Dunn et al.* (1979) showed that XY population in the blood of 22 chimeric bulls ranged from 5 to 95%. *Jaszczak et al.* (1988) found one fertile bull originating from different sex twins, in which only one lymphocyte line with 60,XX karyotype was present.

The results shown in *Table 1.* demonstrate changes in the XY cell line level. Of 25 bulls, 17 animals showed the tendency to increase the XY cell line level and in 8 bulls the level of their own XY cell line decreased. Also the differences between the first and last observation were greater in the bulls in which the level of XY cell line increased. The mean difference between the results of the first and last examination was 25.6 in the group of bulls with the tendency to eliminate the sister (XX) cell line and 10.3 in the group of bulls with the tendency to decrease the level of their own line.

The results described by *Teplitz et al.* (1967) showed that the proportion of XX:XY lines is rather stable, with a small tendency for XY line to increase. *Dunn et al.* (1979) investigated the proportions of the two cell lines in 14 chimeric bulls at 1.5–5.2 year intervals. The proportions have changed; however, the direction of change was almost random. The XY line increased in 7 bulls, was stable in 1 bull and decreased in 6 bulls. In the population of 14 bulls the mean difference was established at the level +1.2 in relation to XY line. In the experiment presented by *Stranzinger et al.* (1981) in the blood of two bulls, originated from dizygotic twins the increase of XY population in the period of 10 months has been found.

The results obtained indicate that the tendency to eliminate the XX line (obtained from the sister of the twin) is very individual and can be compared to individual immunological tolerance on skin grafts between dizygotic twins (*Stone et al.*, 1960).

Meiotic chromosomes at different division stages were analysed in 24 bulls. Only in one bull (no. 1523) has the possible germ cell chimerism been found (*Rejduch et al.*, 1998). Probably, even if the germ cell migration occurs in the early stages of intra-uterine life in other bulls, the XX cell line can be eliminated in the period from birth to the maturation.

This study was conducted as part of research project no 5 PO6D 003 10, financed by the State Committee for Scientific Research.

Table 1.

Changes of XY cell line level in chimeric bulls

No of the bull	Level of 60,XY cell line (%)						Difference between 1–6
	obs. 1	obs. 2	obs. 3	obs. 4	obs. 5	obs. 6	
1342	55	57	53	70	80	84	+29
1423	78	78	79	90	90	92	+14
70251	87	93	96	100	100	100	+13
1473	40	45	50	62	68	71	+31
1523	14	25	39	37	44	54	+40
47048	27	64	72	76	78	86	+59
47915	45	72	69	88	90	78	+33
90315	15	27	40	36	40	52	+37
40053	29	31	34	34	54	68	+39
1639	45	32	54	58	60	65	+20
1673	48	82	84	88	92	92	+44
1681	16	30	26	30	20	19	+3
37947	26	28	36	34	40	47	+21
37332	86	90	92	90	93	96	+10
70924	92	97	96	98	97	100	+8
1524	85	89	88	89	92	90	+5
17675	19	20	16	22	24	48	+29
1690	7	5	6	8	6	6	–1
35152	52	62	60	54	46	44	–8
36142	15	13	18	4	6	8	–7
04273	55	54	54	48	44	44	–11
1640	38	40	37	28	28	37	–1
1675	25	13	10	8	8	12	–13
35901b	98	76	76	80	73	75	–23
35901c	90	80	76	76	70	72	–18

No correlation was found between the XY/XX ratio and reproductive performance.

REFERENCES

Basrur, P.K. – Stoltz, D.R.(1966): Chromosome studies in bovine quintuplets. Chromosoma, 19. 176–187.p.

Dunn, H.O. – Kenney, R.M. – Lein, D.H.(1968): XX/XY chimerism in a bovine true hermaphrodite: an insight into the understanding of freemartinism. Cytogenetics, 7. 390–402.p.

Dunn, H.O. – McEntee, K. – Hall, C.E. – Johnson, R.H. – Stone, W.H.(1979): Cytogenetic and reproductive studies of bulls bom co-twin with freemartins. J. Reprod. Fert., 57. 21–30.p.

Jaszczak, K. – Parada, R. – Boryczko, Z. – Romanowicz, K. – Wijas, B.(1988): A cytogenetic analysis and reproductive ability of bull having karyotype with only XX sex chromosomes in the blood lymphocytes. Genet. Pol., 29 3–4. 368–376.p.

Jost, A. – Prepin, J.(1966): Migration des cellules germinales. Arch. Anat. Micr. Morph. Exp., 55. 161–186.p.

Marcum, J.B.(1974): The freemartin syndrome. Anim. Breed. Abstr., 42. 6. 227–238.p.

Ohno, S. – Trujillo, J.M. – Stenius, C. – Christian, L.C. – Teplitz, R.L.(1962): Possible germ cell chimeras among new bom dizygotic twin calves (*Bos taurus*), Cytogenetics, 1. 258–265.p.

Plante, Y. – Schmutz, S.M. – Lang, K.D.M. – Moker, J.S.(1992): Detection of leucochimerism in bovine twins by the DNA fingerprinting. Anim. Genet., 23. 295–302.p.

Rejduch, B. – Słota, E. – Danielak-Czech, B. – Kozubska-Sobocińska, A.(1998): Preliminary identification of germ cell chimerism in the bull from different sex twins (in press)

Stone, W.H. – Berman, D.T. – Tyler, W.J. – Irwin, M.R.(1960): Blood types of the progeny of a pair of cattle twins showing erythrocyte mosaicism. J. Hered., 51. 136–140.p.

Stranzinger, G. – Dolf, G. – Fries, R. – Stocker, H.(1981): Some rare cases of chimerism in twin cattle and their proposed use in determining germinal cell migration. J. Hered., 72. 360–362.p.

Teplitz, R.L. – Moon, Y.S. – Basrur, P.K.(1967): Further studies of chimerism in heterosexual cattle twins. Chromosoma, 22. 202–209.p.

Authors' address: *Słota E. – Rejduch B. – Kozubska-Sobocinska A. – Danielak-Czech B.:*
National Research Institute of Animal Production,
Department of Immuno and Cytogenetics,
32-083 Balice/Kraków, Poland
Kwaczynska A.: Experimental Station of Animal Production
64-122 Pawłowice, Poland

SEX CHROMOSOME ANALYSIS AS A TOOL IN REPRODUCTIVE STUDIES IN MARES

MÄKINEN, A. — KATILA, T. — MÄKILÄ, M.

The number, structure and function of sex chromosomes of mares influence their reproductive success. Sex determination in mammals is primarily dependent on the presence or absence of the Y chromosome.

When one of the two X chromosomes in females is inactivated some of the genes in the inactivated X chromosome still remain active. Cases of X chromosome inactivation gives some evidence why X chromosome aneuploidy is the most common numerical chromosome alteration in mammals.

Two mares selected for cytogenetical study because of their reproductive problems. The sex chromosomes of these mares were identified using their GTG and CBG staining properties.

A 5-year-old Standardbred trotter mare had an exceptional XY sex chromosome complement in all its lymphocytes.

She had exhibited strong oestrus behaviour but her ovaries were very small (length<2cm) and devoid of follicles. Her cervical opening and external genitalia were normal. Her serum progesterone levels were always<1 nmol/1 over a period of 30 days. An endometrial biopsy specimen revealed that the majority of her glands were small and atrophic, however some other glands were cystically distended and surrounded by a slight periglandular fibrosis.

The phenotypical appearance of the mare was normal, but she was very thin and had painful hocks at the beginning of the first breeding season after her retirement following a very successful racing career.

In this XY sex reversal male the sex determination function of genes on Y chromosome had been altered producing a female like phenotype and underdeveloped female reproduction organs.

All the lymphocytes studied from another 5-year-old riding mare had an XXX sex chromosome constitution.

The mare had very small ovaries: 2x0.5x0.5 cm and 1x0.5x0.5 cm. She had a history of having shown oestrus behaviour. She was phenotypically normal and had normal external genitalia. In this 2n=65,XXX mare the genes in the additional X chromosome, which had escaped the inactivation process interfered with the normal development of the female reproductive organs.

Authors' address: *Mäkinen, A.*: University of Helsinki, Department of Animal Science
P.O.B. 28. 00014, Finland
Katila, T.: University of Helsinki, Department of Clinical Veterinary Sciences, Animal Reproduction, 04290 Saari, Finland
Mäkilä, M.: Veterinary Practice, 37 470 Vesilahti, Finland

DECREASE OF PERCENTAGE OF CARRIERS OF BOVINE HEREDITARY CHROMOSOME ABNORMALITIES

HÁZAS, G. — KOVÁCS, A. — KARAKAS, P.

SUMMARY

The first cases of 1;29 and 14;21 translocations in A.I. bulls were diagnosed in our lab in 1975. The 1;29 was found in a high number of cattle belonging to five breeds (Simmental, Swedish Red and White, Hungarian Grey, Blonde d'Aquitaine, Charolais), the 14;21 in a single Simmental buli and among its offspring. Until 1987 all of the A.I. bulls were State-owned and investigated, carriers were culled together with their stored semen. The programme is continued at the Hungarian National A.I. Corporation, but the order was eluded by some minor private owners. Two (a homo- and a heterozygous) 1;29 carrier Blonde d'Aquitaine bulls were imported and their semen was used, beef bulls in natural service are mostly not investigated. As a result of the programme, the Simmental and Hungarian Grey breeds are considered to be practically free of hereditary chromosome abnormalities, the 1;29 translocation occurs sporadically among beef cattle.

INTRODUCTION

Reduced fertility of heterozygote carriers of the 1;29 translocation was described by *Gustavsson* (1969, 1979), *Cossec* (1973 cit. *Foulley at al.* 1985), *Popescu* (1974), *Queinnec et al.* (1974). The eradication programme was iniciated in Sweden followed by other countries.

MATERIAL AND METHODS

We started with chromosome investigation from cattle blood in 1972. The first carrier Simmental heifer was found by *Sellyei et al.* (1974), the first buli by us in 1975; followed by others belonging to the Simmental, Hungarian Grey, Blonde d'Aquitaine and Charolais breeds. In the Swedish Red and White breed the 1;29 translocation was found in imported cows, while another centric fusion described as 14;21 was diagnosed in a single Simmental bull and among its offspring. Our eradication programme started in 1975 by investigating A.I., and candidate bulls and culling the carriers of hereditary abnormalities.

RESULTS

Changes of the number and proportion of 1;29 translocation carriers belonging to four breeds are demonstrated in *Table 1*. In the first periods old A.I. bulls, later younger bulls and candidates were investigated. The reduction of the total numbers is caused by the growing proportion of Holstein-Friesians not included in the table, as we found no hereditary chromosome abnormalities in this breed. The excess of carrier frequency in the periode 1985–87 in the Simmental breed was caused by nine young candidate bulls bom in a farm, where the cytogenetic results of dams were kept as our secret in the interest of the lifetime evaluations. In the Hungarian Grey breed all of the carriers were descendats of a single Maremma bull imported in 1971. Natural service is dominating in this breed, but semen is taken for storage and limited use. The three Hungarian Grey carriers found in the last periode were detected as young candidates.

DISCUSSION

Until 1987 all of the A.I. bulls were State-owned and investigated, carriers were culled together with their stored semen. The programme is continued at the Hungarian National A.I. Corporation and its results can be demonstrated by the reduction of the percentage of carriers, but the order was eluded by some minor private owners. Two (a homo- and a heterozygous) 1;29 carrier Blonde d'Aquitaine bulls

were imported and their semen was used, beef bulls in natural service are mostly not investigated. As a result of the programme, the Simmental and Hungarian Grey breeds are considered to be practically free of hereditary chromosome abnormalities, the 1;29 translocation occurs sporadically among beef cattle.

Table 1.

Changes of the frequency of 1;29 translocation carrier bulls (all/carriers)

	1972–79	1980–84	1985–87	1988–97
Simmental	395/13 (3.29%)	161/3 (1.86%)	119/9** (7.56%)	70/0 (0.00%)
Hungarian Grey*	25/2 (8.00%)	52/5 (9.62%)	42/1 (2.38%)	101/3 (2.97%)
Charolais	—	27/0 (0.00%)	15/1 (6.67%)	8/0 (0.00%)
Blonde d'Aquitaine	—	—	4/2 (50.00%)	7/0 (0.00%)

* All the carriers were descendants of the same Maremma buli imported from Italy in 1971
** All the 9 candidates were bom from carrier dams on the same State Farm

REFERENCES

Foulley, J.L. – Frebling, J.– Chambeyron, J.J.(1985): La translocation 1-29 chez les bovins: distribution, effects, procédure d'éradication. Bull. Tech. C.R.Z.V. Theix, I.N.R.A., 62. 93–102.p.

Gustavsson, I.(1969): Cytogenetics, distribution and phenotypic effects of a translocation in Swedish cattle. Hereditas, 63. 68–169.p.

Gustavsson, I.(1979): Distribution and effects of the 1/29 translocation in cattle. J. Dairy Sci., 62. 825–835.p.

Popescu, C.P.(1974): Étude cytogénétique du reproducteur. Rapport d'activité des services techniques année. (U.N.C.E.I.A.), 111–122.p.

Queinnec, G. – Darré, R. – Berland, H.M. – Raynaud, J.C.(1974): Étude de la translocation 1-29 dans la population bovine du Sud-Ouest de la France: consequences zootechniques. 1st World Congr. Genet. Appl. Livestock Prod., Madrid, 3. Symposia, 131–151.p.

Sellyei, M.(1974): Personal communicatior

Authors' address: Research Institute for Animal Breeding
H-2053 Herceghalom

APHIDICOLIN-INDUCED FRAGILE SITES IN *BOS TAURUS* LYMPHOCYTE CULTURES (A PRELIMINARY STUDY)

LLAMBÍ, S. — GUEVARA, K. — RINCÓN, G. —
NUNEZ, R. — ARRUGA, M.V. — POSTIGLIONI, A.

SUMMARY

Aphidicolin is a specific inhibitor of eukaryotic DNA polymerase α which is primarily associated with chromosome DNA replication. Cytogenetic studies in Holstein-Friesian breed, have determined different expression of spontaneous fragile sites Xq 3.1 (FRA Xq 3.1). Cytogenetic studies were carried out in two Holstein-Friesian cows with normal karyotypes (2n=60,XX) and two Uruguayan Creole cows with normal and 59/rob(1;29) karyotypes each. We studied different concentrations of aphidicolin. The best concentrations to induced fragile sitesin cattle (X chromosomes, autosomes and t1;29), ranged to 0.24–0.3 μM. This is the first preliminary report of induction of aphidicolin common fragile sites in chromosomes cattle.

INTRODUCTION

Aphidicolin (APC) is a specific inhibitor of eukaryotic DNA polymerase α which is primarily associated with chromosome DNA replication. It was found to induce chromosomal gaps and breaks in a highly nonrandom manner in cultured lymphocytes (*Shutherland and Hecht,* 1985). The distribution and frequency of APC-induced common fragile sites (cFS) may vary among different cell types and the prevalence of them was dependent of transformed human cells (ovarian carcinoma, neuroblastomas, etc., *Glover et al.,* 1984; *Tedeschi et al.,* 1992).

Total and site-specific damage was dose dependent and greatly increased when folic acid was removed from the medium. Concentrations that not necessarily affect either chromosome structure nor mitotic index, have been optimizated in human lymphocyte cultures RPMI 1640, without folic acid, suggesting a final concentration in the medium no greater than 0.2% (*Glover et al,* 1984). Through the mechanism of DNA polymerase inhibition, sites most sensitive to aphidicolin, damage detection of hot spots on human chromosomes (*Shutherland and Hecht,* 1985).

Cytogenetic studies in Holstein-Friesian breed, determined different expression of spontaneous fragile sites Xq 3.1 (FRA Xq 3.1) from culture conditions, finding an enhanced expression in RPMI 1640 (1.8%) from TC199 (0.9%) (*Rincón et al,* 1997). Similar spontaneous frequency expression of FRA Xq 3.1, in different bovine breed blood samples (Holstein-Friesian and Creole catlle) was also reported (Llambí and Postiglioni, 1994; *Llambí and Postiglioni.,* 1997; *Postiglioni et al.,* 1997).

Since the molecular mechanisms underlying fragile site expression are still unknown, this study was aimed to primarily find an optimal concentration and time of induction of the APC-inducible type, to advance in the possible causes of expression of the fragile site in the cattle X chromosome and a better knowledge of the bovine genome .

So, in this preliminary study the results obtained with different concentration of aphidicolin-induced fragile sites in lymphocyte RPMI 1640 with folic acid concentration of 1×10^{-3} mg/mL of two bovine-breeds (Holstein-Friesian and Uruguayan Creole cattle) have been presented.

MATERIAL AND METHODS

Cytogenetic studies were carried out in two Holstein-Friesian cows with normal karyotypes (2n=60,XX) and two Uruguayan Creole cows with normal and 59/rob(1;29) karyotypes each.

Lymphocytes were cultured in RPMI 1640 (Sigma Co) completed medium, supplemented with 10 percent fetal bovine serum (Gibco Co), additionally supplemented with L-glutamine (200 mM), phytohaemaglutinin (0.2 μg/mL) and penicillin (100 IU/mL), streptomycin (100 μg/mL), fungizone (2.5 μg/mL). Aphidicolin was prepared from a initial solution (30 μM), testing the following curves of induction: a) 0.24 μM; b) 0.3 μM; c) 0.36 μM; d) 0.5 μM to final concentrations (*Riggs et al,* 1993).

It was added to culture, 24 h prior to harvest. Finally, colchicine (0.004 mg/mM) was added 2 h before culture harvesting. Cells were incubated for 72 h at 38 °C. Four animals control cultures without aphidicolin were tested.

We have stablished 3 principal groups to better fragile sites evaluation: A) expresion of fragile sites in X chromosomes, B) expresion of fragile sites in rob. 1;29, C) expresion of fragile sites in autosomes. Chromosome image treatments have been obtained by Zeiss laser scan microscope and Adobe photoDeluxe programme (HJW101B7100259-364).

RESULTS

The results demostrated that concentrations of APC-induced type between 0.24–0.3 μM was the best to induced fragile sites, as high aphidicolin concentration (more than 0.36 μM) desintegrated chromosomes material. It was observed specially on one or the two X chromosomes. Over concentration of 0.36 μM we scored 36/63 metaphases without X-chromosome. This results was supported to those finding in human induced lymphocytes cultures where aphidicolin concentrations above 0.4 μM in folic acid medium induced so many breaks and gaps that cells could not be accurately scored. *Table 1.*, shows the relationship among the two samples respect to the X fragile sites (FRA X).

Table 1.

X chromosome APC fragility induction in two cattle breeds

Breed	APC Concentration	Number of methaphases	X fragility (%)
Creole Cattle	0.3 μM	149	50 (33.55)
Holstein-Friesian	0.3 μM	98	30 (30.60)

Robertsonian translocation 1;29 showed 13.3% of fragile sites with 0.24 μM and 30.2% with 0.3 μM, while higher concentrations not permit to distinguish this submetacentric chromosome. Autosome fragile sites showed 39.8% from 0.2 μM, 56.1 in 0.3 μM and 63.5% with 0.36 μM. The increasing of fragile site positively correlated with a higher concentration of aphidicolin is showed in the *Table* 2. Furthermore, the cytomorphological expression of common aphidicolin fragile sites in these breeds, evidenced a prevalence of chromosomal and chromatid breaks (*Fig. 1)*.

Table 2.

Cytogenetic results with different concentrations of APC in two cattle breeds

Animal sample	APC Concentration	Number of methaphases	X fragility (%)	T1;29 fragility (%)	Autosome fragility (%)
Creole Cattle A 2n=59,XXt1;29	Culture control without APC	100	7 (7.0)	2 (2)	10 (10.0)
	0.24 μM	98	17 (17.4)	13 (13.3)	39 (39.8)
	0.3 μM	139	43 (30.9)	25 (18.0)	78 (56.1)
	0.36 μM	63	49 (77.8)	19 (30.2)	40 (63.5)
Creole Cattle B 2n=60,XX	Culture control without APC	100	2 (2.0)	—	6 (6.0)
	0.24 μM	37	20 (54.1)	—	25 (67.6)
	0.3 μM	10	7 (70.0)	—	6 (60.0)
Holstein-Friesian female 307 2n=60,XX	Culture control without APC	28	0 (0.0)	—	0 (0.0)
	0.3 μM	51	6 (11.8)	—	34 (66.7)
	0.5 μM	78	17 (21.8)	—	30 (38.5)
Holstein-Friesian female 349 2n=60,XX	Culture control without APC	60	0 (0.0)	—	1 (1.7)
	0.3 μM	47	24 (51.1)	—	40 (85.1)
	0.5 μM	27	24 (88.9)	—	26 (96.3)

Fig. 1.: **Different types of aphidicolin induced chromosomes alterations**

a: arrows indicate fragile sites in autosomesl and X chromosomes of Holstein-Friesian breed;
b: arrow indicates a telomeric fragile site in the X chromosome of Uruguayan Creole Cattle;
c: arrow indicates fragile site in t1;29 and autosomal of Uruguayan Creole Cattle;
d: arrows indicate double fragile sites in the X chromosome of Holstein-Friesian breed

DISCUSSION

Induced fragile sites have been reported in domestic animals (*DiBerardino et al.*, 1983; *Lopez-Corrales and Arruga*, 1996). Common aphidicolin fragile sites induced have been described in pig using from 0,05 to 0, 35 μM concentrations of Aphidicolin (*Riggs et al.*, 1993).

In our cultures when we used concentration of more than 0,3 μM of Aphidicolin, we obtained chromosome fragility with aggressive damage on chromosome structure. We identified fragmentation on one of the X chromosomes, on autosomes and on the 1;29 translocation chromosome in carrier animal. *DiBerardino et al.* (1983), obtained cattle fragile induction map with BrdU at different concentrations and they identified four fragile sites on X chromosome.

Our experimental results, we observed gradual fragmentation in almost one of the X chromosomes with concentrations upper of 0.3 μM of APC. Five different common fragile sites have been identified on both arms of the X chromosome (Xp and Xq) . The existence of fragile sites may provide a better understanding of how environmental pressures result in genetics damage (*Sutherland and Hecht*, 1985; *Riggs et al.*, 1993). However our results (33.5% of FRA X with 0.3 μM of APC) in the native rustic breed (Uruguayan Creole Cattle) and amount of 30.6% of FRA X in Holstein-Friesian breed highly selected for milk production, do not evidence significant differences between the two breeds submitted to different selection pressure.

In conclusion aphidicolin induces common fragile sites in catlle. We studied different concentrations of aphidicolin (0.24, 0.3, 0.36, 0.5 μM) and all of them have been able to induce fragile sites in catlle (X chromosomes, autosomes and t1;29). Concentrations upper than 0.3 μM produce agresive damage on chromosome structure.

This is the first preliminary report of induction of aphidicolin common fragile sites in the genome of the domestic cattle.

ACKNOWLEDGMENTS

We wish to thank laboratory technician, Mrs. Iris Hernandez. This work was supported by grants of the AGF97-1072, Comisión Interministerial de Ciencia y Tecnología (CICYT), Espa a and CIDEC. Universidad de la República Oriental del Uruguay.

REFERENCES

Di Berardino, D. – Iannuzzi, L. – Di Meo, G.P.(1983): Localization of BrdU induced break sites in bovine chromosomes. Caryologia, 36. 3. 285–292.p.

Glover, T.W. – Berger, C. – Coyle, J. – Echo, B.(1984): DNA polymerase α inhibition by aphidicolin induces gaps and breaks at common fragile sites in human chromosomes. Hum. Genet., 67. 136–142.p.

Llambí, S. – Postiglioni, A.(1994): Localization of the fragile X chromosome break points in Holstein-Friesian cattle. (*Bos taurus*). Theriogenology, 42. 5. 789–794.p.

Llambí,S. – Postiglioni, A.(1997): Frequencies and cytomorphological manifestation of sexual X-chromosome fragility (FRA Xq3.1) in Holstein Friesian cattle. Archiv. Zootec., 45. 203–208.p.

Lopez-Corrales, N.L. – Arruga, M.V.(1996): Induction of chromosomal fragile sites in goats: a preliminary study. Genet. Sel. Evol., 28. 129–139.p.

Postiglioni, A. – Llambí, S. – Gagliardi, R. – de Bethencourt, M.(1996): Genetic characterization of a sample of Uruguayan Creole cattle. I. Cytogenetic characterization of a sample of Uruguayan Creole cattle. Archiv. Zootec., 45. 70–71. 209–213.p.

Riggs, P.K. – Kuczek; T. – Christman; C.L. – Bidwell, C.A.(1993): Analysis of aphidicolin-induced chromosome fragility in the domestic pig (*Sus scrofa*). Cytogenet. Cell Genet., 62. 110–116.p.

Rincón, G. – Llambí; S. – Postiglioni, A.(1997): Expression of X chromosome fragility in Holstein-Friesian cattle: a preliminary study. Genet. Sel. Evol, 29. 395–401. p.

Sutherland, G. – Hecht, F.(1985): Fragile sites in human chromosomes. New York: Oxford University Press, 13.p.

Tedeschi, R. – Vernole, P. – Sanna, M. – Nicoletti, B.(1992): Population cytogenetics of aphidicolin-induced fragile sites. Hum. Genet., 89. 5. 543–547.p.

Authors' address: *Llambi, S. – Guevara, K. – Rincón, G. – Nuñez, R. – Postiglioni, A.*: Laboratory of Cytogenetics Domestics Animals. Area Genetics. Faculty of Veterinary. A. Lasplaces 1550-CP 11600 Montevideo, Uruguay

Arruga, M.V.: Laboratory of Cytogenetics and Molecular Genetics. Faculty of Veterinary. Miguel Servet, 177-50013 Zaragoza, Spain

X TRISOMY IN THE DOG — A CASE REPORT

SWITONSKI, M. — PIENKOWSKA, A. — GOLINSKI, P. — BUKOWSKA, D. — BERESZYNSKI, A.

A five years old crossbred female dog was subjected to cytogenetic investigation due to infertility. The bitch had a normal phenotype, and external reproductive organs appeared to be normal as well. It is known from the owners information that she has never been pregnant, in spite of frequent matings.

Cytogenetic evaluation was carried out with the use of GTG, CBG and Ag-I banding techniques. It was found that in all studied metaphase spreads, an extra X chromosome was present. The karyotype was designated 79, XXX. To our knowledge, it is. the first case of X chromosome trisomy diagnosed in the dog.

Authors' address: Agricultural University of Poznan
60-637 Poznan, Poland

THE EVENT OF MEIOTIC SYNAPSIS IN FEMALE RABBITS

KOVÁCS, B.ZS. — SWITONSKI, M. — WENT, D. — STRANZINGER, G.

SUMMARY

In female mammals, germ cells develop at different stages during fetal life. In rabbits the meiotic synapsis occurs postnatal.

An electron microscopic study was made on developmental stages of the synaptonemal complexes (SC) in oocytes of these animals.

In the presented investigations the different stages of early ovarian differentiation are demonstrated.

The analysis showed that the activity of the meiotic cell division is at its maximum between 10 and 13 days postnatal. After 20 days, the oocytes get into the diplotene stage of the meiosis. These observations are important for the selection of material for SC studies in female rabbits being used as model species. The great differences in the mammalian meiotic developments are discussed.

INTRODUCTION

Female rabbits belong to the species, where meiotic synapsis occurs postnatal. This means, that their gonads are unripe at the time of birth. Oogonies, follicles, but no oocytes can be detected at birth.

In the presented investigation different stages of early ovarian differentiation were demonstrated. Cells at different stages of meiosis were viewed under the light microscope and synaptonemal complexes (SC) were then observed with the electron microscope. Synaptonemal complexes are proteinaceous structures which appear along the homolgous chromosomes in meiotic zygotene and last until the end of pachytene substage of meiotic phophase I (*Switonski et al.*, 1997).

The aim of this study was to determine the day when maximum number of the cells in the stages of meiosis were present after birth in the female rabbits.

MATERIAL AND METHODS

Two mature female rabbits (New Zealand White) were inseminated with semen from a normal buck. After the period of pregnancy, 19 offspring were born, of which 9 were females and 10 males. Only the females were used for the following study. The ovaries of 4-day-old to 24-day-old rabbits were collected.

A well established technique for the preparation of SC was used. This method was based on procedures developed by *Counce et al.* (1973) and adapted by *Switonski et al.* (1987). The oocytes, stained with phosphotungstic acid hydrate (PTA), were observed under a light microscope. Having placed the oocytes on 150 mesh copper grids, they were viewed with the Philips EM 301 (Philips Electron Optics HD-5600) at varying magnifications (1,900 X to 15,000 X). Good quality samples, cells with apparent SC, were photographed with the integrated camera.

RESULTS

Using phasecontrast, slides were first screened for prophases under the light microscope. *Fig. 1.* shows cells of 10-day-old rabbit ovaries in interphase and in meiosis. In 24-day-old animals there does not appear to be any more cell division taking piace in the ovaries (*Fig. 2.*).

In addition an electron microscope study was made on the development stages of the SC in oocytes of these animals.

This study can be an infoimative approach which allows the general observation of the pairing behaviour of chromosomes and in particular if chromosome aberrations are present. Different amounts of cells were observed in the ovaries of each animal. The exact numbers of cells in prophase in comparison to the total number of cells present were determined. These data were given for each animal to the different age categories (*Table 1.*).

Fig. 1.: **Division phase in 10-day-old rabbit ovaries**

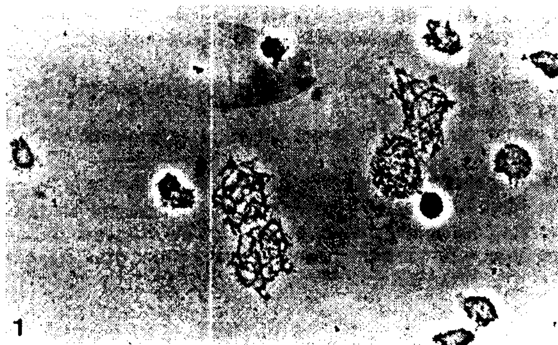
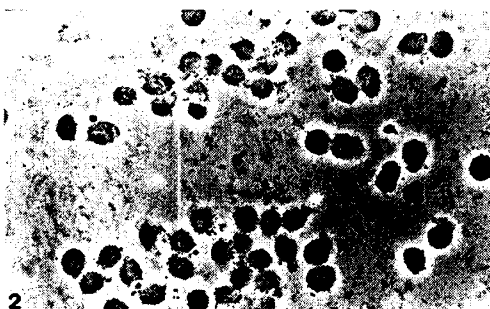


Fig.2.: **Division phase in 24-day-old rabbit ovaries**



The activity of the meiotic cell division was at its maximum between 10 and 13 days postnatal, with approximately 1/3 of all present cells being in prophase. *Gottschewski et al.* (1973) reported that the maximum of the activity is on the 12th day after birth. Between day 13 and 15 there is a rapid decrease of cells in division. After 20 days, most oocytes are in the diplotene stage of meiosis (*Fig. 3.*).

Table 1.

Proportion of cells in prophase to cells in interphase at different ages of animals

Age of animal (days)	4	7	8	10	13	15	20	22	24
Cells in pro-phase/total number of cells	32/345	40/281	68/372	98/330	143/439	50/656	15/265	9/374	5/453

As an example, some selected photographs of different development stages of cells gone through meiosis presented (*Fig. 4.*).

These observations are important for the selection of material for SC studies in female rabbits being used as model species for meiotic studies.

The pairing behaviour of the chromosomes was normal and no abberations were found.

Fig. 3.: **Cells in prophase / total number of cells (%) to age of animals (days)**

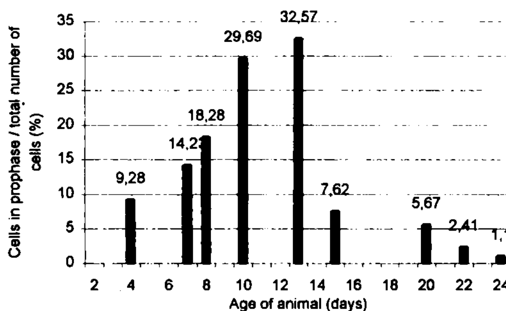
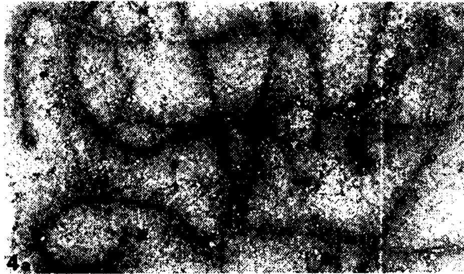
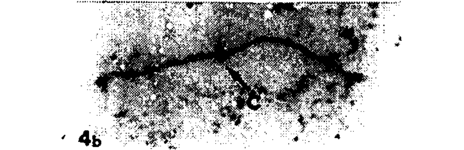


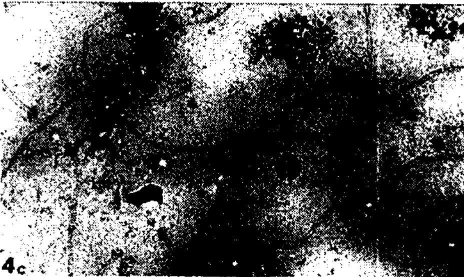
Fig. 4.: **Selected pictures at different development stages**



a) Zygothene-pachytene stage in a cell from a 8 day-old rabbit. Magnification: 1,900 X



b) A metacentric bivalent in a cell from a 10-day-old rabbit Magnification: 3,400 X C indicates the centromere position



c) Pachytene stage in a cell from a 10-day-old rabbit Magnification: 4,500 X

REFERENCES

Counce, S.J. – Meyer, G.F. (1973): Differentation of the synaptonemal complex and the kinetochore in Locusta spermatocytes studied by whole mount electron microskopy. Chromosoma, 44. 231–253.p.

Gottschewski, G.H.M. – Zimmermann, W.(1973): Die Embryonalentwicklung des Hauskaninchens. Normogenese und Teratogenese. Verlag M. & Schapper. Hannover, 5–39.p.

Switonski, M. – Gustavsson, I. – Ploen, L.(1987): The nature of the 1;29 translocation in cattle as revealed by synaptonemal complex analysis using electron microscopy. Cytogenet. Cell Genet., 44. 103–111.p.

Switonski, M. – Stranzinger, G.(1997): Studies of synaptonemal complexes in farm mammals-a review. J. Heredity. (in press)

Authors' address: *Kovács, B.Zs. – Went, D. – Stranzinger, G.:* Department of Animal Science, Swiss Federal Institute of Technology, Zürich, Switzerland
Switonski, M.: Department of Genetics and Animal Breeding, Agricultural University of Poznan, Poznan, Poland

INCIDENCE OF DISOMY IN MINK SPERM BY STRONG CENTROMERE REPEAT PROBES FOR THE CHROMOSOME 2, 5, 8, 9, 11 AND Y

CHRISTENSEN, K. — BRUUSGAARD, K.

SUMMARY

Strong repeat probes have been developed for 6 mink chromosomes. They can be utilized to estimate the rate of disomy in sperm cells by means of in flourescent *in situ* hybridization (FISH). This can initially give an estimate of the non disjunction rate in normal males and it can be developed for studying animals with fertility problems. The disomy rate has preliminary been estimated to 0.51, 0.54 and 0.27 per cent for chromosome 8, 9 and 11, respectively.

INTRODUCTION

The America mink (*Mustela vison*) belongs to the order of Carnivora, and the economic aspects of this animal is indicated by the extensive mink fur production in the Scandinavian countries, USA, Canada and Russia. The gene map for the American mink (*Serov and Pack*, 1993) is not yet well developed compared to other species of domestic animals. However ZOO-FISH mapping for the mink has been carried out (*Hameister et al.*, 1997), showing that 34 human chromosome segments cover the entire mink genome and have syntenic relationship with human chromosomes.

In human there have been several reports on the frequency of disomy on a number of individual chromosomes. The frequencies of disomy for normal males has been estimated to 0.2–0.3 per cent for each chromosome (*Pellestor et al.*, 1996). Disomy frequencies has been used to the estimate effects of environmental hazards or diseases on the production of abnormal sperms in human males (*Monteil et al.*, 1997; and *Robbins et al.*, 1997).

The present report deals with mink cosmids with repeats which showed strong FISH signal on interphase cells. The cosmids are described by *Christensen et al.* (1997), and chromosome specific cosmids were available for the mink chromosomes No 8, 9, 11 and Y. Another cosmid containing nuclear organizer sequences hybridize to chromosome 2 and 5. The five probes has been used individually on sperms from normal males mink to evaluate the frequency of disomy.

MATERIAL AND METHODS

The animals: The mink were from a normal production line of the Wild mink or Pastel colour type. They were kept under normal farming condition and sperm were collected from the epididymus at the end of the mating season at the 24 Marts and at the 1st April after pelting.

Sperm preparation: After collection the semen was washed 3 times in PBS and finally stored in 70% ethanol at 5 °C. The sperm were dropped on a wet slide using the fixative (methanol-acetic acid 3:1) and washed with two drops of fixative-put on the top of the tilted slide, the fixative were sucked of at the edges and the rest was air dried. The slides were kept for one week at room temperature. Finally the slides were treated with dithiotreitol (DTT) (200 mM KOH, 50 mM DTT) under a cover slip, puliing the cover slip over the slide to give a varying exposure time of 5–2 min. The cover slips were washed of with distill water and the slide were washed in the neutralizing buffer (900mM Tris-HCl, pH 8.3, 300mM KCl, 200 mM HCl) and finally washed with distil water and air dried.

DNA probes: Five probes, AG25 (chromosome Y), AG32 (chromosome 11), AG34 (chromosome 2 and 5), AG63 (chromosome 9) and AG64 (chromosome 8) were double labelled with nick translation with biotin-14-dCTP and biotin-14-dATP.

FISH: Denaturation of the DNA in the sperms were done with 70% formamide/2xSSC, pH 7.0, at 65 °C for 2 min and thereafter washed in an ethanol series on ice 70% 90% 100% each for 2 min. There was used one µg biotinylated probe in 30 µl of hybridization solution containing 45% formamide, 2 x SSC, 10% dextran sulfate. The hybridization mix was supplemented with 6 µg sheared genomic mink DNA; denaturation at 70 °C for 5 min and incubated for 20 min. at 37 °C. *In situ*

hybridization was carried out by incubating the slides with denatured sperms and hybridization mix at 42 °C for 20 hours. After hybridization, the slides were washed two times in 45% formamide, and three times in 2 x SSC at 39-42 °C. Visualization of the biotinylated probe was achieved with fluorescein isothiocyanate (FITC) conjugated to avidin, and the signal was amplified with one layer of biotinylated anti-avidin antibody (Vector Laboratories). For more details see *Thomsen et al.* (1996).

Microscopic observations: Only well delineated sperm cells were counted and double spots were scored when an extra signal spot occurred being of the same size as the normal ones, and the two spots separated by at least one spot diameter.

RESULTS AND DISCUSSION

In *Fig. 1.* can be seen sperm cells with the strong hybridization signal, the probe for chromosome 8 gives very well demarked spots. More details are given by *Christensen* (1997).

Fig. 1.: **Hybridization signals of the cosmids AG64 (chromosome 8) in mink sperm cells**

In *Table 1.* are given the disomy counting results for two animals with a probe for chromosome 8, 9 and 11. The disomy rates for the three chromosomes are estimated to 0.51, 0.54 and 0.27 per cent, respectively.

Until now very few strong centromere repeat probes has been developed for the domestic animal species covering single chromosomes. For the domestic pig there exist a chromosome specific strong repeat probe for chromosome 1 (*Jantsch et al.*, 1990) and the Y chromosome (*McGraw et al.*, 1988), which have been used for estimating the frequency of Y bearing sperms (*Kawarasaki et al.*, 1996). In human there has been developed-satellite centromere probes for almost every chromosome and they have been used to estimate the rate of disomy in both normal and abnormal human males.

Table 1.

Preliminary counts of disomy in mink sperm cells for chromosome 8 and 9 and 11

Animal	Probe	Normal	Disomic	No signal
9236	AG64 (chr 8)	2340	12	58
9238	AG63 (chr 9)	2040	11	93
9238	AG32 (chr 11)	1127	3	55

There have been some difficulties with the evaluation of disomy. The probe for chromosome 8 gives very distinct spots as can be seen in Figure 1 whereas the probes for 9 and 11 give more long and narrow spots which might be interrupted with no signal. Until now we were not able to set the conditions for counting the signal from the nuclear organizer probe, chromosome 2 and 5, as the two chromosomes often lie close together and the signal fuse into one. The use of the Y chromosome probe also needs refinements before data can be given.

The frequency of disomy per chromosome in humans range in the neighbourhood of 0.2 per cent In this study in the mink the frequency are around the double. To study the frequency of diploid sperms it is necessary to use two probes with multicolour FISH. In this study only one probe has been used per experiment, so it has not been possible to separate disomic and diploid sperms which might overestimate the rate of disomy. With these reservations the results for the mink here given should be taken as preliminary, more cells will be counted and the technique refined. The technique is worth while to develop for all other domestic animals species. It should be a mean to understand and thereby improve aspects of fertility.

ACKNOWLEDGEMENT

Thank to Inger H. Christensen for excellent technical assistance. The work was supported by the Ministry of Agriculture.

REFERENCES

Christensen, K.(1997): http://www.husdyr.kvl.dk/htm/kc/mink/sperma/sperma.htm.

Christensen, K. – Brusgaard, K. – Malchenko, S. – Lohi, O. – Serov, O.(1997): Arch. Zootec, 45. 259-265.p.

Hameister, H. – Klett, C. – Bruch, J. – Vogel, W. – Christensen, K.(1997): Chromosome Research, 5. 5-11.p.

Jantsch, M. – Hamilton, B. – Mayr, B. – Schweizer, D.(1990): Chromosoma, (Berl) 99. 330-335.p.

Kawarasaki, T. – Sone, M. – Yoshida, M. – Bamba, K.(1996): Mol. Reprod. Dev., 43. 548-553.p.

McGraw, R.A. – Jacobson, R.J. – Akamatsu, M.(1988): Nucl. Acids Res., 16. 10389.p.

Monteil, M. – Rousseaux, S. – Chervet, E. – Pelletier, R. – Cozzi, J. – Sele, B.(1997): Cytogenet. Cell Genet,. 76. 134-138.p.

Pellestor, F. – Girardet, A. – Coignet, L. – Andreo, B. – Charlieu J.P.(1996): Am. J. Hum. Genet., 58. 797-802.p.

Robbins, W.A. – Meistrich, M.L. – Moore, D. – Hagemeister, F. – Weier, H.W – Cassel, M.J. – Wilson, G. – Eskenazi, B. – Wyrobek, A.J.(1997): Nature Genet., 16. 74-78.p.

Serov, O.L. – Pack S.D.(1993): In Genetic Maps, ed. SJ O'Brian, 279-281.p.

Thomsen, P.D. – H yheim, B. – Christensen, K.(1996): Cytogenet. Cell Genet, .73. 203-208.p.

Authors' address: *Christensen, K.*: Division of Animal Genetics, Department of Animal Science and Animal Health, The Royal Veterinary and Agricultural University Bülowsvej 13, DK-1870 Frederiksberg C, Denmark

Bruusgaard, K.: Department of Biochemical and Molecular Genetics, Odense University Hospital, DK-5000 Sdr. Bouleward 29, Odense C, Denmark

SIMULTANEOUS DETECTION OF X- AND Y-BEARING BULL SPERMATOZOA BY DOUBLE COLOUR FLUORESCENCE *IN SITU* HYBRIDIZATION

HASSANANE, M. — KOVÁCS, A. — LAURENT, P. — LINDBLAD, K. — GUSTAVSSON, I.

Double colour fluorescence *in situ* hybridization of the sex chromosomes was applied on spermatozoa of five Swedish Holstein-Friesian bulls. It was demonstrated that cosmids with strong fluorescence signals and scraped chromosomes can successfully be used as markers in this type of studies.

X and Y segregated as expected according to a 1:1 ratio, and there were no interindividual variations. Disomic spermatozoa occurred with a frequency of more than 0.1% (0.067% XX, 0.029% YY and 0.029% XY), which is considerably lower than in man. Diploid spermatozoa occurred with a frequency of 0.5%.

Authors' address: *Hassanane, M. – Lindblad, K. – Gustavsson, I.:* Centre of Reproduction, Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, Uppsala, Sweden
Laurent P.: Laboratoire de Génétique Biochimique et Cytogénétique, INRA, Centre de Recherche de Jouy en Josas, Cedex, France
Hassanane, M.: Unit of Cell Biology, National Research Center Dokki, Giza, Egypt
Kovács, A.: Research Institute for Animal Breeding and Nutrition H-2053 Herceghalom, Hungary

FIRST RESULTS OF SEXING BOVINE SPERMATOZOA BY FISH IN HUNGARY

TARDY, E.P. — SZALAI, G. — GUSTAVSSON, I. — HASSANANE, M. —
LINDBLAD, K. — KOVÁCS, A. — HÁZAS, G. — TÓTH, A. — DOHY, J.

Objective: The aim of the proceeding study was to set up a quick and cheap protocol to define the sex of bovine spermatozoa. Sexing gametes and embrios of domestic animals is an issuc of great interest, becausc breeding centres can influence the sex ratio as required by appling simple and reliable molecular genetic techniques. Our preliminary results are summarised here.

Study design: Fluorescence *in situ* hybridization (FISH) with bovine Y specific probe was performed on metaphase chromosomes from peripheral lymphocyte cultures, and on smears of thawed and decondensed semen of *Bos taurus*. Sex ration and disomic frequency were evaluated. Simultaneously, experiments are in progress to prepare X and Y specific probes by scraping sex chromosomes from metaphase spreads.

METHODS

Metaphase chromosome spreads were prepared according to standard cytogenetic techniques. Semen samples were treated with papain-dithiothreitol solution in order to get maximum probe penetration. Chromatin decondensation was optimised to have observable signal intensity.

FISH with biotin-labelled Y-probe (a kind gift from *Dr. C. Jörgensen*) was performed as described by *Pinkel et al.* (1988) with slight modifications. Hybridized probe-target helix was detected with FITC-avidin.

Concurrently, X and Y specific probes are being prepared as follows: chromosome fragments were scraped from metaphase spreads. DNA was treated topoisomerase I. to serve as template for random PCR amlification. 400–600 bp framents were cloned into E. coli. After overnight incubation bovin DNA was cut out from the isolated vector, and labelled with biotin-dUTP.

RESULTS

Hybridization efficiency and signal intensity of the probes were evaluated on metaphase chromosomes and interphase nuclei. Sex ration and disomic frequency for chromosome Y were calculated upon examining 1440 spermatozoa. 49.58% of the spermatozoa bore no signals (potential X-bearing cell), while 50.35% of the sperms exhibited one compact signal. Our results are in concordance with the expected 1:1 sex ratio in cattle.

Out of the 1440 cells only 3 contained two Y chromosomes, i.e. YY disomy rate was 0.21%, that is apparently higher than the results of *Hassanane et al.* (in press).

CONCLUSIONS

Sexing spermatozoa by *in situ* hybridization with sex chromosome specific probes is easy to perform, large number of semen cuclei can be analysed in a short time. Further effort must be made to prepare X chromosome specific probes suitable for simultancous hybridization with Y (dual-colour FISH) to obtain XX and XY disomy rate, and XX, XY, YY diploidy rate considering the size of the cells.

ACKNOWLEDGEMENTS

We would like to express our sincere gratitude to *Dr. Claus Jörgensen* (The Royal Veterinary and Agricultural University, Coppenhagen, Denmark) for the bovine Y-specific probe and for the technical support in the semen decondensation.

Authors' address: *Tardy, E.P. – Tóth, A.:* Haynal Imre Medical University Budapest, Hungary
Szalai, G.: Technical University of Budapest, Hungary
Gustavsson, I. – Lindblad, K.: Swedish University of Agricultural Sciences Uppsala, Sweden
Hassanane, M.: National Research Center, Dokki, Giza, Egypt
Kovács, A. – Házas, G.: Research Institute for Animal Breeding and Nutrition H-2053 Herceghalom
Dohy, J.: Gödöllő University of Agricultural Sciences H-2101 Gödöllő, Páter K. u. 1.

COAT COLOUR AND COMPLEX TRAITS: A GENETIC APPROACH TO MELANOMA IN GREY HORSES

RIEDER, S. — CHECA-CORTES, M.L. — STRICKER, CH. —
JOERG, H. — MEIJERINK, E. — STRANZINGER, G.

SUMMARY

Grey horses in different breeds and populations are primarily affected by skin melanoma and internal cancer. This susceptibility of grey horses in particular to melanoma is in fact striking and has been known for a long time. Nevertheless, unaffected old grey horses of different sex, families and breeds have been found. Phenotypic data in two complex pedigrees of Camargue horses from the south of France were analysed. The likelihood of a simple two locus model (liability model) explained the phenomenon 10^{11} times better than an environmental based model. A genetic influence on the development of this tumour has been shown in humans as well as in other animal species.

Starting with cross-species-PCR, genes which are specific to the melanocyte or involved in its differentiation and proliferation, were analysed. One of them is c-KIT proto oncogene (KIT) a receptor for tyrosine kinase and its ligand Stem Cell Factor (SCF). KIT is structurally related to platelet-derived growth factor receptor. Mutations in KIT are linked to human piebaldism, a pigmentation disorder, as well as white spotting in the mouse and the dominant white coat colour in pigs. The KIT-ligand SCF has a major influence on proliferation and differentiation of hematopoietic cells, germ cells and melanocytes. A homolog of human, mouse and bovine KIT, as well as a homolog of human, bovine and porcine SCF on horse cDNA and genomic DNA level, was partly cloned and sequenced. The homolog of human, mouse and bovine c-KIT proto oncogene was preliminary mapped to horse chromosome 3q13-21 by fluoresence *in situ* hybridization (FISH).

Further, an equine homolog of human Melanoma Growth Stimulatory Activity (MGSA) was cloned and partly sequenced. The product of MGSA acts as an autocrine growth factor, which was first detected in human melanoma cells and belongs to a superfamily of proteins which includes interleukine-8 and platelet factor 4. In human and mouse, MGSA maps to the same region as c-KIT proto oncogene. Yet, in all these genes no informative sequence difference was discovered.

No association was found between microsatellite markers ASB23, SGCV33 and melanoma or greying in the horse families. Linkage analysis with the same markers was computationally too demanding.

Nevertheless equine chromosome 3q remains an interesting candidate region for pigmentation phenomenons and related complex traits.

MATERIAL AND METHODS

The animal model and segregation analysis

During an annual round-up, blood-samples from a total of 80 Camargue horses from the south of France were collected and stabilized in EDTA for DNA extraction. Each animal was individually controlled and phenotypic data was established. Two complex pedigrees including 65 animals were selected for a segregation analysis with the Pedigree Analysis Package (PAP) from *Hasstedt,* 1994.

Candidate gene approach

The obvious relation between grey horses and their unique susceptibility to melanoma, lead to a candidate gene approach, which included genes from the "melanocyte-cascade" — coat colour patterns, on one hand and growth factors, tumour suppressors — tumour development, on the other hand (*Barsh,* 1996). In a first period during 1996 KIT and its ligand SCF were analysed, together with the autocrine growth factor MGSA (*Giebel et al.*, 1991; *Johansson et al.,* 1992; *Williams et al.,* 1992; *Richmond et al.,* 1988).

DNA and RNA extraction

Genomic DNA was extracted from the mentioned blood-samples according to standard protocols. The RNA resource was horse skin. A CsCl extraction based on standard protocols was carried out. The quality and quantity of DNA and RNA was determined on agarose gels and by photospectrometry.

Cross-species and RT-PCR

Oligonucleotide primers were designed based on sequence information from GenBank. With these primers — specific, for highly conserved regions of the particular genes (KIT, SCF, MGSA), either direct cross-species PCR on genomic DNA, or RT-PCR was carried out. For RT-PCR, single stranded cDNA synthesis with a gene specific cross-species reverse primer, followed by an amplification step, composed of a gene specific cross-sprecies forward and reverse primer, was performed. Depending on the size of the expected fragment, PCR conditions were modified.

A basic amplification protocol started with a denaturation step at 95 °C for 5 minutes, followed by 30 to 35 cycle steps — denaturation at 95 °C for 30 seconds, annealing at 56–60°C for 30 seconds and extension at 72 °C for 30 seconds up to 2 minutes.

RT-PCR started with a denaturation at 70 °C for 5 minutes followed by annealing during a 10 minutes ramp down to 25 °C. The extension step contained 40 minutes at 42 °C, 20 minutes at 50 °C and a desactivation step at 70 °C for 15 minutes.

A reaction contained 200–400ng of genomic DNA or 2,5ug of RNA respectively. Primers were diluted to a 20uM concentration. 200uM of each nucleotide was added as well as a commercially available 10 times Taq-polymerase buffer and about one unit of Taq-polymerase itself. For RT-PCR an AMV enzyme was used. The water was DEPC treated and RNasin served as a RNAse inhibitor.

The PCR products were electrophoretically separated in agarose gels. If the gel contained prominent bands of the expected size, these bands were cut from the gel, followed by DNA extraction. The optained DNA served as template DNA for sequencing.

DNA sequencing and genescan

Sequencing and genescan was performed based on Perkin Elmer standard protocols for dye terminator or dye primer systems respectively with an ABI Prism 373 and additionnal software packages. The received sequence data was analysed and processed with the Wisconsin Package (GCG) from *Devreux et al.* 1984. Microsatellite markers SGCV33 (*Godard et al.*, 1997) on ECA 3q12 and ASB23 (*Lear et al.*, 1998) on ECA 3q22, were analysed in the Camargue pedigrees and processed by genescan. With this data, association and linkage analysis was performed with PAP.

Fluorescence in situ hybridization (FISH)

FISH was performed according to *Joerg*, 1994. PCR products based on cDNA of up to 3000bp were biotin-labeled with random primer labeling. The probe was then hybridized to horse metaphase chromosomes from leukocyte cultures. QFQ-staining enabled individual chromosome identification. Signal detection was processed with a Leitz microscope supported by a CCD camera, computer and software.

RESULTS AND DISCUSSION

Segregation analysis with phenotypic data

The data was analyzed using 6 different liability based threshold models as summarized in *Table 1.* Age of onset was modeled with three classes: 0 to 4, 5 to 10 and 10 to 30 years. Prevalences were set 10%, 25% and 60% respectively. Model 1 was a single locus model with 2 alleles assuming Hardy-Weinberg equilibrium. Model 2 was identical with model 1 except that the transmission of genes from parents to offspring was set to 1.0 for all possible pairs of parental and offspring genes, forming an environmental model (no Hardy-Weinberg equilibrium assumed). Model 3 additionally accounted for a polygenic component, whereas model 4 used regressive coefficients instead. Model 5 is identical to model 2 but modeled with an additional polygenic component, whereas model 6 is a pure polygenic model.

Table 1.

Parameter	Model					
	1	2	3	4	5	6
Gene frequency	0.68	*	0.68	0.68	*	
Dominance	0.37	0.37	0.48	0.5	1.0	
Displacement	17.5	17.5	17.5	17.5	17.5	
Polygenic heritability			1.0		1.0	0.81
Corr. between mates				0.89		
Corr. parent offspring				0.93		
Ln likelihood	53.97	82.38	53.13	52.69	53.36	56.41

* No Hardy-Weinberg equilibrium; 3 type frequencies as parameters.

All genetic models fit the data better than the non-genetic model 2 by at least a factor of 1.8 10^{11}. The results of the genetic models are inconclusive. A comparision of model 5 and 6 indicates some skewness in the data. Allowing for such skewness and accounting for the inheritance of a polygenic component segregating, model 5 seems to explain the data equally well as the mixed inheritance model (3) or the single locus model (1). The mixed model of inheritance (model 3) reveals a slightly better likelihood with one parameter more than model 1, but with this additional parameter, the polygenic heritability goes to the boundary of its parameter space. In order to eventually conclude the mode of inheritance present in this data, further investigation is needed.

However, the analysis indicate a strong genetic influence on melanoma in the tested families. A genetic predisposition for this cancer has already been demonstrated in pigs and fish as well as in humans (*Blangero et al.,* 1996; *Schartl,* 1995; *Lynch et al.*, 1991).

Sequence data for KIT, SCF and MGSA

On cDNA level, no sequence difference could be found in the yet analysed parts of the equine homologs of KIT, SCF and MGSA between affected and unaffected grey individuals and unaffected control animals of other coat colour. The analysed sequence data is available on GenBank through accession no. AF053497 for MGSA, AF053498 for SCF and AF055037 for KIT.

Mapping data for KIT

A PCR product of approximately 3000bp based on cDNA, coding for the major part of KIT, served as a probe for FISH. Thus the equine homolog of KIT was preliminary mapped to ECA 3q13–21. This result corresponds quite well to that of *Lear et al.*, 1997.

Microsatellite data for SGCV33 and ASB23

Association of marker ASB23 with the phenomenon was studied using "McNemar's test" (*Elston*, 1995). No association between the alleles of ASB23 and melanoma nor greying in the horse families could be found. Marker SGCV33 was considered not informative in this association analysis, due to its low heterozygosity. A linkage analysis using ASB23 was computationally too demanding due to the complexity of the pedigree (nested inbreeding loops) and the number of genotypes involved (63 genotypes for a linkage analysis with ASB23 and a single locus with two alleles underlying the liability-based threshold model).

CONCLUSIONS

Cutanous malignant melanoma among grey horses is a unique phenomenon (*Valentine*, 1995). Its comparability to human melanoma is still unclear (*Rodriguez et al.*, 1997). There seems to be a strong genetic background for this character in the investigated population. But no susceptible genotype in the mentioned genes and markers could yet be found. Present research is focused on additional genes from the melanocyte/melanin cascade together with genes for cell cycle regulation. Nevertheless the investigated region on horse chromosome 3q remains an interesting candidate region for other coat colour phenomenons and related complex traits (*Marklund et al.*, 1998). Fur-

ther comparative histological and enzymatic investigation on equine and human tumour nodules, should allow to determine to what extend these neoplasms are in fact identical in the two species. Due to the increase of melanoma in humans, research in this field is of great importance. A "natural" model like the phenomenon in the grey horse, might lead to a better understanding of the genetics of inherited predisposition of this cancer in the future.

REFERENCES

Barsh, G.S.(1996): The genetics of pigmentation: from fancy genes to complex traits. Trends in Genetics, 12. 8. 299–305.p.

Blangero, J., et al.(1996): Genetic determinants of cutaneous malignant melanoma in Sinclair swine. Br. J. Cancer, 73. 5. 667–671.p.

Devereux, J. – Haeberli, P. – Smithies, O.(1984): A comprehensive set of sequence analysis programs for the VAX. Nucleic Acids Res., 1. 387–395.p.

Elston, R.(1995): Linkage and Association to Genetic Markers. Exp. Clin. Immunogenet., 12. 129–140.p.

Giebel, L.B. – Spritz, R.A.(1991): Mutation of the KIT (mast/stem cell growth factor receptor) protooncogene in human piebaldism. Proc. Natl. Acad. Sci., USA, 88. 8696–8699.p.

Godard, S., et al.(1997): Characterization, genetic and physical mapping analysis of 36 horse plasmid and cosmid-derived microsatellites. Mammalian Genome, 8. 745–750.p.

Hasstedt, S.J.(1994): Pedigree Analysis Package. Revision 4.0, Department of Human Genetics University of Utah, Salt Lake City

Joerg, H.(1994): Hochauflösende Chromosomenkartierung beim Rind mittels Zwei-Farben-Fluoreszenz In-Situ-Hybridisierung. Diplomawork ETHZ

Johansson, M. – Ellegren, H. et al.(1992): The gene for dominant white color in the pig is closely linked to ALB and PDGFRA on chromosome 8. Genomics, 14. 965.p.

Lear, T.L., et al.(1997): Chromosomal location of genes for ESR, ETS2, GOT2, KIT, MX1 and PGR in the horse, Equus caballus. In: Proceedings of the 10th North American Coloquium on domestic Animal Cytogenetics and Gene Mapping, Apalachicola, FL (abstract)

Lear, T.L., et al.(1998): Physical assignment of microsatellite markers to horse chromosomes using FISH. Plant and Animal Genome VI Abstracts

Lynch, H.T. – Fusaro, R.M.(1991): Hereditary malignant melanoma. CRC Press Inc., Florida, USA

Marklund, S., et al.(1998): Close association between sequence polymorphism in the KIT gene and the roan coat color in horses. Plant and Animal Genome VI Abstracts

Richmond, A., et al.(1988): Molecular characterization and chromosomal mapping of melanoma growth stimulatory activity, a growth factor structurally related to beta-thromboglobulin. The EMBO Journal, 7. 7. 2025–2033.p.

Rodriguez, M., et al.(1997): Grey horse melanotic condition: A pigmentary disorder. Journal of Equine Veterinary Science, 17. 12. 677–681.p.

Schartl, M.(1995): Platyfish and swordtails: A genetic system for the analysis of molecular mechanisms in tumor formation. Trends in Genetics, 11. 5. 185–189.p.

Valentine, B.A.(1995): Equine Melanocytic Tumours: A retrospective study of 53 horses (1988 to 1991). Journal of Veterinary Internal Medicine, 9. 5. 291–297.p.

Williams, D.E. – De Vries, P., et al.(1992): The steel factor. Developmental Biology, 151. 2. 368–376.p.

Authors' address: *Rieder, S. – Stricker, Ch. – Joerg, H. – Meijerink, E. – Stranzinger, G.*:
Department of Animal Science, Federal Institute of Technology
8092 Zürich, Switzerland
Checa-Cortes, M.L.: Laboratorio de Genética, Dpto. Producción Animal,
Facultad de Veterinaria U.C.M, Madrid, Spain

HETEROGENEOUS CYTOGENETIC FINDINGS IN FELINE FIBROSARCOMAS

MAYR, B. — REIFINGER, M. — ALTON, K. — JUGL, M. — BREM, G.

SUMMARY

Short term cultures of six feline fibrosarcomas were subjected to cytogenetic investigations. A remarkable genetic heterogeneity was revealed between the neoplasms of the six patients. Each of the tumours showed different clonal abnormalities. Cytogenetic abnormalities observed were e.g. reciprocal translocations t(D3q;E1q) and t(A1q;B3q), deleted A1, A2 and B1 and trisomy F1.

INTRODUCTION

An understanding of the genetics of soft tissue tumours including fibrosarcomas, malignant fibrous histiocytomas and sarcomas is vitai in understanding the disease process. These disorders are rather common in cats and associations to different causes including different vaccine types have found high interest. Nonetheless, there are only very few cytogenetic data about feline fibrosarcomas so far (*Mayr et al.*, 1991, 1994, 1996, 1998).

MATERIALS AND METHODS

Neoplasms were observed in six cats. Two of them were female (cats 1and 2). Both were 9 years old. The localisation of their tumours were the thigh (cat 1) and the ear (cat 2). The other four patients (cats 3 to 6) were male and 6, 11, 10 and 7 years old. The locatisation of their tumours were the shoulder (cats 3 and 4), the ear (cat 5) and the mammary region (cat 6).

Histopathologically, the six tumours (cats 1 to 6) proved to be fibrosarcomas (Figs. 4a and 5a). The tumours were removed surgically in the Veterinary University and general practices in Vienna in February 1997. Their regional lymph nodes were unaffected and no recurrences occured so far.

Primary explant cell cultures were performed by cutting the tumour material in small pieces. The fragments were given to sterile flasks containing 10 ml RPMI 1640 medium with L-glutamine, antibiotics (50 iu penicillin and 50 µg streptomycin/ml^{-1}) and 10 percent fetal calf serum (Gibco). Culturing war performed in 5 percent carbon dioxid in air for 14 days until harvesting.

Thirty metaphases were karyotyped in all cats by Trypsin-G-banding according to the method of *Wang and Fedoroff* (1972). Chromosome numbering followed the Reading Conference proposals (1980).

RESULTS

In cat **1**, three of the metaphases presented with a reciprocal translocation 38,t (D3q;E1q), (*Fig. 1.*). The other cells showed the normal karyotype (2n=38).

In cat **2**, six of the metaphases showed a deleted chromosome B1, 38(delB1q), (*Fig. 2.*). Three other cells showed the karyotype 36(delB1,-D4,-E2), because they additionally lost one chromosome D4 and E3. The major part of the short arm of the E1-chromosome appeared to be shortened in several cells. All other cells were free of alterations.

In cat **3**, three of the cells showed a deleted chromosome A2, 38(delA2q), (*Fig. 3.*). Two of these and one additional cells showed a deletion on the long arm of chromosome A 38(delA2q). Moreover, hyperdiploidy with A3- and E2-trisomy was observed in two cells. The other cells appeared to be free of alterations.

In cat **4**, in two of the cells a deletion 38,(delA1q) was present. Three other cells showed addtionaly to the deleted A1q a deleted B3q, giving rise to 38(delA1q; del B3q). Size differences between chromosomes C2 were detected in 2 cells (*Fig. 4.*). No other abnormalities were detected.

In cat **5**, trisomy F1, 39(+F1) was present in five cells (*Fig. 5.*). Moreover, additional size differences between chromosome C1 were observed in 2 cells. No other abnormalities were seen.

In **cat 6**, breakages on the long arm of chromosome A1 (A1q) were visible in four cells (*Fig. 6.*). Moreover, monosomy F2 and/or B1 were found in two cells. The other cells appeared free of alterations.

Fig. 1.: **Karyotype of cat 1 (fibrosarcoma). Note the reciprocal translocation 38,t(D3q;E1q), (arrows)**

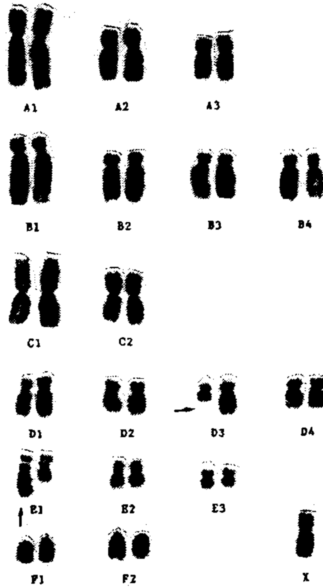


Fig. 2.: **Karyotype of cat 2 (fibrosarcoma). Note the deleted chromosome B1, 38(delB1q), (arrow)**

Fig. 3.: **Karyotype of cat 3 (fibrosarcoma). Note the deleted chromosome A2, 38(delA2q), (arrow)**

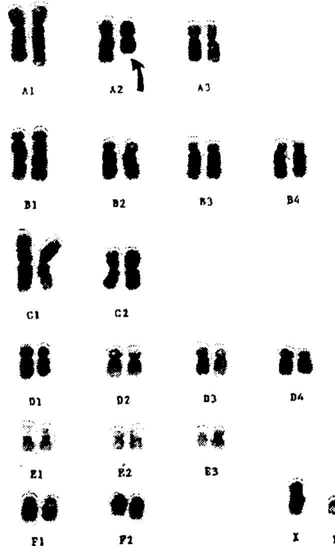


Fig. 4.: **Karyotype of cat 4 (fibrosarcoma). Note the deleted chromosomes A1 and B3, giving rise to 38(delA1q; delB3q) and also differences between chromosomes C2 were detected (arrows)**

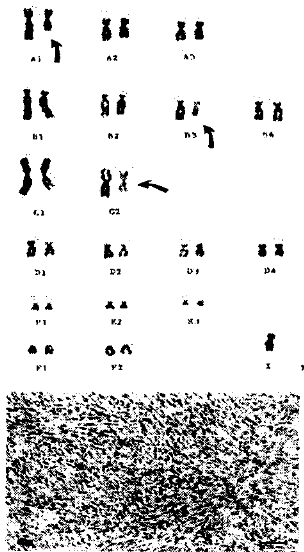


Fig. 4a.: **Fibrosarcoma of cat 4; comparatively good differentiation, median mitotic activity. Bar = 60 µm**

Fig. 5.: **Karyotype of cat 5 (fibrosarcoma). Note the trisomy F1, 39(+F1) and the size differences between chromosomes C1 (arrows)**

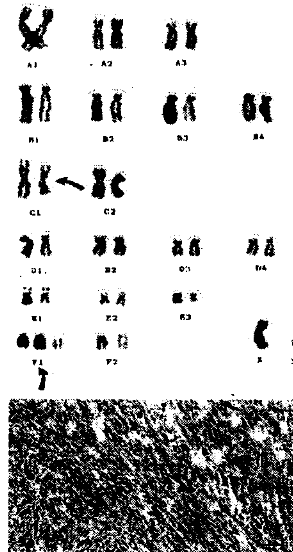
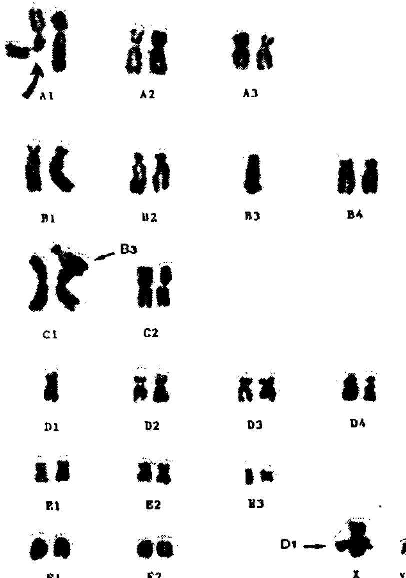


Fig. 5a.: **Fibrosarcoma of cat 5; comparatively good differentiation, moderate mitotic activity, marked inflammatory reaction. Bar = 60 µm**



DISCUSSION

The present investigation including six mesenchymal tumours clearly demonstrates the occurence of different aberrations in different patients. Thus, the presence of distinct deletions (concerning e.g. regions of chromosomes A1, A2, B1, B2, B4 or E1 or translocations e.g. involving chromosome E1) suggests the absence of a unique specific aberration as a general characteristics in the majority or in all feline soft tissue tumours. Nonetheless, several of the chromosomes mentioned above are repeatedly observed in soft tissue neoplasms. Another chromosome frequently affected is chromosome F1. All these data fully corroborate and expand data our earlier studies concerning fibrosarcomas and malignant fibrous histiocytomas (*Mayr et al.*, 1991, 1994, 1996, 1998).

In general, our data are in good correspondence with human data, where no specific abnormalities have been found in malignant fibrous histiocytomas, but the karyotypic profiles are clearly nonrandom (*Örndal et al.*, 1994; *Heim and Mitelman*, 1995).

The high percentage of normal diploid cells without detected aberrations could represent tumour cells really without or with submicroscopic changes or contaminating stromal cells. This phenomenon is widespread in primary short term cell cultures and awaits an alleviation by the use of higher resolving and more informative molecular cytogenetic techniques in the near future. Examples are the use of fluorescence *in situ* hybridization (e.g. FISH), comparative genomic hybridization (CGH) and primed *in situ* hybridization (PRINS).

At present, the number of localized loci on the genetic map in domestic animals including cats is low compared to man and mouse (*Lyons et al.*, 1997). However, the feline and human karyotypes seem to possess remarkable banding similarities and syngenies (*Nash and O'Brien*, 1982; *Dutrillaux and Couturier*, 1983; v. *Kiel et al.*, 1985; *O'Brien*, 1992; *O'Brien et al.*, 1993; *Rettenberger et al.*, 1995).

ACKNOWLEDGEMENTS

The work was supported by the project „Vergleichende genetische Studien an Neoplasmen" of the Ludwig Boltzmann Institute for immuno-, cyto- and moleculargenetic Research by the Austrian Ministry for Health and Protection of Consuments.

REFERENCES

Dutrillaux, B. – Couturier, J.(1983): The ancestral karyotype of carnivora: comparison with that of platyrrhine monkeys. Cytogenet. Cell Genet., 35. 200–208.p.

Heim, S. – Mitelman, F.(1995): Tumors of Bone and Soft Tissue. In: Heim, S. - Mitelman, F. (eds.), Cancer Cytogenetics, Wiley-Liss, N.Y., 483.p.

Kiel, K. – Hameister, H. – Somssich, I.E. – Adolph, S.(1985): Early replication banding reveals a strongly conserved functional pattern in mammalian chromosomes. Chromosoma, 93. 69–76.p.

Lyons, L.A. – Laughlin, T.F. – Copeland, N.G. – Jenkins, N.A. – Womack, J.E. – O'Brien, S.J. (1997): Comparative anchor tagged sequences (CATS) for integrative mapping of mammalian genomes. Nature Gen., 15. 47–56.p.

Mayr, B. – Eschborn, U. – Kalat, M.(1991): Near triploidy in a feline fibrosarcoma. J. Vet. Med. A., 38. 617–620.p.

Mayr, B. – Hofstadler, E. – Schleger, W. – Reifinger, M. – Eisenmenger, E.(1994): Trisomy D1, Marker F1: New cytogenetic findings in two cases of feline fibrosarcoma. J. Vet. Med. A., 41. 197–201.p.

Mayr, B. – Bockstahler, B. – Loupal, G. – Reifinger, M. – Schleger, W.(1996): Cytogenetic variation between four cases of feline fibrosarcoma. Res. Vet. Sci., 61. 268–270.p.

Mayr, B. – Wegscheider, H. – Reifinger, M. – Jugl, M.(1998): Cytogenetic alterations in four feline soft-tissue tumours. Vet. Res. Comm., 22. 21–29.p.

Nash, W.G. – O'Brien, St.J.(1982): Conserved regions of homologous G-banded chromosomes between orders in mammalian evolution: carnivores and primates. Proc. Natl. Acad. Sci., USA, 79. 6631–6635.p.

O'Brien, St.J.(1992): Genetic Maps. Nonhuman Vertebrates. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

O'Brien, St.J. – Womack, J.W. – Lyons, L.A. et al.(1993): Anchored reference loci for comparative genome mapping in mammals. Nature Genet., 3. 103–112.p.

Örndal, C. – Rydholm, A. – Willen, H. – Mitelman, F. – Mandahl, N.(1994): Cytogenetic heterogeneity in soft tissue tumours. Cancer Gen. Cytogen., 68. 1–21.p.

Reading Conference(1980): Standardisation of banded karyotypes of domestic animals. Hereditas 92. 145–162.p.

Rettenberger, G. – Klett, Ch. – Zechner, U. – Bruch, J. – Just, W. – Vogel, W. – Hameister, H. (1995): ZOO-FISH analysis: cat and humane karyotypes closely resemble the putative ancestral mammalian karyotype. Chromosome Res., 3. 479–486.p.

Wang, H.C. – Fedoroff, S.(1972): Banding in human chromosomes treated with trypsin. Nature New Biol., 235. 52–54.p.

Authors' address: *Mayr, B. – Jugl, M. – Brem, G.*: Institute for Animal Breeding and Genetics, Veterinary University, A-1210 Vienna, Veterinärplatz 1.
Reifinger, M. – Alton, K: Institute for Pathology and Forensic Veterinary Medicine, A-1210 Vienna, Veterinärplatz 1.

ANALYSIS OF NUMERICAL CHROMOSOME ABNORMALITIES IN BOAR SPERM BY MEANS OF FISH

RUBES, J. — VOZDOVÁ, M. — KUBÍCKOVÁ, S.

SUMMARY

The objective of the current research is to develop chromosome-specific probes for use in evaluating aneuploidy in boar spermatozoa using FISH technology. A multicolor FISH method was developed to detect aneuploidy in sperm of boars using DNA probes specific for small regions of chromosomes Y, 1 and 10. The average frequencies of disomy for the specific chromosomes were: 1-1, 0.078%; 10-10, 0.072%; and YY, 0.083%. The incidence of disomy did not differ significantly by chromosome. The average frequencies of diploidy were 0.175% for 1-1-10-10, and 0.021% for Y-Y-10-10. Thus the incidence of diploidy was significantly higher than that of disomy for the chromosomes examined (P<0.01). The newly described 2-chromosome FISH method appears to be reliable and sensitive for detecting aneuploidy in pig sperm. The observed level of numerical chromosome aberrations in pig sperm appear to be within the range of the baseline frequencies reported so far in men.

INTRODUCTION

Aneuploidies rank with the most serious and most frequently occurring chromosomal aberrations in man (*Hassold*, 1998; *Hecht and Hecht*, 1987; *Martin et al.*, 1991). They are associated with infertility, spontaneous abortions, perinatal mortality, and mental retardation in man and with embryonic and fetal mortality in farm animals. Recent advances in molecular cytogenetics, provide a number of new approaches for investigating chromosomal disorders in gametes (reviewed by *Hassold*, 1998). The first technique for evaluating human sperm chromosomes was the human sperm-hamster egg fusion assay. This labor-intensive assay allows karyotypic analysis of a limited number of human sperm after they fertilize zona-free hamster oocytes. During the first 15 years of its use in

of human sperm after they fertilize zona-free hamster oocytes. During the first 15 years of its use in multiple laboratories world-wide, about 20,000 sperm karyotypes were analyzed (*Martin et al.*, 1993). More recently, the use of fluorescence *in situ* hybridization (FISH) using chromosome-specific probes has permitted the detection of aneuploidy in large numbers of human and animal sperm. Typically 10,000 sperm per donor or per animal are evaluated to give a good estimate of the incidence of aneuploidy in the sample.

Many authors have expressed the opinion that the incidence of aneuploidy is much higher in humans than in other species under study. However, almost no data are available regarding the incidence of sperm chromosomal aberrations in species other than humans and laboratory rodents. Although the animal sperm-hamster oocyte fusion assay was also used for investigations in farm animals, most of the papers deal with methodology and the total number of tested spermatozoa has not exceeded several hundreds (*Creighton and Houghton*, 1987; *Kovacs and Foote*, 1989; *Bird and Houghton*, 1990; *Benkhalifa et al.*, 1991). Nevertheless, the limited data available from this method suggests that the percentage of aneuploid sperm is similar in boars and man (*Bird and Houghton*, 1990). Apart from methodological papers by *Kawarasaki et al.* (1995,1996); the potential of FISH as a method of investigating aneuploidy in sperm of farm animals has yet to be exploited .

Therefore, the objective of the current research is to develop chromosome-specific probes for use in evaluating aneuploidy in boar spermatozoa using FISH technology. We describe methods for detection of aneuploidy in boar sperm hybridized with DNA probes specific for two or three chromosomes simultaneously, and we establish baseline frequencies of sperm aneuploidy in this domestic species.

MATERIALS AND METHODS

Ejaculates were collected from 15 fertile boars on a regular semen collection schedule at a large artificial breeding center (Czech Republic, Brno).

Decondensation of sperm nuclei was performed by a modification of the method of *Kawarasaki et al.* (1996). Briefly, semen samples were washed once with an equal volume of phosphate-buffered saline (PBS, pH 7.4) containing 6 mM EDTA for 3 min. Washed spermatozoa were resuspended in 300μl of PBS containing 2mM dithiothreitol (DTT) and incubated for 40 min. After washing with PBS, the samples were fixed for 10 min with acetic alcohol (methanol and acetic acid, 3:1) and dropped on clean microscope slides.

Probes for chromosomes 1 and Y were prepared on the basis of DNA sequence data from GeneBank Nucleotide Sequence Database. Primers 5`-GTTGCACTTTCACGGACGCAGC-3` and 5`-CTAGCCCATTGCTCGCCATAGC-3` were designed according to the Mc1 satellite DNA (X51555) and subjected to PCR to amplify a 244-bp chromosome 1-specific fragment. A Y- chromosome specific primer pair 5`-AATCCACCATACCTCATGGACC-3`, 5`-TTTCTCCTGTATCCTCCTGC-3`, predicted 377-bp amplification product, was designed according to the male-specific sequence (X12696). The cosmid S0045 (Yerle, at al.1994) was used as probe for the centromeric region of chromosome 10. For two-color FISH experiments, the probes were labeled with either Fluorescein-11-dUTP or Cy3-dUTP (Amersham).

Two color FISH was performed on duplicate slides to probe simultaneously for chromosomes 1 and 10, or 10 and Y. Following hybridization and post-hybridization washes, sperm were counterstained with DAPI added to the antifade mounting medium (Vectashield). Slides were examined with an Olympus BX60 fluorescent microscope equipped with a DAPI/FITC/Texas Red triple-band filter (Chroma). Strict scoring criteria were used to discriminate normal from abnormal cells based on their fluorescent-domain phenotypes (*Robbins at al.*, 1993; *Rubeš at al.*, 1998). For cells to be recorded as having an additional chromosome 1, 10 or Y, the two fluorescent domains of the same color had to be separated by a distance of more than 1 the diameter of the respective fluorescence domains within that cell. If two or more fluorescence domains for a particular target were seen in one cell, the domains had to be of equal size and intensity to be scored as multiple domains. Additionally, the extra chromosome fluorescence domains had to appear similar to those in neighboring cells.

Specimens were scored in duplicate by two scorers who were blinded to each other's scoring results. Ten thousand sperm nuclei were examined from each boar for each combination of probes, Comparisons among the sets were done by the Mann-Whitney and Kruskal-Wallis tests using the STATGRAPHICS, version 4.0 (STCS, Inc.) software.

RESULTS AND CONCLUSIONS

A multicolor FISH method was developed to detect aneuploidy in sperm of boars using DNA probes specific for small regions of chromosomes Y, 1 and 10. The DNA probes showed excellent specificity and sensitivity in pig sperm nuclei. The number of sperm without any hybridization signal was consistently very low (<1%). We examined baseline frequencies of aneuploid sperm. The FITC-dUTP-labeled Y probe gave a single green domain in about half the cells which is consistent with half of the sperm carrying a Y chromosome and the other half carrying an unlabeled X chromosome. Sperm with extra fluorescent domains were assigned to the following categories: a) hyperhaploidies for chromosome Y and 10, namely: 10-Y-Y, 10-10-Y or 10-10-, or, b) meiosis II errors leading to diploidy: 10-10-Y-Y. The second analysis of each sample used combined DNA probes for chromosomes 1 and 10. Again sperm with extra colored domains were categorized as hyperhaploid (1-1-10 and 10-10-1) or diploid (1-1-10-10).

The results are summarized in *Table 1*. The average frequencies of disomy for the specific chromosomes were: 1-1, 0.078%; 10-10, 0.072%; and YY, 0.083%. The incidence of disomy did not differ significantly by chromosome. The average frequencies of diploidy were 0.175% for 1-1-10-10, and 0.021% for Y-Y-10-10. Thus the incidence of diploidy was significantly higher than that of disomy for the chromosomes examined ($p < 0.01$).

Table 1.

Sperm disomy and diploidy analyses by FISH

	Chromosome	Freq. per 10^4 sperm ($\bar{x} \pm SD$)	Range	%
Sperm disomy				
	1	7.8±4.75	1–18	0.078
	10	7.2±3.14	4–16	0.072
	Y	8.3±5.06	1–18	0.083
Sperm diploidy				
	Y-Y-10-10	2.1±1.17	0–8	0.021
	1-1-10-10	17.46±7.85	6–35	0.175

In *Table 2.*, the frequencies of sperm aneuploidy in pigs (this study) are compared with those obtained for young men (in our laboratory , *Rubeš at al.*,1998) and for mice (*Lowe et al.*, 1996) and rats (*Lowe et al.*, 1998). The average frequencies of aneuploidy in pig sperm are in good agreement with those in humans with respect to all the hyperhaploid and diploid categories. Thus for pig sperm, frequencies for autosomal disomy per 10,000 sperm are: 7.8, or 7.2, as compared with 4.7 for humans). For disomy of the Y chromosome, the frequency in pigs is 8.3 compared with 2.2 in humans. For diploidy YYAA the frequency is 2.1 for pigs vs 2.6 for humans, and for total diploidy the frequency is 17.5 for pigs vs 23.9 for humans. Based on limited data, the frequencies of Y-Y disomy and total diploidy may be lower in rodents.

Table 2.

Comparison of frequencies of sperm with aneuploidy among pig, rat, mouse and human using two- or three-chromosome FISH

Species	Pig	Rat	Mouse	Human
Reference	current study	Lowe et al., 1998	Lowe et al., 1996	Rubeš at al., 1998
No. of individuals	15	14	2	15
Probes used in FISH	1, 10, Y	Y, 4	X, Y, 8	X, Y, 8
Disomy autosomes	7.8, 7.2	2.88	8	4.7
Disomy Y	8.3	1.05	0.5	2.2
Diploidy - total	17.46		8	23.9
Y-Y-A-A	2.1	0.36		2.6

The newly described 2-chromosome FISH method appears to be reliable and sensitive for detecting aneuploidy in pig sperm. The observed level of numerical chromosome aberrations in pig sperm appear to be within the range of the baseline frequencies reported so far in men. Thus FISH shows great promise for detecting and measuring cytogenetic damage in sperm of domestic animals and should be applicable for similar applications in oocytes and embryos.

ACKNOWLEDGMENT

We are indebted Dr. M.Yerle (INRA Castanet-Tolosan, France) for providing us with the cosmid S0045. Supported by the Grant Agency of the Czech Republic (grant No 523/98/0261) and the Ministry of Agriculture (grant No. EP0960006094).

REFERENCES

Benkhalifa, M. – Bonneau, M.– Popescu, P. et al.(1992): A method for cytogenetic analysis of boar spermatozoa using hamster oocytes. Ann. Génét., 35. 61–64.p.

Bird, J. M. – Houghton, J.A.(1990): Cytogenetics of porcine sperm chromosomes following IVF of zona-free hamster oocytes. Arch. Androl., 25. 45–57.p.

Creighton, P. – Houghton, J.A.(1987): Visualization of pig sperm chromosomes by in-vitro penetration of zona-free hamster ova. J. Reprod. Fert., 80. 619–622.p.

Hassold, T.J.(1998), Nondisjunction in the Human Male. In: Handel, M. A., Meiosis and Gametogenesis, Academic Press, NY., 383–406.p.

Hecht, F. – Hecht, B.K.(1987): Aneuploidy in humans: dimensions, demography, and dangers of abnormal numbers of chromosomes. In Vig BK, Sandberg AA (eds) Aneuploidy, Part A: Incidence and etiology. Alan R. Liss, Inc., New York, 9-49.p.

Kawarasaki, T. – Sone, M. – Yoshida, M. et al.(1996): Rapid and simultaneous detection of chromosome Y- and 1-bearing porcine spermatozoa by fluorescence *in situ* hybridization. Mol. Reprod. Dev., 43. 548–553.p.

Kovács, A. – Foote, R.H.(1989): Chromosome preparation from bovine spermatozoa. Theriogenology, 31. 213.p.

Lowe, X.R. – de Stoppelaar, J.M. – Bishop, J. – Cassel, M. – Hoebee, B. – Moore II, D. – Wyrobek, A.J.(1998): Epididymal Sperm Aneuploidies in Three Strains of Rats Detected by Multicolor Fluorescence In Situ Hybridization. Environ. Mol. Mutagen., 31. 125–132.p.

Lowe, X. – Hogan, S.O. – Moore II, D. – Bishop, J. – Wyrobek, A.(1996), Aneuploid epididymal sperm detected in chromosomally normal and Robertsonian translocation-bearing mice using a new three-chromosome FISH method. Chromosoma, 105. 204–210.p.

Martin, R.H. – Ko, E. – Chan, K.(1993): Detection of aneuploidy in human interphase spermatozoa by fluorescence *in situ* hybridization (FISH). Cytogenet. Cell Genet., 64. 23-26.p.

Martin, R.H. – Ko, E. – Rademeker, A.(1991): Distribution of aneuploidy in human gametes: comparison between human sperm and oocytes. Am. J. Med. Genet., 39. 321-331.p.

Robbins W.A. – Segraves R. – Pinkel D. – Wyrobek A.J.(1993): Detection of aneuploid human sperm by fluorescence *in situ* hybridization: Evidence for a donor difference in frequency of sperm disomic for chromosomes 1 and Y. Am. J. Hum. Genet., 52. 799-807.p.

Rubeš, J. – Lowe, X. – Moore, D. – Pereault, S. – Slott, V. – Evenson, D. – Selevan, S. – Wyrobek, A.J.(1998): Cigarette smoking lifestyle and sperm disomy. Fertil. Steril., (in press)

Yerle, M. – Goureau, A. – Gellin, J. – Le Tissier, P. – Moran, C.(1994): Rapid mapping of cosmid clones on pig chromosomes by fluorescence *in situ* hybridization. Mammalian Genome, 5. 34-37.p.

Authors' address: Veterinary Research Institute
Hudcova 70, 621 32 Brno, Czech Republic

THE INCIDENCE OF ANEUPLOIDY IN BOVINE OOCYTES MATURED *IN VITRO*

LECHNIAK, D — SWITONSKI, M.

Aneuploidy is a common phenomenon observed in mammalian gametes. It is well documented especially in humans, where the rate of aneuploid germ cells ranges from 1.5 to 51.9% in oocytes, and from 3.9 to 10% in spermatozoa. As it has alread been shown, the overall nondisjunction rate in oocytes of domestic animals ranges between 2 and 7% put the number of analyzed cells is relatively low. Aneuploid oocytes — when fertilized — give rise to aneuploid embryos that usually die during early pregnancy. The frequency of aneuploid embryos in domestic animals does not exceed 2% (*King*, 1990). The aim of the present study was to evaluate the rate of aneuploidy in bovine oocytes matured *in vitro*.

Oocyte collection and *in vitro* maturation was carried out according to the protocol described by *Lechniak et al.* (1996). Briefly: ovaries were collected at the local slaughterhouse and cumulus-oocyte complexes (COCs) were aspirated, washed and matured *in vitro* in TCM 199 medium supplemented by ECS and hormones for 24h at 39 °C in a humid 5% CO_2 atmosphere. After maturation, the cumulus cells surrounding oocytes were removed by both incubation in 0.25% trypsin + 0.02% EDTA solution and pipetting . Air-dried chromosome slides (*Tarkowski*, 1966) were stained in 5% Giemsa.

Cytogenetic analysis was carried out on 226 secondary bovine oocytes. A haploid chromosome set has been identifed in 186 cells. The group of aneuploid oocytes was comprised of hypo- and hyperhaploid cells with an excess of hypohaploidy.

Metaphase spreads comprising less than 29 chromosomes were not included into the calculation and considered to be a technical artifact. The rate of aneuploid calculated on the number of hyperhaploid oocytes reached 7.1%. The X chromosome was identif ed in all analyzed oocytes, thus only autosomal aneuploidies were found.

REFERENCES

King, W.A.(1990): Adv. Vet. Sci. Comp. Med., 23. 229–250.p.
Lechniak, D. et al.(1996): Theriogenology, 46. 267–277.p.
Tarkowski, A.K.(1966): Cytogenetics, 5. 394–400.p.

Authors' address: Agricultural University of Poznan
60-637 Poznan, Poland

HIGH INCIDENCE OF DIPLOID SECONDARY OOCYTES MATURED *IN VITRO* IN THE PIG

SOSNOWSKI, J. — WAROCZYK, M. — LECHNIAK, D. — SWITONSKI, M.

Mortality of embryos at early developmental stages is frequently recorded in the early pregnancy in the pig. Polyploidy, as an effect of polyspermy or the fertilization of unreduced (diploid) secondary oocytes, seems to be one of the factors causing the mortality. The aim of this study was to estimate the rate of diploid oocytes collected from individual sows and matured *in vitro* (IVM).

Fifty pairs of ovaries, collected within 15–20 min after slaughter, were used as a source of primary oocytes. Cumulus-oocyte complexes were aspirated from visible follicles (2–5 mm in diameter) using low pressure (about minus 0.1 bar). Only oocytes with an evenly granulated ooplasm and compact, intact cumulus cells were selected for maturation.

Groups of oocytes selected from a pair of ovaries from a single donor were matured in 100 μl droplets of medium (TCM-199 + 20% ECS + 75 iu/ml HCG + 1 μg/ml 17-ß oestradiol + 50 μg/ml gentamycin + 100 iu/ml penicillin) under paraffin oil at 38.5 °C in a humid 5% CO_2 atmosphere for 40–44 hours. Chromosome slides were prepared according to the procedure described by *Tarkowski* (1966).

Altogether 2275 oocytes were recovered from 50 pairs of ovaries (45.5 oocytes per pair, ranging between 12 and 191). Among them 949 oocytes were selected for IVM (41.7%, 949/2275). The number of selected oocytes for IVM from a single pair of ovaries ranged between 7 and 87, with an average of 19. Chromosome slides were prepared from 870 oocytes (91.7%, 870/949), but only 540 oocytes were useful for cytogenetic investigation (62.1%, 540/870) and 529 of them (98.0%, 529/540) reached telophase I (9 oocytes) or metaphase II (520 oocytes). A very high rate of diploid oocytes was found in metaphase II chromosome spreads. Altogether, 168 spreads (32.3%, 168/520) demonstrated diploid number of chromosomes. Diploid oocytes were observed for 36 donor sows. The individual percentage of diploid metaphases II ranged from 7.1% to 76.9%.

REFERENCE

Tarkowski, A.K.(1966): Cytogenetics, 5. 394–400.p.

Authors' address: Agricultural University of Poznan
60-637 Poznan, Poland

GROWTH HORMONE POLYMORPHISM (RFLP) IN DAY-10 PIG EMBRYOS

LECHNIAK, D. — LONG, S.E. — NISSEN, A.K.

The aim of the present study was to investigate whether there is a relationship between embryonic genotype at the pGH locus and litter size, embryonic perimeter and sex in Day-10 pig embryos.

MATERIAL AND METHODS

Seven litters od Day-10 embriyos from Landrace x Yorkshire sows inseminated with mixed Duroc semen were collected *post morten* and analysed (Day 0=day of insemination). Embryos were photographed and individually frozen (*Nissen et al.*, 1997). Afterwards DNA was extracted and embryos were sexed by means of PCR using SRY primers. A total of 93 Day-10 embryos were genotyped at the pGH locus using PCR-RFLP analysis. The pair of primers corresponding to the pGH gene with hte sequence published by *Kirkpatrick* (1992) as well as two restriction enzymes: Msp I and Hae II have been used in the present study. The PCR protocol was that described by *Kirkpatrick* (1992). PCR was carried out in a final volume of 30μl. Half of each PCR product was submitted for Msp I (4U/15T1 of PCR product) and the other for Hae II digestion (2.5 U/15T1). The pGH genotypes were visualized on 2% agarose gel. After digestion of PCR product with Msp I, three different genotypes could be identified according to the DNA fragments produced: (++) genotype –222bp,147bp and 137bp; (+–) genotype — 284bp,222bp,147bp and 137bp; (– –) genotype — 284bp and 222bp. Amplified DNA digested with Hae II yields three possible fragment combinations: (++) genotype — 333bp and 173 bp; (+–) genotype — 506bp,333bp and 173bp; (– –) genotype — 506bp fragment only.

RESULTS

The genotypes and alleles distribution in the analysed pool of Day-10 pig embryos are presented in the *Tables 1 and 2*. The Msp I (– –) genotype has not been found.

A possible relationship between the pGH genotypes within and between litters and embryonic characteristics (perimeter, sex) as well as litter sizes will be discussed.

Table 1.

The pGH genotype and allele variants distribution in the analysed Day-10 pig embryos

Litter	total	Msp I genotypes			Hae II genotypes		
		(++)	(+–)	(– –)	(++)	(+–)	(– –)
I	24	21	3	—	2	10	12
II	26	26	—	—	—	13	13
II	15	7	8	—	—	15	—
IV	13	13	—	—	5	8	—
V	7	—	7	—	—	7	—
VI	5	5	—	—	4	1	—
VII	3	3	—	—	—	—	3
total	93	75	18	—	11	54	28
genotype frequencies		0.81	0.19	0.0	0.12	0.58	0.3
alele frequencies	0.9 (+)	0.1 (–)		0.41 (+)		0.59 (–)	

Table 2.

The frequency of Msp I / Hae II genotype variants

Litter	Msp I/Hae II genotype combinations					
	++/+–	++/– –	+–/++	+–/+–	+–/–	+–/– –
I	1	8	12	1	2	—
II	—	13	13	—	—	—
III	—	7	—	—	8	—
IV	5	8	—	—	—	—
V	—	—	—	—	7	—
VI	4	1	—	—	—	—
VII	—	—	3	—	—	—
total	10	37	28	1	17	—
frequencies	0.1	0.4	0.3	0.01	0.19	0

Authors' address: *Lechniak, D.*: August Cieszkowski Agricultural University of Poznan
Dept. of Genetics and Animal Breeding, Poland
Long, S.E.: University of Bristol, Dept. of Clinical Veterinary Science
Langford House, England
Nissen, A.K.: Royal Veterinary and Agricultural University, Coppenhagen
Dept. for Clin. Studies, Reproduction, Denmark

MELANIN INFLUENCE ON CHRONIC AND FRACTIONATED IRRADIATION IN GERM AND SOMATIC CELLS OF MICE

MOSSE, I.B. — PLOTNIKOVA, S.I. — KOSTROVA, L.N. — DUBOVIC, B.V. — MOLOPHEI, V.P.

SUMMARY

Pigment melanin influence on mutagenic effect of ionizing radiation has been investigated in mice germ and somatic cells. Melanin was injected into stomach or intraperitoneally. Chronic gamma irradiation at the exposure rate of 0.007 Gy/h was used. Melanin was shown to decrease the mutation rate induced by chronic irradiation in germ cells even more effectively than the genetic effect of acute irradiation. Radioadaptive response has been found in bone marrow cells — the genetic effect of the divided radiation dose (0.2 Gy+1.5 Gy) was about half as much as the effect of single 1.7 Gy dose. Melanin has been shown to be able to remove completely the low radiation dose effect — if pigment was used before the first conditioning dose, adaptive response didn't appear. Melanin application after the first conditioning dose led to 4-fold decrease in the aberration level, because both radioadaptive reaction and protection have been observed.

INTRODUCTION

Long-term exposure of large population groups to ionizing radiation at small doses due to environmental contamination with radionuclides has set radiobiologists the task of searching for radioprotective substances of a new type — capable to reduce remote effects of long-term irradiation.

Conventional radioprotectors are known to be effective only during single irradiation and to display their radioprotective properties only when being applied in high-toxic concentrations.

Application of the most effective radioprotectors for reducing genetic effect of irradiation has shown that they are either unable to protect hereditary structures or are less effective than in protection against radiation death.

In this respect melanin pigment created by the nature itself for organism protection against UV-radiation and generated in human skin under sun ray influence ("sun tan") is worth attention.

Melanin is not toxic, it is in microorganisms, plants, animals and people (melanin dyes hair, eyelashes, iris of the eye, skin). Some foodstuffs (e.g. tea, coffee, cocoa, chocolate, some mushrooms) contain melanin.

We carried out experiments for studying melanin effect in animals (drosophila, mice) and cultured human cells upon remote genetic consequences of irradiation which have shown that this pigment considerably reduces frequency of all types of mutation induced by irradiation in animal germ cells and human lymphocytes by 2–4 times (*Mosse*, 1982; *Savchenko et al.*, 1985; *Mosse*, 1990).

Melanin ability to reduce the frequency of genetic lesions inherited from generation to generation and accumulated in populations as "genetic load" is especially valuable (*Mosse and Lyakh*, 1994). For the first time we have shown, by means of melanin, principal possibility of effective protection of animal populations under irradiation within many generations (*Mosse et al.*, 1986).

The investigation of melanin possibility to influence chronic irradiation effects was very important and urgent. Besides, it was very interesting to investigate melanin influence on radioadaptive response.

It's known that one of mechanisms of adaptive response is cell repair system stimulation (*Barquinero et al.*, 1996; *Boothman et al.*, 1996; *Joiner et al.*, 1996; *Salone et al.*, 1996;*Tedeschi et al*, 1996), so some repair inhibitors are able to stop or to decrease adaptive reaction. We decided to try another modification way — to use effective radioprotector, which is capable to remove conditioning radiation dose. Earlier we found that the less radiation dose, the higher pigment melanin protection, and its mechanism is not related with repair system stimulation (*Mosse et al.*, 1997).

METHODS

The influence of melanin isolated from animal hair on genetic effects of acute and chronic irradiation in mice and human lymphocytes has been studied. Mice males of 2.5 months and 22g weight were used. The starch gel or its melanin suspension was injected into stomach every day with a special needle. Melanin was supplied in concentrations from 0.3 to 30 mg/kg. Mice were exposed to 1–3 Gy of γ-rays of Cs^{137} at the dose rate of 0.007Gy/h (chronic irradiation) and 420Gy/h (acute one). Animals were killed 2.5–3.0 months after the exposure was stopped. This interval was necessary for repairing irradiated spermatogonia. The levels of reciprocal translocations in metaphase of spermatocytes were analysed cytologically after Iven's method. Mice Af were irradiated with 1.7 Gy or 0.2+1.5 Gy X-rays with 4 hours interval. Melanin (3 mg/kg) was injected intraperitoneally 2 hours before the first or second radiation dose. The rate of chromosomal aberrations in bone marrow cells was analysed cytologically. Statistical significance of the experimental data was confirmed with t-criterium, where "n" was equal to the number of analysed mice.

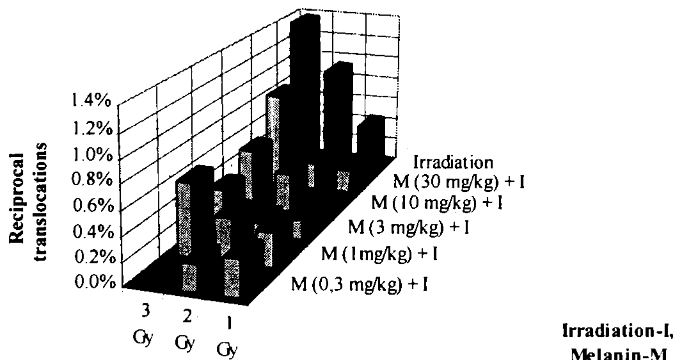
RESULTS AND DISCUSSION

Melanin influence on mutagenic action of chronic irradiation in germ cells.

Investigations of melanin influence on the spontaneous mutation level has demonstrated that melanin itself doesn't possess a mutagenic activity in all concentrations used, even being supplied for 30 days.

Melanin in all concentrations was shown to reduce effectively mutagenic action of acute γ-irradiation. The melanin influence on genetic effect of chronic irradiation was even more effective. The data presented in *Fig.1.* show that the pigment in all used concentrations greatly reduced the percentage of induced mutations at different doses of chronic irradiation.

Fig. 1.: **The melanin influence on chronic irradiation induced mutation frequency in mice germ cells**



It's very difficult to compare antimutagenic activity of melanin under acute and chronic irradiation because in the first case only one injection of melanin has been used, but in the second case melanin has been injected many times (once a day for 10-20 days). Nevertheless it is possible to draw a conclusion that melanin is no less and even more effective under chronic irradiation than under acute one.

It was revealed that melanin activity doesn't depend on concentration used. There is evidence that only small amount of melanin can penetrate into cells, and melanin quantity inside cells doesn't increase with rise in outside melanin concentration — this fact can explain the absence of such correlation.

Radioprotective action of this pigment is associated with its ability to accept and to release electrons and with anti-radical activity. It's clear that when low-dose irradiation is used, the possibility for melanin to catch free radicals or electrons is better.

So, by means of cytogenetic analysis of mice germ cells, we have demonstrated the possibility to decrease genetic effects of chronic irradiation using pigment melanin.

Melanin influence on adaptive response in bone marrow cells

Irradiation of mice in vivo with 1.7 Gy induced 22.0% of aberrations in bone marrow cells. Melanin injection before irradiation significantly decreased mutation rate (*Table 1.*). So, melanin was found to be an effective protector for bone marrow cells. Irradiation of mice with a fractionated dose (0.2 Gy+1.5 Gy with 4 hours interval) led to a significant decrease of the mutation level in comparison with single 1.7 Gy dose effect (*Table 1.*), it means we have found an adaptive response in mice bone marrow cells in vivo. Pigment melanin was used for this adaptive reaction modification.

Table 1.

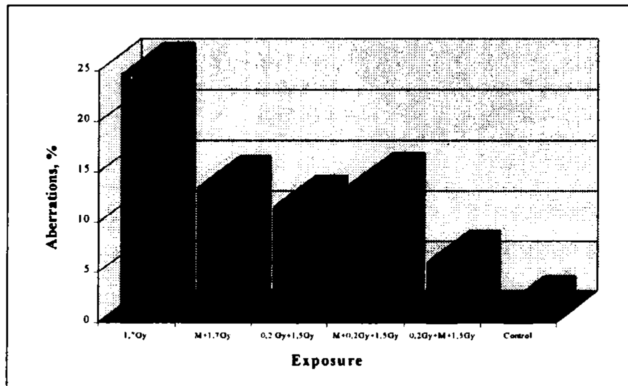
Melanin influence on adaptive response in bone marrow cells

Exposure	Mice number	Cell number	Cells with aberrations, %	Aberrations, %
1.7 Gy	15	1200	16.84	24.58 ± 1.24
M+1.7 Gy	12	800	9.86	13.38 ± 1.20
0.2 Gy+1.5 Gy	16	1200	10.83	11.50 ± 0.92
M + 0.2 Gy+1.5 Gy	8	600	11.33	13.83 ± 1.41
0.2 Gy+M+1.5 Gy	4	200	5.50	6.00 ± 1.68

If melanin was injected before the first conditional dose the mutation level was the same, as after the pigment application before single 17 Gy dose.

This fact can be explained by the absence of an adaptive response. Obviously, melanin removes 0.2 Gy effect completely, that's why an adaptive reaction doesn't appear. But it is possible to propose another explanation that melanin is not capable to decrease mutagenic action of fractionated irradiation. In order to prove, what version is correct melanin application between the first conditioning and the second basic dose was tested. In this case chromosomal aberration level was 4-fold lower than mutagenic effect of single 1.7 Gy dose (table). Thus, both adaptive response and melanin protection has been observed (*Fig. 2.*).

Fig. 2. **Melanin(M) influence on adaptive response in mice bone marrow cells**



These results are unique and make it possible to draw the following conclusions:

1). Melanin protection against chronic irradiation with low dose rate is even more effective than against acute one.
2). Adaptive response can be prevented by radioprotector.
3). Melanin is able to remove completely low radiation dose effect.
4). Adaptive response — the excellent possibility to investigate low doses effects.
5). Mechanism of melanin protection differs from repair system stimulation.

REFERENCES

Barquinero, J.F. – Barrios, L. – Caballin, M.R. – Miro, R. – Ribas, M. – Subias, A. – Egozeue, J. (1996): Decreased sensitivity to the cytogenetic effects of bleomycin in individuals occupationally exposed to ionizing radiation // Mutation Research, V. 354. 81–86.p.

Boothman, D.A. – Meyers, M. – Odegaard, E. – Wang, M.(1996): Altered G_1 checkpoint control determines adaptive survival responses to ionizing radiation. Mutation Research, V. 358. 143–153.p.

Joiner, M.C. – Lambin, P. – Malaise, E.P. – Robson, T. – Arrand, J.E. – Skov, K.A. – Marples, B.(1996): Hypersensitivity to very-low single radiation doses: Its relationship to the adaptive response and induced radioresistance. Mutation Research, V. 358. 171–183.p.

Mosse, I.B.(1982): Modification of mutagenic influence of ionizing radiation in animal sex cells. Advances of Modern Genetics, N. 10. P. 70–103.p.

Mosse, I.B.(1990): Radiation and Heredity. Genetic aspect of antiradiation protection. Minsk, University, 208p.

Mosse, I.B. – Lyakh, I.P.(1994): Influence of melanin on mutation load in Drosophila population after long-term irradiation. Radiation Research, V. 139. N. 3. P. 356–358.p.

Mosse, I. – Plotnikova, S. – Kostrova, L. – Maksimenya, I. – Dubovic, B.(1997): Genetic effects of melanin. 7th Congress of the European Society for Photobiology. Congress Centre, Stresa, Italy, 66.p.

Salone B. – Preyazzoli V. – Bosi A. – Olivieri G.(1996): Interaction of low-dose irradiation with subsequent mutagenic treatment: role of mitotic delay. Mutation Research V. 358, P. 155–160.p.

Savchenko, V.K. – Kasinskaya, S.I. – Dobina, A.I. – Mosse, I.B. – Kuzhir, T.D. – Mikhalevich, L.S. (1985): Selection and mutation process in populations. Minsk, Nauka i tekhnika, 224p. (in Russian)

Tedeschi, B. – Caporossi, D. – Vernole, P. – Padovani, L. – Mauro, F.(1996): Do human lymphocytes exposed to the fallout of the Chernobyl accident exhibit an adaptive response? III.

Challenge with bleomycin in lymphocytes from children hit by the initial acute dose of ionizing radiation. Mutation Research, V. 354. 77–80.p

Authors' address: *Mosse I.B. – Plotnikova S.I. – Kostrova L.N. – Molophei V.P.*: Institute of Genetics and Cytology of the National Academy of Sciences, Akademicheskaya St. 27, 220072, Minsk, Belarus

Dubovic B.V.: Scientific Medical Radiological Centre, Russian Academy of Med. Sciences, Obninsk, Russia

CHROMOSOME ABNORMALITIES IN A CANINE MAMMARY CARCINOMA CELL LINE ANALYZED USING CANINE CHROMOSOME-SPECIFIC PAINTS

TAP, O.T. — RUTTEMAN, G.R. — ZIJLSTRA, C. — DE HAAN, N.A. — BOSMA, A.A.

In a previous study (*Mellink et al.*, 1989), we analyzed three cell lines derived from two metastases of a mammary carcinoma in a 12 years old female dog with the use of conventional cytogenetic techniques (Giemsa staining and GTG-banding). The modal chromosome number in these cell lines was 76, the normal number of chromosomes of the dog being 2n=78. In addition to the two biarmed chromosomes that we considered to be the two X chromosomes, we found a small metacentric marker chromosome. From its symmetrical banding pattern, we concluded that this chromosome might be an isochromosome, without being able to detect its origin.

Recently, we have reinvestigated one cell line by fluorescence *in situ* hybridization with canine chromosome-specific paints. We received these paints from the Sanger Centre (Hinxton, Cambridgeshire, U.K.), where they have been produced from normal flow-sorted canine chromosomes (*Langford et al.*, 1996). All 33 paints available (A-Z and aa-ff) were applied.

The cell line, during further passages, showed a modal chromosome number of 77. Ninety percent of the cells contained three biarmed chromosomes, and 10% of the cells contained four biarmed chromosomes. We observed aberrations with the following paints: paint H (hybridizes to chromosomes 8 and 11; *Langford et al.*, 1996), paint L (hybridizes to chromosomes 13 and 15; *Langford et al.*), paint dd (hybridizes to chromosome 37; *Breen et al.*, this issue), paint ee (hybridizes to chromosome 38; *Breen et al.*), and paint X (hybridizes to the X chromosome; *Langford et al.*, 1996). These aberrations, all involving whole chromosomes, are: — loss of an X chromosome, — formation of an isochromosome 8 or 11 (in 10% of the cells), — formation of an isochromosome 13 or 15, and — centric fusion of a chromosome 37 and a chromosome 38 (probably the marker chromosome of *Mellink et al.*).

For the dog, mapping data are available with respect to three genes that are known to be related to breast cancer development in humans: BRCA1, ERBB2, and TP53. None of these genes is located on a chromosome found to be abnormal in our cell line.

The results of this study show that chromosome painting is a most useful tool for the cytogenetic analysis of canine tumor cells.

REFERENCES

Breen, M. – Reimann, N. – Bosma, A.A. – Ladon, D. – Zijlstra, C. – Bartnitzke, S. – Switonski, M.– Long, S.E. – de Haan, N.A. – Binns, M.M. – Bullerdiek, J. – Langford, C.F.(1998): Committee for the Standardized Karyotype of the Dog *(Canis familiaris)*: Standardization of chromosomes nos. 22–38 of the dog *(Canis familiaris)* with the use of canine painting probes (this issue).

Langford, C.F. – Fischer, P.E. – Binns, M.M., – Holmes, N.G. – Carter, N.P.(1996): Chromosome-specific paints from a high-resolution flow karyotype of the dog. Chrom. Res., 4. 115–123.p.

Mellink, C.H.M. – Bosma, A.A. – Rutteman, G.R.(1989): Cytogenetic analysis of cell lines derived from metastases of a mammary carcinoma in a dog. Anticancer Res., 9. 1241–1244.p.

Authors' Address: *Tap, O.T. – Zijlstra, C. – De Haan,' N.A. – Bosma, A.A.*: Department of Cell Biology and Histology, Faculty of Veterinary Medicine, Utrecht University, P.O. Box 80.157. 3508 TD Utrecht, The Netherlands

Rutteman, G.R.: Department of Clinical Sciences of Companion Animals, Faculty of Veterinary Medicine, Utrecht University, P.O. Box 80.154, 3508 TD Utrecht, The Netherlands

DETECTION OF CHROMOSOME BREAKS AND GENETIC PATHOLOGY BY MOLECULAR GENETICS

MONTEAGUDO, L.V. — POSTIGLIONI, A. — LLAMBI, S. — ARRUGA, M.V.

SUMMARY

Human and animal chromosome fragile sites have been associated to a large variety of anomalies, including oncological diseases and different metabolic and functional disturbances. In the human species, trinucleotides repeats are known since 1991 to be in the origin of such fragile sites. Unequal meiotic crossing-over expands such repeats. When a certain amount of repeats accumulate, chromosome instability arises together with its phenotipical alterations. By using the RED methodology, it's now possible to detect not only instable repeats but "premutated" individuals, carrying a potentially dangerous amount of repeats. Our main goal is the development of standard procedures to evaluate candidates to reproduction. In this way, not only chromosomally instable animals but also premutated individuals could be erradicated. Future genetic degradation in those populations should be thus avoided by not using premutated animals in Artificial Insemination.

INTRODUCTION

Chromosomal unstability has been observed in man and animals. Human Fragile X chromosome is associated with mentai retardation, the most common form of inherited mentai retardation. The expansion of CGG repeats in the FRM1 locus is involved in the mutation responsible for this syndrome (*Kremer et al.*, 1991). In the light of molecular analysis (*Hecimovic et al.*, 1997), human individuals can be normal (from 6 to 50 CGG repeats), premutated (from 50 to 200 CGG copies) or mutated (more than 200 CGG). In the last years, other trinucleotid expansions have been found to

be associated to different diseases while potentially dangerous expansions have been identified in different human genome positions (*Haaf et al.,* 1996). As they appear in exons, their expansions alterate normal transcription and give place to different phenotype modifications (*Austin et al.,* 1991).

Animal chromosome fragile sites have been widely studied. Some of them must be induced by additives in culture medium, while other appears in a spontaneous way (*Llambi et al.,* 1998).

The aim of the present study is to detect possible trinucleotide expansions in bovine chromosomes by using a cytogenetic and molecular approach. The X chromosome is used as a model. Several fragile sites have been detected in this chromosome.

MATERIAL AND METHODS

Individual genomic dot slots were obtained by using Bio Rad Bio-Dot SF Microfiltration Apparatus 200 ng of DNA per individual. Digoxigenin-5'-$(CGG)_5$ was hybridized to the slots following *Hecimovic et al.* (1997). Stringent washing (20 minutes at 48 °C in 2 x SSC,0.1% SDS; 20 minutes at 48 °C in 1 x SSC,0.1% SDS; 20 minutes at RT in 0.1 x SSC,0.1% SDS) is then applied. Slot blots size is constant, so that signal intensity is accurately determined. Hybridization signals are analysed using a Bio-Rad Gel Doc 1000 Gel Documentation System provided with density quantification software.

Bovine chromosome spreads were obtained following *Moorhead et al.* (1968). For fragile sites (spontaneous or induced) obtention (*Llambi et al.,* 1998).

FISH was performed using bio-5'-$(CGG)_5$–3'-bio as a probe. Hybridization medium contains 50% formamide and 10% dextran sulfate in 2 x SSC. Stringence washing is performed in 50% Formamide in 2 x SSC at 45 °C (three times for three minutes each time) followed by 5 washings in 2 x SSC at 45 °C (two minutes each) Biotine signal detection is based on a double round of signal amplification by FITC-avidine and antiavidine biotinilated antibodies (*Pinkel et al.,* 1986). Propidium iodide is used as a counterstain.

FISH results were analysed using a Zeiss LSM confocal microscope. Argon laser and two different photomultipliers for fluoresceine and propidium iodide emissions were used. Background is eliminated from final images by multiple average laser scanning.

RESULTS AND DISCUSSION

Slot dot hybridization with $(CGG)_5$

Clear individual differences are obtained, since optical density is determined to be up to three times higher in some animals than in others (*Fig. 1.*). As equal amounts of undigested DNA are present in each slot, a different amount and/or size of CGG expansions can be blamed for such differences. This result points to the existence of (CGG) expansions in some individuals. At least, a potential risk of expansions exists.

Fig. 1.: **Individual bovine DNA slot blots after hybridization to $(CGG)_5$. The first individual shows a three times more intense signal**

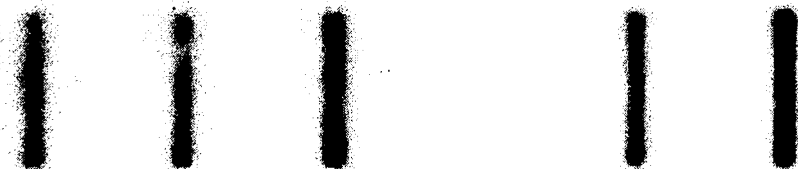
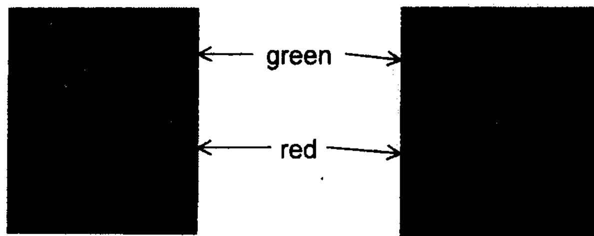


Fig. 2a., 2b.: **Bovine X chromosomes showing fluoresceine hybridization signal with $(CGG)_5$ Propidium Iodide counterstained chromosomes are presented in red colour**



FISH studies

As seen in figures 2a and 2b, three different points on bovine X chromosome show positive signals. Two of them are located close to both telomeres while the last one is in the long arm. These three loci are coincident with some of the previously detected fragile sites in this chromosome.

CONCLUSIONS

— Different bovine individuals carry different amounts or sizes of CGG expansions.
— $(CGG)_5$ hybridizes to three points on bovine X chromosome. These points are coincident with fragile sites.

ACKNOWLEDGMENTS

This work supported by grants of the AGF97-1072, Comisión Interministerial de Ciencia y Tecnología (CICYT), Espana and CIDEC. Universidad de la República Oriental del Uruguay.

REFERENCES

Austin, M.J.(1991): Cancer Genetics & Cytogenetics, 54. 71–76.p.
Haaf,T. – Sirugo, G. – Kidd, K.K – Ward, D.C.(1996): Nature Genetics, 12. 183–185.p.
Hecimovic, S. – Barisic, I. – Muller, A. – Petkovic, I. – Barie, I. – Ligutic, I. – Pavelic, K.(1997): Clinic. Genet., 52. 147–154.p.
Kremer, E.J. – Pritchard, M. – Lynch, M. – Yu, S. – Holman, K. – Baker, E. – Warren, S. – Schlessinger, D. – Sutherland, G. – Richard, R.(1991): Science, 252. 1711–1714.p.
Llambi, S. – Guevara, K. – Rincón, G. – Nunez, R. – Arruga. M.V. – Postiglioni, A.(1998): XIIIth Europ. Colloq. Cytogenet. Domest. Anim., Budapest
Moorhead, P.J. – Newell, P.C. – Mellman, W.J. – Battips, D.A – Hungerford, D.A.(1960): Exp. Cell Res., 20. 613–616.p.
Pinkel, D. – Straume, T. – Gray, J.W.(1986): Proc. Natl. Acad. Sci., USA, May, 83. 9. 2934–2938.p.

Authors' address: *Monteagudo, L.V. – Arruga, M.V.*: Laboratory of Cytogenetics and Molecular Genetics. Faculty of Veterinary
Miguel Servet 177. 50.013-Zaragoza (Spain)
Postiglioni, A. – Llambi, S.: Laboratorio de Citogenética de Animales Domésticos. Area de Genética. Facultad de Veterinaria. Universidad de la República, Montevideo, (Uruguay)

ELJÖTT AZ IDŐ A KLINIKAI CITOGENETIKA ÚJJÁÉLEDÉSÉRE?

GUSTAVSSON, I.

Sok évvel ezelőtt a háziállat citogenetika fejlődése éppen a kromoszóma rendellenességeknek a fertilitásban, a torzfejlődésben játszott szerepének leírásával indult meg. Ennek következtében számos szarvasmarha állományban kiszelektálták az 1;29 centrikus fúziót, a sertésben talált sok reciprok transzlokáció miatt, pl. Franciaországban, bevezették a mesterséges termékenyítésben használt kanok citogenetikai szűrését. A tudományág az utóbbi időben elsősorban a kromoszóma térképezésre koncentrált. A molekuláris citogenetika korszerű eszközeinek klinikai bevezetése azonban egyre sürgetőbb. A sertésnél megfigyelt sok reciprok transzlokáció a magas mutációs rátára utal. A szarvasmarhában igen kevés hasonló rendellenességet írtak le eddig, feltehetően főleg a nehezen értékelhető kariotípusa miatt. Különösen ebben a fajban igen veszélyes lehet az esetleges rendellenességek átörökítése a lehetséges nagy utódpopuláció miatt.

NATE FECHHEIMER ÉS AZ ŐT KÖVETŐ GENETIKAI KUTATÁSOK KORSZAKA

STRANZINGER, G.F.

A Háziállat Citogenetika Kollokviumok gyakori résztvevője, a szakterület egyik legkiemelkedőbb alakja és az Ohio Állami Egyetem (Columbus, USA) professzora hunyt el 1992-ben. Munkássága sok fiatal kutató számára például szolgál. Pályáján végigjárta a kvantitatív és kvalitatív genetika (citogenetika, molekuláris genetika) számos területét. Publikációinak (több mint 150) végigtekintése igen tanulságos lehet a következő kutatók generációja számára: a szarvasmarha színöröklődése, az embrionális elhalások okai, heteroploidia az emlősök és házityúk gamétáiban és embrióiban, kromoszómaszerkezeti átrendeződések és polimorfizmusok következményei.

Azon ritka kutatók közé tartozott, akik át tudták tekinteni a történelem, a tudomány és mezőgazdaság területeit, így igen komplex képet és jóslatokat adva a diákoknak és pályatársaknak. Az őt követő korszak technikai vívmányai (különösen a géntérképezés) oly mértékben megváltoztatta a tudományág arculatát, hogy az új diszciplínák szintézise napjainkban elkerülhetetlenné válik (pl. új tenyészértékbecslési módszerek).

A SZARVASMARHA KROMOSZÓMÁLIS NÓMENKLATÚRÁJÁBAN LÉVŐ ELLENTMONDÁSOK FELOLDÁSA

IANNUZZI, L.

Már számos tanulmány rámutatott arra, hogy a marha, a juh és a kecske több alkalommal módosított standard kariotípusában több hiba van, ami abból fakad, hogy a felállításkor viszonylag alacsony felbontású sávozott kariogrammok álltak rendelkezésre, másrészt az eltérő sávozási technikákat alkalmaztak (G- ill. R-sáv). A problémás kromoszómák biztos azonosítására a következő módszereket alkalmazták:

a./ A vitatott kromoszómák nagy felbontású sávozással történő összehasonlítása a szarvasmarhafélék markerkromoszómáival (a bivaly és a marha centrikus fúziós kétkarú kromoszómái).

b./ Marker próbák *in situ* hibridizációja a bivaly markerkromoszómáihoz

c./ Az U8 marker próba hibridizációja és NOR (nukleólusz organizáló régió) festés a marha 27. kromoszómáján és a bivaly azzal homológ 24. kromoszómáján.

d./ ZOO-FISH (humán kromoszóma-specifikus könyvtár próbákkal történő hibridizálás) a bivaly 1p. 4p, 5p és 24. kromoszómákra. Ezekkel a vizsgálatokkal sikerült egyértelműen feloldani az eddigi ellentmondásokat.

ELTÉRŐ EXPRESSZIÓT MUTATÓ CDNS SZEKVENCIÁK *IN SITU* HIBRIDIZÁCIÓJA SZARVASMARHA KROMOSZÓMÁKON

SCHWERIN, M. — GOLDAMMER, T. — BRUNNER, R.M.

Az állatgenetika jelenlegi egyik legnagyobb törekvése a mennyiségi tulajdonságokat meghatározó gének (QTL) térképezése a különböző háziállatokban. Az eddigi vizsgálatok ígéretesek, bár a térképezés csak több tíz cM pontosságot nyújt. Gyakorlati hasznosításra csak az ad lehetőséget, ha nagyobb pontosságú térképezésre van lehetőség. Ebben a vizsgálatban egy alternatív lehetőséget alkalmaztak a fenotípusos varianciát meghatározó különböző expressziót mutató gének azonosítására. Adott tulajdonságban eltérő fenotípust (tejelő ill. hús-tej típus) mutató egyedektől a feltehetően eltérő génexpresszió mintázatot mutató szövetekből izolált mRNS-ből cDNS könyvtárakat hoztak létre, amelyekkel szubtraktív hibridizációt végeztek: az egyik fenotípusból származó jelölt cDNS-t előhibridizálták a másik változat cDNS-ével (kompetitorként), majd *in situ* hibridizációval azonosíthatták azokat a kromoszómaszakaszokat, amelyek nagy valószínűséggel hordozzák a keresett QTL-eket.

PÉLDA A FELLAZÍTOTT KROMATINROSTOKON VÉGZETT *IN SITU* HIBRIDIZÁCIÓ (FIBER-FISH) ALKALMAZÁSÁRA A GENOM FINOMSZERKEZETI VIZSGÁLATÁBAN: A MARHA CYP19 LOKUSZA

BRUNNER, R.M. — FÜRBASS, R. — GOLDAMMER, T. — VANSELOW, J. — SCHWERIN, M.

A CYP-19 lokusz az aromatáz citokróm P450 gént (CYP-19) és egy homológ pszeudogént (CYP19φ) hordoz. Mindkét gén a 10q26 kromoszómaszakaszon található. A restrikciós analízis szerint a CYP19 60 kb méretű és távolsága a pszeudogéntől több mint 15 kb. Az érintett terület analízisére a fiber-FISH módszert alkalmazták. Ezzel az interfázisos sejtmag anyagát lazítják fel annyira, hogy a felszabadult kromatinszálon a közeli gének igen finom térképezése és egymáshoz viszonyított helyzetük meghatározása lehetséges. A megfigyelések arra utalnak, hogy a CYP19 gén és a pszeudogén 130 kb körüli helyet foglalnak el és a két exon távolsága 20 kb.

A SZARVASMARHA 23. KROMOSZÓMÁJÁN ELHELYEZKEDŐ NAGY HISZTOKOMPATIBILITÁSI KOMPLEX (MHC) FIZIKAI TÉRKÉPEZÉSE

GOLDAMMER, T. — HESS, M. — BRUNNER, R.M. —
BISHOP, M.D. — GELHAUS A. — SCHWERIN, M.

Korábbi genetikai és fizikai térképezéssel kimutatták, hogy a szarvasmarha 23. kromoszómáján elhelyezkedő MHC I., II., és III. osztályai homológok a más emlősök kromoszómáival, mint pl. az egér 17. és az ember 6p kromoszómájával. Ezekkel ellentétben azonban a marha MHC génjei két külön régióba csoportosulnak, amint a fizikai és genetikai térképezési eredmények mutatták. Jelen vizsgálatok szerint a marha MHC II.b osztálya a 23q12–13 kromoszómális régióban van elkülönülve, míg a II.a osztály génjei az I. és III. osztály szoros közelségében vannak a 23q21–22 régióban.

ROBERTSON TRANSZLOKÁCIÓK MOLEKULÁRIS ANALÍZISE

JOERG, H. — RIEDER, S. — SUWATTANA, D. — STRANZINGER, G.

A szarvasmarhafélék leggyakoribb kromoszómális átrendeződései az akrocentrikus kromoszómák fúziója (Robertsoni transzlokáció). Általában ezeknek a transzlokációknak nincs fenotípusos hatása és szegregálódásuk megfigyelhető az egymást követő nemzedékekben. A heterozigóta hordozóktól származó utódoknál azonban eltérő arányok találhatók a különböző termékenyülési és embriómortalitási mutatók miatt. A transzlokációs kromoszómák szegregálódásának tanulmányozására különböző hordozó egyedeket párosítottak. Egy állatban két transzlokáció (1;29 és 14;20) együttes előfordulását találták. A Robertsoni transzlokációk vizsgálatára *in situ* hibridizációt végeztek alphoid szatellit DNS (a centromerikus régió repetitív szekvenciái) próbával. A szarvasmarha 1,715 szatellit DNS próbája az összes akrocentrikus kromoszómához hibridizált, míg az X, Y és az 1;29 transzlokációs kromoszómák nem mutattak jelet. A 14;20 transzlokációs kromoszóma erős kettős jelet adott és két centromer jelenlétére utal. Az alphoid DNS nem szükséges a centromer funkcióhoz, de a húzófonalak tapadási helyének feltételezhetően eltérő száma okozhatja a gyakoribb nondiszjunkciót (kromoszómák szétválási zavara az osztódás során) és a csökkent termékenységet. A fúziós kromoszóma kapcsoltsági csoportjába tartozó mikroszatellit markerekkel kimutatható az 1;29 transzlokációt hordozó bika. A Brown Swiss és a Simmental fajtákban vizsgálva a homo- és heterozigóta hordozókat, egy informatív allélkombináció segítségével megállapították, hogy az 1;29 transzlokáció azonos eredetű a két fajtában, ezért valószínűleg ezen fajták kialakulása előtt lépett fel.

BOVIDÁK TCR GÉNJEINEK MOLEKULÁRIS ANALÍZISE ÉS FISH-TÉRKÉPEZÉSE

LIPSI, M.R. — ANTONACCI, R. — MASSARI, S. —
DE CARO, F. — MICCOLI, M. — CICCARESE, S.

A szerzők a juh TCRA, TCRD és TCRG régióit reprezentáló öt genomiális klónt izolálták és jellemeztek. Ezek mindegyikét felhasználták limfocita-tenyészetekből preparált juh kromoszómákon végzett fluoreszcens *in situ* hibridizációs kísérletekben. Ha a lambda-LVAI7 (TCRA) és a lambdaMCD (TCRD) klónokat együtt hibridizáltatták, specifikus jeleket találtak a juh 7. kromoszóma hosszú karjának 14–22. sávjában. A két genomiális klón együttes térképezése a 7. juh kromoszómán az első FISH-bizonyíték amely a TCRA és TCRD gének együttes elhelyezését mutatja *Bovidákban.*

Ha a lambda-6A3(TCRG I) és a lambda-17A5, vagy lambda-18A6 (TCRG2) klónokat használták az együttes hibridizációs kísérletekben, a juh 4. kromoszómáján két különálló jelet észleltek. Azt is demonstrálták, hogy a TCRG 1 és a TCRG 2 gének a kecske, a szarvasmarha és a házibivaly esetében is a homológ kromoszómákon oszlanak meg. Feltételezhető egy átrendeződés, mely a 4. kromoszómán a *Bovidae* család szétválása előtt történt.

HUMÁN KROMOSZÓMÁLIS RÉGIÓK NAGYMÉRVŰ KONZERVÁCIÓJÁNAK KIMUTATÁSA BIVALY KROMOSZÓMÁKON ZOO-FISH TÉRKÉPEZÉSSEL ÉS R-SÁVOZÁSSAL

IANNUZZI, L. — DI MEO, G.P. — PERUCATTI, A. — BARDARO, T. — FERRARA, L.

Humán kromoszóma-specifikus könyvtár próbákat hibridizálva bivaly replikációs R-sávozott kromoszómákon megfigyelhető volt, hogy az evolúció során történt számos szerkezeti átrendeződés ellenére, a humán kromoszómaszakaszok nagymértékben konzerválódtak a bivaly kromoszómáin.

HUMÁN EXPRESSZÁLÓDÓ SZEKVENCIA AZONOSÍTÓK (EST-K) ALKALMAZÁSA A HATÉKONY ÖSSZEHASONLÍTÓ GÉNTÉRKÉPEZÉSHEZ

LAHBIB-MANSAIS, Y. — DALIAS, G. — MILAN, D. —
YERLE, M. — ROBIC, A. — GYAPAY, G. — GELLIN, J.

A QTL-eken (mennyiségi tulajdonságokat meghatározó lokuszok) előforduló gének azonosításához ezeken a területeken minél több kódoló szekvenciát kell lokalizálni. A kódoló szekvenciák általános keresési módszere a szövetspecifikus cDNS könyvtárakból származó kiónok izolálása és jellemzése. Akkor is érdemes ezt a megközelítést alkalmazni a sertésben, ha ez óriási munkát is jelent, miként az volt az emberi genom esetében is. Az emlős fajok között a kromoszómaszakaszok konzerválódása lehetővé teszi a humán adatok felhasználását. A sertés és a humán kromoszómák közötti nagy homológiát mutatja a két géntérkép. Egyik jellemző példája a halotán gén esete, amit a humán 19. és a sertés 6. kromoszómájának összehasonlító géntérképezése alapján izoláltak. Hasonló módszert alkalmaznak jelenleg az RN (húsminőséget befolyásoló) génre, ami a sertés 15. kromoszómáján található. A humán genomban expresszálódó szekvenciák azonosítóinak (EST) meghatározására a *Généthon* program besugárzással előállított szomatikus sejthibrid paneleket alkalmaz, amelyeket ugyanakkor sertés, marha és kutya DNS alkalmazásával is tesztelnek PCR-ral. Ez az ún. ZOO-PCR azt mutatja, hogy az eddig tesztelt 10.00 EST kb. 10%-a képes DNS-t amplifikálni legalább egy másik fajban, ezért az eredeti humán primereket alkalmazzák a különböző fajokban. A heterológ PCR-ral eddig 344 humán EST-t teszteltek a sertéssel és közülük 186 primer pár sokszorozott a sertésre specifikusan. Ezt követően 65 EST-t lokalizáltak sertés kromoszómákon PCR-ral a sejthibrid paneleken.

SERTÉS SZOMATIKUS SEJTHIBRID PANEL ELŐÁLLÍTÁSA BESUGÁRZÁSSAL: A SERTÉS GÉNTÉRKÉPEZÉS ÚJ ESZKÖZE

YERLE, M. — PINTON, P. — ROBIC, A. — HAWKEN, R. —
ALEXANDER, L. — BEATTIE, C. — SCHOOK, L. — MILAN, D. — GELLIN, J.

A sertés szomatikus sejthibrideket elsősorban a genetikai és citogenetikai térképek összekapcsolására alkalmazzák. Bár a sertés géntérkép még nem olyan részletes, mint a humán, de bizonyos kromoszómális régiókban hatékonyabb eszközre van szükség a gének sorrendjének vizsgálatára ill. új markerek kimutatására. Újabban, a humán géntérképezésben, a besugárzással előállított szomatikus sejthibridek (RH) jelentettek előrelépést. Specifikus DNS szekvenciák jelenléte vagy hiánya főleg PCR-ral kerül kimutatásra. A gének helyzete ill. a markerek közötti távolság statisztikai módszerekkel állapítható meg a közöttük fellépő kromoszómatörések gyakorisága alapján. Besugárzott sertés limfocita vagy fibroblaszt sejtek recipiens sejtekkel történő fúziójával 152 hibrid kiónt állítottak elő. A vonalak citogenetikai jellemzése többféle módszerrel történt: 1./ Fluoreszcens *in situ* hibridizáció SINE (a sertés genomban elszórtan helyeződő repetitív szekvenciák) próbával a sertés kromoszóma töredékek számának és méretének megállapítására. 2./ Kromoszóma-painting (egy vagy több kromoszómára specifikus próbakeverék, amely egész kromoszómán ad jelet) fordított módszere: interSINE PCR — a genomban egyenletesen elszórtan elhelyezkedő repetitív szekvenciák közötti egyes kromoszómákra specifikus szakaszok PCR-ral történő felsokszorozása és hibridizálása sertés kromoszómákhoz. 3./ Dupla PRINS (primerekkel indított *in situ* DNS szintézis) a sertés kromoszómák két csoportjára annak a megállapítására, hogy a kromoszómatöredékek saját centromerjükkel vannak jelen vagy kromoszóma átrendeződésekben vettek részt. Ezen túlmenően, 32 markeren alapuló PCR-ral vizsgálták a sertés genom arányát a kiónokban: a 152 vonal közül 38-ban ez az arány 10% alatti, 19-ben 10 és 20% közötti és 95-nél 20% feletti. Ezen vizsgálatok alapján 126 klónt tartottak meg a térképezési célokra.

A JELZETT DNS-PRÓBÁK FELISMERÉSÉNEK ALTERNATÍV MÓDSZERE AZ *IN SITU* HIBRIDIZÁCIÓVAL VÉGZETT SERTÉS-GÉNTÉRKÉPEZÉSBEN

MUSILOVA, P. — KUBICKOVA, S. — VOZDOVA, M. — RUBES, J.

A nem-rádioaktív, nem-fluoreszcens *in situ* hibridizációt, mint a sertés géntérképezés lehetséges módszerét ismertetik. A megközelítés a hibridizált biotinnel jelzett próba peroxidázhoz kötött avidinnel történő megtalálásán és a peroxidáz diaminobenzidin szubsztráttal történő, sötét csapadékot eredményező reakcióján alapul. Egyetlen gén térképezése esetén a módszer érzékenységének növelésére a peroxidáz-jel erősségét kétszeresen fokozták: immunológiai módszerrel, biotin-avidinnel, majd a peroxidázzal katalizáltatva biotin-tiramid válik ki. Ezzel a módszerrel a sertés ciklin-függő kináz 4 (CDK4) két 2 kb hosszúságú próbáját és a c-MOS proto-onkogént a sertés 5p12, illetve 4q14-15 kromoszóma régiókra térképezték. Ennek a nem-rádioaktív, nem-fluoreszcens *in situ* hibridizációs módszernek az az előnye, hogy gyors, biztonságos, érzékeny és pontos.

KROMOSZÓMÁLIS MARKEREK ALKALMAZÁSA A SERTÉS HÚSTERMELÉSÉT ÉS A HÚSMINŐSÉGÉT MEGHATÁROZÓ QTL-EK (MENNYISÉGI TULAJDONSÁGOKAT MEGHATÁROZÓ LOKUSZOK) AZONOSÍTÁSÁRA

KOMISAREK, J. — SZYDLOWSKI, M. — SWITONSKI, M. — KURYL, J.

A hústermelést, és -minőséget meghatározó QTL-ek azonosítása egyik része a Lengyel Sertés Géntérképezési Programnak. A három generációs referenciacsalád lengyel nagy fehér kocák és zlotnickai pettyes kanok keresztezésén alapul. 40 mennyiségi tulajdonságot analizáltak az F2 kanokon párhuzamosan az I., II. típusú genetikai markerek és kromoszómális markerek szegregációs vizsgálatával.

A sertés kariotípusában az akrocentrikus (13–18.) kromoszómák konstitutív heterokromatinjának és a nukleolusz organizáló régió — NOR — (8., 10. kromoszómákon) méretbeli polimorfizmusa szolgált kromoszómális markerként. Ezeket a polimorfizmusokat az F1 és F2 generációkban vizsgálták. Két méretbeli variáns a nagy (++) és kicsi (+) volt megállapítható a 10., 15., és 17. kromoszómákon. A markerek szegregációs analízise az F1 generáció informatív párosításaiból származó utódokon történt. 52, 60 és 60 F2 utód állt rendelkezésre, erre a célra a 10., 15., és 17. kromoszómákat illetően. A vizsgálatok analízise azt mutatta, hogy a 15. kromoszóma C-sáv polimorfizmusa összefüggésben van a hosszú hátizom súlyával. Hasonlóképpen kapcsolatot találtak a 10. kromoszóma NOR polimorfizmusa és a sonka mérete, valamint a 150. napos kori testsúly között. Ezek a megfigyelések a vizsgált tulajdonságokra ható QTL-ek jelenlétét sugallják a 10. és 15. kromoszómákon.

MARHA KROMOSZÓMA-FRAGMENT SPECIFIKUS KÖNYVTÁRAK — A NAGY FELOLDÁSÚ FRAGMENT-SPECIFIKUS MARKER-TÉRKÉPEK MEGALKOTÁSÁNAK ESZKÖZEI

GOLDAMMER, T. — BRUNNER, R.M. — KÜHN, C. — WEIKARD, R. — SCHWERIN, M.

Módszert írnak le a kromoszóma-fragment specifikus könyvtárak létrehozására és jellemzésére. A módszer magában foglalja egyetlen, G-sávozással azonosított kromoszóma-sáv izolálását mikrodisszekcióval, a levágott kromoszóma fragment- DNS sokszorosítását DOP-RCR-ral, az így nyert DNS-szekvenciák plazmid-klónozását, valamint a PCR-termékek faj- és kromoszóma-specifikusságának, illetve a létrehozott könyvtárak fajspecifikusságának ellenőrzését. Az ilyen könyvtárak értékes eszközök kromoszóma-fragment specifikus DNS-markerek izolálására.

A PRION GÉN (PRNP) KROMOSZÓMÁLIS LOKALIZÁCIÓJA SZARVASMARHA, BIVALY, JUH ÉS KECSKE FAJOKBAN, FLUORESZCENS *IN SITU* HIBRIDIZÁCIÓVAL ÉS R-SÁVOZÁSSAL

IANNUZZI, L. — PALOMBA, R. — DI MEO, G.P. — PERUCATTI, A. — FERRARA, L.

A prion gén (PRNP) mutációi összefüggésbe hozhatók számos faj neurodegeneratív betegségével, az ember Creutzfeldt-Jakob kórjával, a marha BSE szindrómájával és a juh scrapie-vel. A gént a humán genomban a 20p12-pter kromoszómaszakaszon térképezték, míg a marhában eddig csak a 13. kromoszómán helyeződő U11 kapcsoltsági csoporthoz tudták rendelni, a pontos lokalizáció hiányzott. A PCR-ral előállított próba *in situ* hibridizációs jelét replikációs R-sávozott kromoszómákon határozták meg. A szarvasmarha, a juh és a kecske 13., a bivaly 14. kromoszómáján mutatkozott hibridizációs jel. Ezek a kromoszómák a 4 faj között homológok. A megfigyelések megerősítik a prion gén U11 kapcsoltsági csoporthoz tartozását. A kecske 13. és a bivaly 14. kromoszómáján ez az első molekuláris marker, amely közvetve biztosítja a marha U11 kapcsoltsági csoportjába tartozó (expresszálódó) gének markerekként való felhasználását.

A PRION GÉN (PRNP) TÉRKÉPEZÉSE SZARVASMARHA, JUH ÉS HUMÁN KROMOSZÓMÁKON

CASTIGLIONI, B. — COMINCINI, S. — DRISALDI, B. —
HILLS, D. — WILLIAMS, J.L. — FERRETTI, L.

A prionok az emlősök idegrendszerének fertőző degenerációját okozó részecskék. Ezek a betegségek a juhban a scrapie, a marhában a BSE és az ember Creutzfeldt-Jakob kórja és az öröklődő, végzetes inszomnia (FFI). A prionok egyetlen génnek a termékei, ami nagymértékben konzerválódott az evolúció során. PCR-ral előállított és jelölt próbával végzett *in situ* hibridizáció a prion gént a marha 13q17 és a juh 13q17/q18 kromoszómaszakaszán mutatta ki. A két fajból származó próbák ugyanoda hibridizáltak, ami a lokusz nagyfokú evolúciós konzervációját mutatja a két faj között. A humán genomban a 20p12/p13 kromoszómaszakaszon jelentkezett a hibridizációs jel. A humán próba fehérjekódoló része (738bp), a juh és szarvasmarha kariotípusban ugyanarra a helyre hibridizálódott, mint a homológ próbák.

A SZARVASMARHA ÉS A JUH PRO C, EN-1, ALPI, TNP1 ÉS IL-1B GÉNJEINEK ÖSSZEHASONLÍTÓ TÉRKÉPEZÉSE

LOPEZ-CORRALES, N.L. — SONSTEGARD, T.S — SMITH, T.P.L

Az 5 gén citogenetikai lokalizációjának az volt a célja, hogy ezeknek a géneknek a humán 2. kromoszómán való együttes öröklődése a juhban ugyanúgy szétvált-e, mint a szarvasmarha esetében. A PRO C, EN-1, ALPI, TNP1 gének próbái juhban a 2. kromoszóma hosszú karján adtak jelet, míg az IL-1b a 3. kromoszóma rövid karján. Szarvasmarhában az EN-1 a 2. kromoszómán, az IL-1b a 11. kromoszómán volt fellelhető. Az 5 vizsgált gén helyeződése a két fajban hasonló rendben figyelhető meg, ami azt mutatja, hogy együttes öröklődésük és a kromoszómális átrendeződések, amelyek humán és szarvasmarha esetekben az eltérést okozták, a juhban is fennmaradt az evolúció során.

A JUH GÉNTÉRKÉPEZÉSE SZOMATIKUS SEJTHIBRIDEKKEL: A 4., 5. ÉS 6. KROMOSZÓMA JELLEMZÉSE

TABET-AOUL, K. — SAIDI-MEHTAR, N. — LANTIER, I. — VAIMAN, D. — LANTIER, F.

A 24 hörcsög-juh szomatikus sejthibrid vonalból álló panelt már korábban is használták géntérképezésre. Mivel a citogenetikai vizsgálat nem volt lehetséges, a panel juh-kromoszóma tartalmát kellett megállapítani. A juh 26 kromoszómáján megoszló 90 juh ill. marha mikroszatellittel vizsgálva a panel a juh minden kromoszómájával összeköthető volt. A következő lépésben azt kellett megállapítani, hogy a juh kromoszómák egészükben vagy csak részleteikben vannak jelen a sejthibrid vonalakban. Erre a célra újabb juh, kecske és marha mikroszatelliteket használtak, amelyeket polimeráz láncreakcióval (PCR) vizsgáltak a sejtvonalakban juh és hörcsög genomiális DNS-t állítva kontrolként. Az új és a már korábban megállapított markerek korrelációja 0,74 volt. Példaként véve a 4., 5. és 6. kromoszóma vizsgálatát megállapították, hogy egyes sejthibrid vonalakban az egész kromoszóma, másokban csak egy részük van jelen. A sejthibrid panel alkalmas lesz a juh regionális térképezésére ill. újabb kromoszómaszakaszra specifikus mikroszatellitek előállítására.

A 18S+28S RDNS LOKALIZÁCIÓJA FLUORESZCENS *IN SITU* HIBRIDIZÁCIÓVAL ÉS A HÁZINYÚL KROMOSZÓMATÉRKÉPÉNEK KIEGÉSZÍTÉSE

ZIJLSTRA, C. — KORSTANJE, R. — DE HAAN, N.A. —
van LITH, H.A. — van ZUTPHEN, L.F.M.— BOSMA, A.A.

G-sávozott limfocitából készült kromoszóma preparátumokon a 13., 16., 20. és 21. kromoszómákon lokalizálták a 18S+28S riboszómális RNS géneket. Ezek a területek egybeesnek a már korábban ezüst-festéssel meghatározott nukleolusz organizáló régiókkal.

A nyúl diploid kromoszómaszáma: 2n=44. A G-sávozott standard kariotípust 1981-ben állították fel. A sávmintázat nagyfokú homológiát mutat az ember kariotípusával. Ezt számos génlokalizáció is megerősítette. A nyúl jelenleg felfrissített kromoszómatérképe 50 kromoszómához rendelt lokuszt tartalmaz, amelyek közül 21-nek a kromoszómasávokban elfoglalt helye pontosan ismert.

A SERTÉS 4-ES KROMOSZÓMÁJÁNAK TÉRKÉPEZÉSE BESUGÁRZÁSSAL ELŐÁLLÍTOTT SEJTHIBRIDEKEN

LOPEZ-CORRALES, N.L. — MUNGAL, C. — McCARTHY, L —
McDOWALL, S. — GOODFELLOW, P.N. — ARCHIBALD, A.L.

Az Európában és Amerikában kifejlesztett sertés genetikai térkép 10–20 cM felbontással lefedi a genom 90%-át, 1500, főleg mikroszatellit, marker felhasználásával. Ez a térkép jelenleg a mennyiségi tulajdonságokért felelős gének (QTL) térképezésére használatos a 20–30 cM intervallumokban. Ezeknek az azonosításához szükséges a minél nagyobb felbontású géntérkép elkészítése. Ehhez szolgáltat eszközt a besugárzással előállított sejthibridek alkalmazása. A vizsgált markereket hordozó sejtvonalak, sertés genomikus DNS próbával, *in situ* hibridizálva kerültek elemzésre, annak megállapítására, hogy mely sertés kromoszómák ill. fragmentek vannak jelen a sejtvonalban. A vizsgált 40 mikroszatellit marker közül 18 került genotipizálásra a felállított panelekben. Ezen adatok elemzésével állítható fel a 4-es kromoszóma géntérképe.

A NŐVÉR CHROMATID CSERÉK SPONTÁN GYAKORISÁGAI LOVAKBAN

MURAMATSU, M. — MIYAZAKI, H. — MURAMATSU, S. — YOSHIZAWA, M. — FUKUI, E.

Öt percheron és öt breton fajtájú ló vértenyészeteit BudR-rel kezelték, majd a preparátumokat Giemsával festették. Lovanként legalább 70, a második osztódás metafázisában lévő limfocitát rtékeltek. A két fajta között nem találtak szignifikáns eltérést.

KROMOSZÓMASÁVOZÁSI VIZSGÁLATOK NYESTKUTYA KARIOTÍPUSON

PIENKOWSKA, A. — SWITONSKI, M.

A nyestkutya kariotípusában különböző számú B kromoszóma figyelhető meg. A fajnak két változata létezik: az eurázsiai (*Nyctereutes procyonoides procyonoides*) 2n=54+B (1-4) és a japán (*Nyctereutes procyonoides viverrivus*) 2n=34+B (1-7) kariotípussal). A közölt vizsgálatban az eurázsiai formát tanulmányozták GTG, QFQ, QFQ/CBG és Ag-I /GTG festési technikákkal. A GTG sávozás alapján csoportosították a kromoszómákat és határozták meg a NOR hordozó kromoszómákat (1, 4, 13 autoszóma és az Y). A C-sávozás centromerikus heterokromatint mutatott ki a kromoszómákon. Az X kromoszómán intersticiális (karon helyeződő) heterokromatin jelentkezett, míg a 3-as és a B kromoszómákon nem figyelhető meg heterokromatin.

MORFOLÓGIAILAG KÜLÖNBÖZŐ 4-ES KROMOSZÓMÁK ÖRÖKLŐDÉSE A ZATORSKA LÚD FAJTÁBAN

RABSZTYN, A. — JASZCZAK, K. — JASZCZAK, J. — KAPKOWSKA, E.

A zatorska lúdfajta kialakításában lengyel lúdfajtákat (*Anser anser*) és a bütykös kínai hattyúludat (*Anser cygnoides*) használták fel. A két fajban eltérő, szubmetacentrikus ill. metacentrikus, 4-es kromoszóma, tehát polimorfizmus figyelhető meg a szerkezetében. Célzott párosításokat követően megállapították, hogy a metacentrikus változat a várhatónál jóval ritkábban fordul elő az utódok kariotípusában.

ÚJ MARKEREK ALKALMAZÁSA A HÁZITYÚK FIZIKAI ÉS KAPCSOLTSÁGI GENETIKAI TÉRKÉPÉNEK ÖSSZEKÖTÉSÉRE

FILLON, V. — PITEL, F. — MORISSON, M. — POUZADOUX, A. — BERGÉ, R. — ZOOROB, R. — AUFFRAY, C. — GELLIN, J. — VIGNAL, A.

A házityúk kariotípusa 2n 78 kromoszómából áll az ivari kromoszómákkal együtt (ZZ: kakas, ZW: tojó). A standard kariotípus csak a 8 nagy makrokromoszómára és az ivari kromoszómákra volt felállítható a sávfestési módszerekkel, míg a 30 mikrokromoszóma még azonosíthatatlan csak önkényesen, nagyság szerinti sorrendbe csoportosíthatók. A házityúk genetikai térképe csak néhány nagy kapcsoltsági csoportból (makrokromoszómákon) és számos kisebb, kevés markert tartalmazó kapcsoltsági csoportból (mikrokromoszómákon ill. makrokromoszómák egyes régióiban) tevődik össze. Ezért nagy szükség van minél több, lehetőleg mikrokromoszómán helyeződő marker azonosítására. Az első megközelítésben mikroszatelliteket tartalmazó BAC és PAC klónokat azonosítottak, melyekből a mikroszatelliteket szubklónozták és szekvenálták. A polimorfizmust mutatókat a nemzetközi (Compton és East Lansing) back-cross térképezési referencia populációban térképezték és fluoreszcens *in situ* hibridizációval kromoszómális régiókhoz rendelték. A második megközelítés a meglehetősen alultérképezett mikrokromoszómákra irányult. *In situ* hibridizációval mikrokromoszómákra térképezett BAC és PAC klónokat szubklónoztak SSC polimorfizmus kimutatására. A tizenegy azonosított marker különlegessége, hogy egyben fizikai és genetikai markerek is, ezért kapcsoltsági csoportokat lehet velük mikrokromoszómákhoz rendelni. A megfigyelések szerint igen magas rekombinációs arány fordul elő a mikrokromoszómákon. Számos kapcsoltsági csoport a mikrokromoszómákon 50 cM-nál nagyobb. Az eddigi megfigyelések még hiányosak annak megállapítására, hogy milyen arány van a genetikai és fizikai méretük között.

A HÁZILÚD MOLEKULÁRIS CITOGENETIKAI VIZSGÁLATA

HIDAS, A.

A világon tenyésztett lúdfajták két lúdfajból származnak. Európában a nyárilúd (*Anser anser*), Afrikában és Ázsiában főleg a kínai hattyúlúd (*Anser cygnoides*) háziasított változatait tartják. A két faj (fajtacsoport) citogenetikai érdekessége az, hogy a 4-es kromoszómájuk morfológiailag eltérő. Az *A. anser* esetében szubmetacentrikus, az *A. cygnoides*-ben hasonló méretű, de metacentrikus 4-es kromoszóma figyelhető meg. Korábbi vizsgálatok azt mutatták, hogy a metacentrikus kromoszómaváltozat, ellentétben a szubmetacentrikussal, centromerikusan és interkalárisan (karon helyeződően) is hordoz heterokromatint. Ezeknek a területeknek az eredetére ill. más heterokromatikus régiókkal mutatott rokonságára, a genomikus *in situ* hibridizáció (GISH) alkalmazása nyújtott információt. Megállapítható volt, hogy a metacentrikus kromoszómaváltozat a szubmetacentrikus típustól eltérően tartalmaz egy centromerikus heterokromatint, amely rokon az *A. anser* kariotípusának más kromoszómáin is előforduló szatellit DNS szekvenciákkal és egy interkaláris helyeződésü heterokromatint, amelynek szekvenciái egyáltalán nincsenek jelen az *A. anser* genotípusában. Mindkét terület további vizsgálatokat igényel, különösen, mivel igen jó modellként szolgálhat a heterokromatikus kromoszómális területek (szatellit DNS) megjelenésének, változásainak általánosabb törvényszerűségei megismeréséhez.

ISMÉTLŐDŐ TRISZÓMIÁK CSIRKE EMBRIÓKBAN

HIDAS, A. — VÁRKONYI, E. — LIPTÓI, K. —
SAYAHZADEH, H. — LENNERT, L. — SZALAY, I.

Tojóhibrid vonalak ismert származású embrióinak citogenetikai vizsgálatakor, két tyúktól származó embriók között ugyanannak a kromoszómának (1. ill. 2) a triszómiáját figyelték meg ismételten. Az aneuploidiák meglehetősen ritka típusú anomáliák a csirke embriókban egyébként gyakori kromoszóma rendellenességek között (haploidia, triploidia, poliploidia), ezért ezek ismételt előfordulása aligha lehet véletlenszerű. A korai embriókban előforduló számbeli kromoszóma rendellenességek genetikai háttere korábban is kimutatható volt, ezért a megfigyelt esetek is örökletes meghatározottságra utalnak. A dolog külön érdekessége, hogy az ismétlődő triszómiákkal az érintett kromoszómák is ugyanazok voltak, ami arra utalhat, mintha ezeknek különösen nagy hajlamuk lenne a nondiszjunkcióra (kromoszómapárok szétválási zavara a meiózisban).

KROMOSZÓMÁLIS ÉS EMBRIONÁLIS RENDELLENESSÉGEK VIZSGÁLATA KORÁN ELHALT LÚD EMBRIÓKBAN

LIPTÓI, K. — HIDAS, A. — SZALAY, I.

Három lúdvonal vizsgálata történt Artigueres-ben (Franciaország, INRA). A cél az embrióelhalások típusának megállapítása, a korán elhalt embriók kariotípusának vizsgálata volt, valamint hogy meghatározzák az embrionális és kromoszómális rendellenességek arányának változását a tojóciklus során és ezen vizsgálatok alapján kiválasszanak néhány állatot a következő évre, a rendellenességek öröklődésének tanulmányozására. A minták gyűjtése három alkalommal történt, a tojásrakási ciklus elején (februárban), a közepén (áprilisban) és a végén (júniusban). Megállapították az inkubáció 5. napján kilámpázott tojásokból nyert embriók fenotípusát és kariotípusát. A kromoszóma rendellenességek aránya 7,14% volt a 4-es vonalban, 16,83% a 7-esben és 12,73% a 9-esben. Az összes rendellenesség számbeli eltérést mutatott. Haploid, haploid/diploid, triploid, triploid/diploid, diploid/polyploid kariotípusokat találtank mindhárom vonalban. Megvizsgálták az embrionális rendellenességek arányának változását a különböző időpontokban. Szignifikáns változás csak abban az esetben volt, ha a három vonalat együttesen értékelték. Ekkor a tojóciklus elején az embrionális rendellenességek aránya szignifikánsan magasabb volt a ciklus közepéhez képest. Ez 10,49%, 6,18% és 7,16%, a termékeny tojásokra vetítve. Egyedi vizsgálatok alapján értékelték a teljes és féltestvér családokat. Minden vonalban voltak olyan teljes és féltestvér családok, amelyek szignifikánsan nagyobb arányban mutattak eltérést a populáció átlagtól a kromoszómális, és azembrionális, vagy mindkét rendellenességre nézve (3 a 4-es vonalban, 4 a 7-esben és 2 a 9-esben).

KÉT SZIRTIFOGOLY FAJ (*ALECTORIS RUFA* ÉS *A. GRAECA*) MEGKÜLÖNBÖZTETÉSE GENETIKAI VIZSGÁLATOKKAL

SAZ, J. — ARRUGA, M.V. — TEJEDOR, M.T. — VILLARROEL, M. — SAVVA, D.

A spanyolországi helyi faj (*Alectoris rufa*) genetikai állományába bekeveredett a behurcolt (*A. graeca*) faj ellenőrizetlen tenyésztéssel. A hibridizáció mértékének megállapítására és a két faj biztonságos azonosítására számos módszert (kromoszómavizsgálat, fehérje-elektroforézis, DNS fingerprinting, PCR) próbáltak ki tiszta vérben tenyésztett állományokban. A PCR és DNS mikroszatellit markerek segítségével lehetségesnek bizonyult a fajok biztonságos elkülönítése. A módszer alkalmazásával az egyedek fajának azonosítása, fajtisztasága lehetséges ill. a természetben végbement fajok közötti keveredés mértéke becsülhető.

A *VIPERA URSINII RAKOSIENSIS* KARIOTÍPUSÁNAK VIZSGÁLATA

LIPTÓI, K. — ÚJVÁRI, B. — KORSÓS, Z. — HIDAS, A.

1995-ben egy kutatási program indult a magyarországi *vipera ursinii rakosiensis* populációk vizsgálatára (OTKA-T16608). Ennek a munkának egy részét képezte a faj kromoszóma vizsgálata. A cél az volt, hogy azonosítsuk a kariotípust, valamint összehasonlítsuk a különböző helyekről származó mintákat. Az egyedek Dabason és Bugacon kerültek begyűjtésre. 16 makro- és 20 mikrokromoszóma volt azonosítható. Hét pár makrokromoszóma szubmetacentrikus, egy pár akrocentrikus és mindkét ivari kromoszóma szubmetacentrikus. Egy Dabasról származó nőstény 16 mikrokromoszómát mutatott 20 helyett.

SPONTÁN TRIPLOIDIA AFRIKAI HARCSÁBAN (*CLARIAS GARIEPINUS*)

RÁCZKEVI-RADICS, J. — RADICS, F.

Az afrikai harcsa két, Szarvason fenntartott vonalának kariológiai elemzését végezték el 23 ivadék veséjéből készített kromoszóma preparátumon. 22 egyed, hasonlóan a más vonalakban leírtakkal, 2n=56 kromoszómaszámot mutatott egy szatellit-hordozó és egy, a nőstényekben heteromorf (ZW) kromoszómapárral. A feltételezett ZW rendszerrel ellentétben, a korábbi molekuláris genetikai vizsgálatok XX-XY típust valószínűsítenek. Egy példány triploid (84) kromoszómakészlettel rendelkezett. Az érintett egyed normális fenotípust mutatott, fejlődő herékkel, jóllehet nőivarra jellemző (W) kromoszómát is hordozott. A triploidia feltehetően a petesejt második poláros test visszatartásával és termékenyülésével alakult ki.

A HARCSAIKRA (*SILURUS GLANIS L.*) TÚLÉRÉSÉNEK HATÁSA A CITOGENETIKAI RENDELLENESSÉGEKRE

VÁRKONYI, E. — HORVÁTH, L. — OZOUF-COSTAZ, C. — BILLARD, R.

A halak embrionális fejlődése során mind a keltetőházakban, mind a természetes szaporodási helyeken előfordulhat torzfejlődött lárvák megjelenése. Korábbi vizsgálatok szerint a harcsaikra 6 órás túlérése a torzfejlődött lárvák arányának komoly növekedését (50%) eredményezi. Feltételezhetően a lárvák torzfejlődését okozhatja a meiózis során bekövetkező kromoszóma-szétválási zavar is, tehát a morfológiai rendellenességeknek genetikai oka lehet.

A kísérletekben az ovulált ikrát lefejték, és azonnal termékenyítették (kontroll), illetve 6 órán át az anya hasüregében hagyták, ezt követően termékenyítve. Vizsgálták a termékenyülési és kelési százalékot, a morfológiai és genetikai rendellenességek arányát és típusát az utódpopulációban, valamint a szülők kariotípusát.

Megállapították, hogy a termékenyülési és kelési százalék alacsonyabb, míg a fenotípusos és genotípusos rendellenességek száma szignifikánsan nagyobb a kezelt csoportban. A túlérett ikra termékenyítését követően a genetikai rendellenességek (tetraploid, triploid, aneuploid) aránya 3,33–20% között volt, ami összehasonlítva a kontroll csoporttal, nagyon magas.

AZ EZÜSTKÁRÁSZ (*CARASSIUS AURATUS GIBELIO*) CITOGENETIKAI VIZSGÁLATA

VÁRADI, L. — TÓTH, B.

A behurcolt ezüstkárász populációja óriási növekedést mutatott az elmúlt 10 évben, ami a biodiverzitást veszélyezteti a természetes vizekben és rendkívüli károkat okoz a halgazdaságokban. A nagy szaporaságot a speciális és egyszerű reprodukciós mechanizmus magyarázhatja. Állományaik többnyire triploid nőstényekből állnak, amelyek gynogenezissel szaporodnak. A triploid ikrák fejlődését fajidegen (többnyire ponty) ondósejtek indukálják, a genetikai anyaguk részvétele nélkül. Ezekben a populációkban olykor normál diploid egyedek (hím- és nőivar egyaránt) is találhatók, amelyek a megszokott módon szaporodnak. A jelen vizsgálatban egy hazai populációt elemeztek citogenetikailag. A begyűjtött hímek mindegyike diploid kromoszómaszámot mutatott, a nőstények között triploid és diploid egyedek is előfordultak. Néhány hím mozaicizmust mutatott a kromoszómaszámot illetően: 100, 136, 156, 174, 186 számú kromoszóma volt a különböző sejtekben.

LEPÉNYHALAK (*PLEURONECTIDAE, SCOPTHALMIDAE, SOLEIDAE*) MOLEKULÁRIS CITOGENETIKÁJA

PARDO, B.G. — BOUZA, C. — CASTRO, P. — MARTÍNEZ, J. — SÁNCHEZ, L.

Újabban számos lepényhal faj tenyésztése kezdődött meg Spanyolországban. A citogenetikai ismeretek ebben a fajcsoportban meglehetősen szórványosak. Három család különböző fajain végezték el a kariotípus elemzését. A heterokromatikus régiókat restrikciós endonukleázokkal jellemezték, a nukleolusz organizáló régiókat (NOR) és polimorfizmusukat A9-, CMA3-sávozással és *in situ* hibridizációval mutatták ki. A kariológiai adatok a későbbiekben végzendő kromoszóma-manipulációs kísérletekben lesznek fontosak.

A GENOMIKUS *IN SITU* HIBRIDIZÁCIÓ (GISH) ALKALMAZÁSA HALFAJOK HIBRIDJEINEK AZONOSÍTÁSÁRA

HIDAS, A. — VÁRKONYI, E.

Halak fajhibridizációja és egyéb részleges genomátvitel esetén a különböző fajokból származó kromoszómák elkülönítésére alkalmas módszernek bizonyult a „genomic *in situ* hibridization (GISH)" eljárás. A módszer szerint az egyik faj jelölt totál genomját használták próbaként, míg a másik faj jelöletlen totál genomja volt a kompetítor DNS. Így a kevert genomú hibridek esetében az egyik szülőtől származó kromoszómakészlet erős fluoreszcens jelet adva detektálható volt.

Az izolált DNS jelölése direkt detektálásra lehetőséget nyújtó fluorescein-dUTP-vel illetve rhodamine-dUTP-vel, nick transzlációs módszerrel történt. Ezzel a jelöléssel a ponty kromoszómái piros (rhodamine), a rózsás díszmárnáé zöld (fluorescein) színben fluoreszkáltak.

Megállapították, hogy mindkét vizsgált hibrid-csoportban a pontyból, illetve a Koi pontyból származó kromoszómák száma 50, míg a rózsás díszmárnából származóaké 24–25, egyedektől függően.

MIKROSZATELLIT MARKEREK FIZIKAI TÉRKÉPEZÉSE A KUTYA (*CANIS FAMILIARIS*) KROMOSZÓMÁIN

SWITONSKI, M. — ŁADON, D. — SCHELLING, C. —
SCHLAPFER, J. — STRANZINGER, G. — DOLF, G.

Kilenc mikroszatellit marker próbáját lokalizálták a kutya QFQ-sávozott kromoszómáin. Jelenleg a kutya 21 legnagyobb autoszómája és az ivari kromoszómák szerepelnek csak a standard kariogramban a kariotípus nehézsége (sok és kicsiny kromoszóma) miatt. A kilenc lokalizált marker közül 7 található azonosítható kromoszómákon, kettő pedig kis autoszómákon adott hibridizációs jelet.

A KUTYA (*CANIS FAMILIARIS*) 22–38 SZÁMÚ KROMOSZÓMÁINAK STANDARDIZÁLÁSA CANIN PAINTING PRÓBÁKKAL

BREEN, M. — REIMANN, N. — BOSMA, A.A. — ŁADON, D.—
ZIJLSTRA, C.— BARTNITZKE, S. — SWITONSKI, M. — LONG, S.E. —
DE HAAN, N.A. — BINNS, M.M. — BULLERDIEK, J. — LANGFORD, C.F.

A házikutya (*Canis familiaris*) kromoszóma-készlete jelentős kihívást jelent a részletes kariotípus analízisek szempontjából. Ennek oka nem csak a magas diploid érték (2n=78), hanem még az összes autoszóma pár akrocentrikus alakja is, melyek csupán fokozatos méret csökkenést mutatnak.

Az 1994 augusztusában, Koppenhágában (Dánia) tartott 11. Európai Háziállat Citogenetikai Kollokviumon alakult meg a Kutya (*Canis familiaris*) Kariotípus Standardizálásának Bizottsága.

A Bizottság 1996-ban tette közzé az 1–21, valamint az X és Y kromoszómák standardját (*Switonski és mtsai*, 1996), melyet *Selden és mtsai* (1975) elrendezésére alapoztak. Ez magában foglalja az 1–25. számú autoszómák számozási rendszerét és azok G-sávokra alapozott ideogramjait, számozott régiókkal és sávokkal. A Bizottság megállapította, hogy a kisebb autoszómák (22–38) megbízható azonosítása, csupán sávozottságuk alapján nem lehetséges.

Az Animal Health Trust-tal együttműködve a Sanger Centre áramlásos citometriával osztályozott kutya kromoszómákból painting próbákat állított elő (*Langford és mtsai*, 1996).

A kisebb autoszómához kötődő painting próbákat négy közreműködő laboratóriumban különböző fajtájú egészséges kutyák GTG- és DAPI-sávozású metafázisainak kromoszómáin alkalmazták.

A Bizottság 1997-es utrechti (Hollandia) találkozóján összehasonlítottuk az így kapott eredményeket. Számozási rendszert fejlesztettünk ki a 22-38. számú autoszómákra alapvetően *Selden és mtsai* (1975) elrendezését követve, figyelembe véve annak *Reimann és mtsai* (1996) által bevezetett kisebb változtatásait. Mindazonáltal nagyszámú esetben egy adott kromoszóma azonosításának kérdésében annak painting sajátságai alapján tudtunk egyetértésre jutni.

Az egyik probléma az volt, hogy nem minden próba kötődik csupán egyetlen kromoszóma párhoz (az S, V, W, Z és cc jelűek két-két kromoszóma párhoz hibridizálnak). Laboratóriumaink közül kettőben (az Utrechti és a Poznani Egyetemen) eltérést találtunk a fluoreszcencia intenzitásában az S próba alkalmazásakor, mely a 21. és a 23. kromoszóma párokhoz hibridizálódott (az utóbbi párt fényesebben jelölte).

Az egyes metafázisos kutya kromoszómák azonosítása fontos a géntérképészeti tanulmányokban, veleszületett rendellenességeket mutató kutyák citogenetikai analízisekor és a daganatsejtek

kariotípus rendellenességeinek eseteiben. Előkészületben van a Bizottság ajánlásainak folyóiratban történő közlése. Ezek az ajánlások tartalmazzák a 22–28. kromoszómák számozási rendszerét, mely azok mindkét; GTG- és DAPI-sávozásán, valamint painting jellemzőin alapul. Ezen kromoszómák régióinak és sávjainak számozásában nem tudtunk teljes egyetértésre jutni. Ebben a vonatkozásban a szerzők a *Reimann és mtsai* (1996) által prezentált kiterjesztett ideogramra hivatkozhatnak.

A 22–38. számú kromoszómák a következő paintekkel hibridizálnak (*Langford és mtsai*, 1996) megjelölései:

Kromoszóma sorsz	Paint	Kromoszóma sorsz.	Paint	Kromoszóma sorsz	Paint
22	N	28	V	34	Z
23	S	29	W	35	ff
24	V	30	bb	36	cc
25	T	31	Z	37	dd
26	aa	32	W	38	ee
27	U	33	cc		

A KUTYA MIKROSZATELLITA MARKEREK FIZIKAI LOKALIZÁCIÓJA AZ EZÜSTRÓKA (*VULPES FULVUS DESM.*) KROMOSZÓMÁKON

ŁADON, D. — SWITONSKI, M. — SCHELLING, C. — SCHLAPFER, J. — DOLF, G.

A kutya marker géntérkép előrehaladása lehetőséget ad más Canidae fajok térképeinek kialakítására. Máig 32 markert térképeztek az ezüstróka kromoszómákon a szomatikus sejthibridizációs megközelítés alkalmazásával (Genetic Maps, ed. Stephen J. O'Brien, Cold Spring Harbor Laboratory Press, 1993).

Most öt mikroszatellita marker, a CanBern1, CanBern6, ZuBeCa1, ZuBeCa3 és ZuBeCa4 lokalizációjáról számolunk be az ezüstróka kromoszómákon. Ezeket a próbákat előzőleg a kutya kromoszómákhoz kapcsoltuk. A kozmid próbákat random priming módszerrel biotin-16UTP-vel jelöltük és ezután használtuk fel a FISH kísérletekhez. A FISH előtt a kromoszómákat QFQ sávozással azonosítottuk és az ezüstróka G-sávos kariotípusának nómenklatúráját követtük (*Mäkinen és mtsai*, 1985). A markereket a következő ezüstróka kromoszómákon lokalizáltuk:

CanBern1	5pter
CanBem6	5pter
ZuBeCa1	16q2
ZuBeCa3	2p2
ZuBeCa4	14q1

EGY JUH SZATELLITA I. ÉS II. DNS KÖZÖTTI KAPCSOLATOT TARTALMAZÓ SZEKVENCIA IZOKROMOSZÓMA ÉS MIKROKROMOSZÓMA KIALAKULÁST TÁMOGAT HÖRCSÖG SEJTEKBEN

CICCARESE, S. — D'AIUTO, L. — CSERPÁN, I. — VONGHIA, G. — BARSANTI, P.

A magasabb rendű szervezetekben a teljes centroméra funkció biztosításához szükséges DNS szekvenciák természete és fontosságuk a stabil mesterséges kromoszóma vektorok kialakításához még mindig nem eléggé ismert. Míg újabb kísérletek (*Harrington és mtsai,* 1997) alapvető szerepet tulajdonítanak az alfa-szatellitának a centroméra aktivitásban, más bizonyíték (*Cho*, 1997) arra utal, hogy a centroméra funkcióhoz nincs szükség alfoid DNS-re, illetve az hasonló tulajdonságú más szekvenciákkal pótolható. A házijuh (*Ovis aries*) centromérás heterokromatinja alapvetően két különböző repetitív DNS szekvenciából, szatellita I-ből és szatellita II-ből tevődik össze (*Buckland és mtsai,* 1983, 1985). Újabban kimutattuk, hogy a szatellita I. és II. különálló szomszédos blokkokat alkot a legtöbb heterokromatikus juh kromoszómában (*D'Aiuto és mtsai,* 1997). Az I. és II. típusú szatelliták közötti kapcsolatot tartalmazó fág kiónok további elemzése a különböző juh centromérikus magas ismétlődésű szekvenciák blokkjai közötti régiók váratlan komplexitását mutatták ki. Annak értékelésére, hogy az ilyen régiókból származó szekvenciák előidézhetnek-e de novo centroméra formációt a transzfektált sejtekben, K20 hörcsög sejtekbe a kapcsolatot tartalmazó 17/3pc fágot a puromicin és higromicin rezisztencia markereket hordozó pCEPUR132 vektorral együtt vitték be. Az együttes bevitel kalciumfoszfát DNS precipitációval történt. A juh centromérás szekvenciákat tartalmazó transzformált sejtek szelekcióját úgy biztosítottuk, hogy az F-12 tápfolyadékhoz 14. napra 15 mg/ml puromicint tettünk. Tíz szer-rezisztens koloniát izoláltunk, ezeket felszaporítottuk és Southern biot analízissel elemeztük 17/3pc fág DNS próbával. 17/3pc szekvenciák a K17/3 klón számos kolóniájában voltak. A klónból készült kromoszóma preparátumokat nick-transzlációval biotinált 17/3pc fág DNS-sel *D'Aiuto és mtsai* szerint *in situ* hibridizáltattuk.

A kromoszómákat DAPI festéssel tettük láthatóvá. Komputeres, hűtött CCD kamerával felszerelt mikroszkóppal digitalizált képeket kaptunk. A FITC és DAPI fluoreszcenciákat szürke skála képekként rögzítettük, ezután hamis színeket kaptak és a komputer egyesítette azokat. A FISH kísérletek legalább négy típusba tartozó 17/3pc DNS-t tartalmazó kromoszóma struktúra jelenlétét mutatták ki. Az első típusban a 17/3pc egy eredeti hörcsög kromoszóma hosszú karjába épült be. Más sejtekben a jel egy kisebb kromoszóma végén mutatkozik, mely úgy tűnik, hogy az első strukturális típus q ter régiójának felel meg.

A megfigyelt szerkezetek harmadik típusa egy metacentrikus kromoszóma, melyen a hibridizációs jel az elsődleges konstrikciónál helyeződik; a legvalószínűbb, hogy ez a fentebb leírt telocentrikus kromoszóma duplikációja. Hipotézisünkkel összhangban olyan metafázisokat is megfigyeltünk, melyeken a jel telocentrikus és metacentrikus kromoszómákon egyaránt jelen volt. Végül néhány osztódásban a transzfektált 17/3pc klón egy mikrokromoszómában volt jelen. Ezek az eredmények azt mutatják, hogy a juh centroméra szekvenciák kromoszóma töréseket indukálnak a transzfektált hörcsög sejtvonalban és azt is sugallják, hogy funkcionáló centromérák képződtek de novo.

Az indukált kromoszóma struktúrák stabilitásának és azok kifejezett centromérás sajátosságainak vizsgálata folyamatban van.

A JAK (*BOS GRUNNIENS*) x SZARVASMARHA (*BOS TAURUS*) HIBRIDEK STERILITÁSÁNAK CITOGENETIKAI OKA

TUMENNASAN, Kh. — TUYA, Ts. — TSENDTSESMEE, L. — HOTTA, Y. — TAKASE, H. — SPEED, R.M. — CHANDLEY, A.C.

A jak és a szarvasmarha keresztezése F1 hibridet eredményez, ezt Mongóliában hainag-nak hívják, a nőivar termékeny, de a hímek meddők. Ereje révén a hainag a mongol farmerek hasznos igásbarma és látható, amint a családi kocsit húzza. A *B. taurus* (2n=60) és a *B. grunniens* (2n=60) kariotípusa azonosnak tűnik, mindkettő 58 akrocentrikus, vagy telocentrikus autoszómából, egy nagy szubmetacentrikus X- és egy kis szubmetacentrikus Y-kromoszómából áll, ennél fogva érdekes, hogy az F_1 hibrid hím hainag steril. Ez olyan más interspecifikus hibridekhez hasonlítható, melyek az egyik, vagy mindkét ivarban sterilek annak ellenére, hogy a két szülőtől igen hasonló, vagy éppenséggel azonos kromoszóma készleteket örökölnek.

A hereméretek felvétele, a szövetgyűjtés a szomatikus kromoszóma analízisekre, a herék szövettani vizsgálatára, a meiótikus kromoszóma analízisre légszárítással és mikrokiszórással ismert standard módszerekkel történt.

Két jakból, két szarvasmarhából, két hainagból és a visszakeresztezések közül két ortúm-ból és két uszanguzí-ből vettek anyagot. A két szarvasmarha heréi a többi állatokéinál nagyobbak voltak.

A here szövettana, a nagyobb méretekkel egyezően, csak a két szarvasmarha esetében mutatott, az ondósejt fokozatig eljutó, teljes spermatogenetikus fejlődést. Az összes többi állat ivarilag éretlennek mutatkozott, habár a szövettani metszetekben mindegyikükben voltak ivarsejtek, melyek a spermatocita stádiumig fejlődtek. A két jakban a tubulusok 25, illetve 50%-ában voltak spermatociták; mindegyik hainag és uszanguzí tubulusainak mintegy harmadában voltak spermatociták, míg a két ortúmban kb. a tubulusok 50%-a mutatott érési folyamatot eddig a stádiumig. A többi tubulusban a fejlődés csak a spermatogonális stádiumig jutott. Egészében a leletek azt mutatták, hogy a pubertás jobban előrehaladt a szarvasmarhákban, mint a jakokban, illetve a hibridek bármelyikében. Meiózis analízis légszárított preparátumokból: az ivarsejtek fejlődésének valamennyi stádiuma, beleértve az ondósejteket, megtalálható volt a két szarvasmarhából készült lemezeken, de csak Sertoli-sejtek, spermatogónia és korai spermatociták voltak a jak, a hainag és az uszanguzí lemezeken.

MEGBESZÉLÉS

Először is kitűnt, hogy a szarvasmarha korábban érte el a pubertást, mint a jak, vagy bármely hibrid. Másodszor, le lehet vonni azt a következtetést, hogy a spermatogenezis az utóbbi csoport valamennyi állatában legalább az elsődleges spermatocita stádiumig eljutott. A hainag heréjében a fejlődés csak a spermatogonium stádiumig jut el és a szövettani preparátumokból úgy látszott, hogy a spermatogóniális sejtek száma is kicsi volt. A munka harmadik eredménye volt, hogy a hainag meiótikus profázisában szinapszis rendellenességeket találtunk, annak ellenére, hogy a jak és a marha kromoszómái citológiai módszerekkel nem különböztethetők meg.

A spermatogenézis elakadásának oka ilyen helyzetekben továbbra is magyarázatra szorul.

Mindazonáltal, a meiózis profázisában a kromoszómák párosodásának rendellenességei emlősökben nem okozzák szükségszerűen a spermatogenézis elakadását, az inkább az ivarsejt-vonalat ért bizonyos korábbi zavarok következménye lehet. A hainagban például, úgy tűnt a szövettani metszetekből, hogy a spermatogónia számszerűen csökkent volt a tubulusokban, ami annak a jele, hogy a spermatogenezis zavara megelőzte a kromoszóma-párosodási stádiumokat. Az is nyilvánvaló volt, hogy a hainagban nem minden, mikrokiszórással vizsgált profázisban lévő spermatocita mutatott kromoszóma-párosodási rendellenességeket. Hasonlóképpen a visszakeresztezett állatok, melyek kromoszómálisan egyik, vagy másik szülői típushoz állnak közelebb, jobb kromoszóma-párosodási (és ezáltal bizonyára jobb fertilitási) eredményeket érhetnek el, amint azt az ortúmban megfigyelték.

Az ivari sejtvonal zavarai általában kifejezettebbek a hímekben, mint a nőstényekben, arra utalva, hogy a spermatogenezis az oogenézisnél sokkal érzékenyebb folyamat. A „Haldane-szabály" érvényesül a szarvasmarha x jak hibridek esetében, ahol, amint Haldane megjósolta, a heterogametikus ivar az, mely nagyobb eséllyel lesz meddő. Adataink alapján feltételezzük, hogy az első és második visszakeresztezések egyre inkább termékenyek lesznek. Ennek a természetes modellnek további vizsgálata hozzájárulhat a közeli rokon emlős fajok hibridjeiben észlelt infertilitás mechanizmusainak megértéséhez.

60,XX/60,XY KIMÉRIZMUST HORDOZÓ BIKÁK REPRODUKCIÓS TELJESÍTMÉNYÉNEK ÉRTÉKELÉSE ONDÓVIZSGÁLAT ALAPJÁN

REJDUCH, B. — JAZDZEWSKI, J. — SŁOTA, E. —
KOZUBSKA-SOBOCINSKA, A. — DANIELAK-CZECH, B.

Citogenetikai analízis után, kétpetéjű ikerellésből született 28 bikát választottunk ki, mint a 60,XX/60,XY kimérizmus hordozóit, hogy értékeljük ennek a kromoszóma rendellenességnek a hatását a reprodukciós teljesítményre.

Öt állatot ki kellett zárni ebből a csoportból, a következők miatt:

— azoospermia – két állat,

— igen alacsony növekedési ráta – egy állat,

— gyenge szexuális viselkedés – két állat.

A spermavizsgálat a következő paraméterekre terjed ki: az ejakulátum térfogata (ml), a mozgó ondósejtek (%) és a sperma sűrűsége (millió/ml). Az állatonként 12 ondómintát 12–15. hónapos bikáktól vették.

Az adatokat 23 normális kariotípusú bika ondójának hasonló paramétereivel hasonlították össze (*1. táblázat*).

A megállapított értékek szignifikáns eltéréseket mutattak az ejakulátum mennyisége és magasan szignifikánsakat a mozgó ondósejtek aránya és a sperma sűrűsége között. Ez a három paraméter kisebb értéket mutatott a kimérizmust hordozó bikák csoportjában, mint a kontroll csoportban (normális kariotípusú bikák).

A kapott eredmények azt sugallják, hogy a kiméra bikákat tanácsos eltávolítani a mesterséges termékenyítő állomásokról.

1. táblázat

A kiméra bikák ondóvizsgálatának eredményei

A bika száma	Térfogat (ml)	Motilitás (%)	Sűrűség, millió/ml
1342	4,03	66,7	1 182
1417	4,86	65,0	879
1423	5,03	56,7	724
26254	3,46	66,7	870
1473	5,85	73,3	1 505
70924	4,88	68,3	858
35152	2,26	60,8	504
1524	2,64	53,6	991
1523	3,00	45,0	552
36142	3,95	64,0	971
04273	1,15	60,0	713
47915	3,11	55,8	863
17675	3,54	59,2	549
90315	4,77	61,7	832
40053	5,58	55,8	918
37947	3,03	45,0	606
1639	4,26	46,7	552
1673	2,67	53,3	677
1640	5,21	58,3	647
1681	2,62	48,3	560
1690	2,36	41,7	289
35901c	3,24	53,3	766
35901b	1,30	23,5	398
XX/XY n=23	3,60*	55,8**	757**
kontroll n=23	4,40	69,2	1021

* P<0,05 ** P<0,01

A KÉT LIMFOCITA POPULÁCIÓ ARÁNYA (XX;XY) KIMÉRA BIKÁKBAN

SŁOTA, E. — REJDUCH, B. — KWACZYNSKA, A. — KOZUBSKA-SOBOCINSKA, A.. — DANIELAK-CZECH, B.

25 kétpetéjű ikerellésből született bikát vontak citogenetikai ellenőrzésbe. Az egy hónapos állatokból vért vettünk és azt 10. hetenként megismételtük tizenöt hónapos korukig. A limfocita tenyésztés után nyert kromoszóma preparátumokat, hagyományosan, Giemsával festettük. A két sejtvonal arányát állatonként 100 metafázis alapján állapítottuk meg. Az XY sejtvonal arányának változásait az *1. táblázat* mutatja.

A 25 bika közül 17 mutatta az XY sejtvonal arányának növekedési tendenciáját, míg 8 esetében saját XY sejtvonaluk aránya csökkent. Az első és az utolsó vizsgálat közötti eltérések is nagyobbak voltak azoknál a bikáknál, ahol az XY sejtvonal aránya emelkedett, átlagos eltérés 25,6% volt azon bikák csoportjában, melyek nővérük (XX) sejtvonalának eliminálási tendenciáját mutatták és 10,3% abban a bikacsoportban, mely saját sejtvonalának csökkenési tendenciáját mutatta.

Nem találtak korrelációt az XY/XX arány és a reprodukciós teljesítmény között. Az eredmények arra utalnak, hogy a nőivarú ikertestvértől kapott XX vonal eliminálásának tendenciája nagyon egyéni és az ikrek közötti bőrátültetésnél mutatkozó egyéni immun-toleranciával hasonlítható össze.

1. táblázat

Az XY sejtvonal arányának változásai kiméra bikákban

A bika száma	A 60,XY sejtvonal aránya vizsgálatonként						Eltérés az 1.–6. között
	1.	2.	3.	4.	5.	6.	
1342	55	57	53	70	80	84	+29
1423	78	78	79	90	90	92	+14
70251	87	93	96	100	100	100	+13
1473	40	45	50	62	68	71	+31
1523	14	25	39	37	44	54	+40
47048	27	64	72	76	78	86	+59
47915	45	72	69	88	90	78	+33
90315	15	27	40	36	40	52	+37
40053	29	31	34	34	54	68	+39
1639	45	32	54	58	60	65	+20
1673	48	82	84	88	92	92	+44
1681	16	30	26	30	20	19	+3
37947	26	28	36	34	40	47	+21
37332	86	90	92	90	93	96	+10
70924	92	97	96	98	97	100	+8
1524	85	89	88	89	92	90	+5
17675	19	20	16	22	24	48	+29
1690	7	5	6	8	6	6	–1
35152	52	62	60	54	46	44	–8
36142	15	13	18	4	6	8	–7
04273	55	54	54	48	44	44	–11
1640	38	40	37	28	28	37	–1
1675	25	13	10	8	8	12	–13
35901b	98	76	76	80	73	75	–23
35901c	90	80	76	76	70	72	– 18

SZEX KROMOSZÓMA ANALÍZIS, MINT A KANCÁK SZAPORODÁSI VIZSGÁLATAINAK ESZKÖZE

MÄKINEN, A. — KATILA, T. — MÄKILÄ, M.

A szex kromoszómák száma, struktúrája és funkciója befolyásolja a kancák reproduktív sikerét. Emlősökben az ivari determináció alapvetően az Y kromoszóma jelenlététől, illetve hiányától függ.

Amikor a nőstényekben a két X kromoszóma egyike inaktiválódik, akkor annak génjeiből néhány aktív marad. Ezek az esetek bizonyos magyarázatot adnak arra, hogy miért az X kromoszóma aneuploidia a leggyakoribb számbeli kromoszóma elváltozás az emlősökben.

Reprodukciós problémáik miatt két kanca citogenetikai vizsgálatát végezték el a szerzők, melynek során ivari kromoszómáikat G- és C-sávjaik alapján azonosították.

Az egyik ötéves amerikai ügető kanca minden limfocitájában XY ivari kromoszóma készlettel. Erősen kifejezett sárlási viselkedés mellett petefészkei igen kicsinyek (kevesebb, mint 2 cm hosszúak) voltak, tüszők nélkül. A méhnyak bejárata és a külső nemi szervek normálisak voltak. Szérum progeszteron szintje 30 napon keresztül állandóan 1 mmol/l alatt volt. Az endometrium biopszia azt mutatta, hogy a mirigyek zöme kicsi és atrófiás, míg néhány más mirigy cisztikusan megnagyobbodott és azokat enyhe periglanduláris fibrózis övezte. A kanca normális küllemű, de nagyon sovány volt és érzékeny térdhajlattal első tenyésszezonjában, miután befejezte igen sikeres verseny karrierjét. Ebben az XY sex reversal hímben az Y kromoszómán lévő gének ivardetermináló funkciója megváltozott, és ez nőstényszerű fenotípust és csökkent fejlettségű női ivarszerveket eredményezett.

Egy másik ötéves hátasló kancának XXX ivari kromoszóma összetétele volt. Ennek a kancának igen kicsi petefészkei voltak: 2x0,5x0,5 cm és 1x0,5x0,5 cm. Az anamnézis szerint volt sárlása. Fenotípusosan normális volt és külső nemi szervei is normálisak voltak. Ebben a 2n=65,XXX-kancában a harmadik X kromoszóma elkerülte az inaktivációt és befolyásolta a női nemi szervek normális fejlődését.

AZ ÖRÖKLŐDŐ KROMOSZÓMA RENDELLENESSÉGEKET HORDOZÓ SZARVASMARHÁK ARÁNYÁNAK CSÖKKENÉSE

HÁZAS, G. — KOVÁCS, A. — KARAKAS, P.

Az 1;29 transzlokációt Magyarországon először *Sellyei és mtsai* (1974) találták meg egy hegyitarka üszőben. 1975-ben az 1;29 és a 14;21 transzlokációt diagnosztizálták laboratóriumukban a mesterséges termékenyítésben használt bikákban. Az 1;29 transzlokációt számos fajtában (hegyitarka, svéd vörös és fehér, maremman x magyarszürke, blonde d'Aquitaine, charolais), míg a 14;21-est csak egy hegyitarka bikában és annak utódaiban észlelték. 1987-ig az összes mesterséges termékenyítésben használt bika állami tulajdonban volt és vizsgálatra került, a hordozókat, tárolt spermájukkal együtt, selejtezték. A programot az Országos Mesterséges Termékenyítő Rt-nél folytatják, de néhány kisebb magántulajdonos kijátszotta a rendeletet. Két (egy homo- és egy heterozigóta) blonde d'Aquitaine bikát importáltak és azokat mesterséges termékenyítésben használták. A természetes fedeztetésben használt hústípusú bikák nagy részét nem lehet vizsgálni. A mesterséges termékenyítésben használt bikák többsége holstein-fríz lett, és ebben a fajtában, a szerzők nem találtak öröklődő kromoszóma rendellenességet. A program eredményeként a magyarországi hegyitarka és magyarszürke fajták öröklődő kromoszóma rendellenességektől mentesnek tekinthetők. Az 1;29 transzlokáció sporadikusan fordul elő húsmarhákban.

APHIDICOLIN ÁLTAL ELŐIDÉZETT TÖRÉSI HELYEK *BOS TAURUS* LIMFOCITA TENYÉSZETEKBEN

(ELŐZETES KÖZLEMÉNY)

LLAMBÍ, S. — GUEVARA, K. — RINCÓN, G. — NUNEZ, R. — ARRUGA, M.V. — POSTIGLIONI, A.

Ismert, hogy az aphidicolin az eukarióta alfa DNS-polimeráz specifikus inhibitora, mely kromoszómális elváltozásokat indukál. A kromoszóma struktúrát, illetve a mitózis indexet még nem szükségszerűen befolyásoló optimális koncentrációkat folsav-mentes RPMI 1640 tápfolyadékban tenyésztett humán limfocitákon állapítottuk meg. Az aphidicolinra leginkább érzékeny helyek, a forró pontok károsodásának felismerése, az emberi kromoszómákon, a DNS-polimeráz gátlásával történt. Most a szarvasmarha Xq 3.1 szakasza törési helyeinek tenyésztési körülményektől független kifejeződését határozták meg, fokozott kifejeződést találva az RPMI 1640 tápfolyadékban. Más módszerként a sister chromatid exchange (SCE) tesztet használtuk normális, tejtermelésre erősen szelektált szarvasmarhák kariotípusain, illetve kevésbé szelektált (1;29) Robertson-transzlokációt hordozó tenyészállatain. Az erős szelekciós nyomásnak kitett állatok normális kariotípusaiban alacsonyabb átlagos SCE értékeket kaptunk. A normális és rob. (1;29) genotípusú állatok aphidicolin által indukált kromoszóma-törési helyeinek vizsgálata hozzájárulhat a szarvasmarha genom-rezisztenciájának megismeréséhez.

A szerzők ebben az előzetes közleményben mutatják be az eltérő koncentrációjú aphidicolinnal kapott törési helyeket RPMI 1640 tápfolyadékban létesített limfocita tenyészetekben. Két marhafajtát választottak: a) normális kariotípusú holstein-frízt és b) 1;29 transzlokációt hordozó uruguayi kreolt, hogy később összehasonlítsák ezek populációs citogenetikai eredményeit.

Egy 1;29 Robertson-transzlokációt hordozó uruguayi kreol és egy normális kariotípusú holstein-fríz nőstényből heparinizált vénás vért vettünk. A 10% magzati borjúsavót tartalmazó RPMI 1640 tápfolyadékot még L-glutaminnal (200 mM), phytohaemaglutininnal (0,2 µg/ml), penicillinnel (100 NE/ml), sztreptomicinnel (100 µg/ml) és fungizonnal (2,5 µg/ml) egészítettük ki. Az aphidicolint törzsoldatból (30 µmol) készítettük és a következő végkoncentrációkat teszteltük: a) 0,24 µmol; b) 0,3 µmol; c) 0,36 µmol; d) 0,5 µmol; e) 0,6 µmol.

Az eredmények azt mutatták, hogy az optimális koncentráció az indukált törési helyek szempontjából 0,24–0,3 µmol között volt, mert a magas aphidicolin koncentráció dezintegrálja a kromoszómákat. Ezt a humán limfocita tenyészetekben kapott eredmények is megerősítik. Az 1;29 kromoszóma 3,5% törési helyet mutatott 0,2 µmol-nál, ill. 15,2%-ot 0,3 µmol-nál, míg a magasabb koncentrációknál nem lehetett azonosítani ezt a szubmetacentrikus kromoszómát. Az X kromoszómák 7% törési helyet mutattak 0,2 mikromolnál, ill. 10%-ot 0,3 mikromolnál.

Magas koncentrációknál legalább az egyik X kromoszóma teljesen dezintegrálódott. Az autoszómák 26% törési helyet mutattak 0,2 mikromolnál és 47,3%-ot 0,3 mikromolnál. Tehát az első konklúzió az, hogy az indukált törési helyek vizsgálatára, szarvasmarha genomban, a legjobb aphidicolin végkoncentráció 0,3 mikromol volt. Több limfocitát kell értékelnünk ahhoz, hogy választ adhassunk az aphidicolin által indukált törési helyek és a genom kromoszóma rendellenességek iránti rezisztenciája között.

X TRISZÓMIA KUTYÁBAN

(ESETISMERTETÉS)

SWITONSKI, M. — PIENKOWSKA, A. — GOLINSKI, P. — BUKOWSKA, D. — BERESZYNSKI, A.

Egy ötéves keverék szuka meddőség miatt került kromoszóma vizsgálatra. A szuka normális fenotípusú volt és külső nemi szervei is normálisnak mutatkoztak. Gazdája szerint gyakori párosodások ellenére sohasem vemhesült.

A citogenetikai értékelés a GTG, CBG és Ag-I sávtechnikák alkalmazásával történt. Valamennyi metafázisban egy extra X kromoszómát találtunk. 79,XXX kariotípust írtunk le. Tudomásunk szerint ez az X kromoszóma triszómia első kutyában diagnosztizált esete.

A MEIÓTIKUS SZINAPSZIS LEFOLYÁSA NŐSTÉNY NYULAKBAN

KOVÁCS, B.ZS. — SWITONSKI, M. — WENT, D. — STRANZINGER, G.

A nőivarú emlősökben az ivarsejtek a magzati élet különböző szakaszaiban fejlődnek. A nőstény nyulak abba a csoportba tartoznak, ahol a meiótikus szinapszis születés után következik be. Ez azt jelenti, hogy gonádjaik születésük idején éretlenek. Oogoniumok és follikulusok találhatók, de oociták nem.

Az állatok petesejtjeinek fejlődési szakaszait elektronmikroszkópos synaptonemal analysis (SC) vizsgálatnak vetették alá, ami fontos információkkal szolgálhat a rendellenes kromoszómák párosodási viselkedéséről. A szerzők előadásukban bemutatják a korai petesejt differenciálódás különböző szakaszait.

Két ivarérett újzélandi fehér nyulat termékenyítettek. Normális vemhességi idő után 19 utód, 9 nőstény és 10 bak született. Erre a vizsgálatra csak a nőstényeket használtuk és különböző életkorban áldoztuk fel őket. A teljes petefészkeket a 4 és a 24 napos kor között vettük ki.

Az SC preparáció ismert módszereit alkalmazták kis módosításokkal. A petesejteket 150-es lyukméretű réz gridekre tették és Philips 301 elektronmikroszkóppal vizsgálták. A sejtek többsége a 10–13. napos állatokban volt az információt adó pachyten stádiumban, a szórás a 4–15. napig terjedt.

A vizsgálat szerint a meiotikus sejtosztódás aktivitásának maximuma a születés utáni 10–13. nap között van. A 20. nap után a petesejtek a meiozis diplotén szakaszába kerülnek. Ezek a megfigyelések fontosak a nőstény nyulak, mint modell állatok SC vizsgálatokra történő kiválasztásánál. Tárgyaljuk az egyes emlősök meiotikus fejlődésének óriási eltéréseit.

A DISZÓMIÁK GYAKORISÁGÁNAK BECSLÉSE AMERIKAI NYÉRC SPERMIUMBAN, A 2, 3, 5, 8, 9, 11 ÉS Y-KROMOSZÓ-MÁK ERŐS CENTROMÉR REPEAT PRÓBÁJÁVAL

CHRISTENSEN, K. — BRUUSGAARD, K.

Az amerikai nyérc (*Mustela vison*) a ragadozók rendjébe tartozik, és az állat gazdasági jelentőségét mutatja a skandináv országok, az USA, Kanada és Oroszország extenzív nyércprém termelése.

Több beszámoló foglalkozik az egyes kromoszómák diszómiáinak gyakoriságával emberben. A normális férfiak diszómiás gyakoriságát, minden egyes kromoszómára vonatkozóan 0,2–0,3 százalékra becsülték. A diszómiák gyakoriságának a meghatározása a környezeti ártalmaknak, vagy betegségeknek az abnormális állati spermiumok termelésére való hatásának becslésére használható.

Olyan repeat nyérc cosmidokkal dolgoztunk, melyek interfázisos sejtekben erős FISH jelet adtak, illetve kromoszóma-specifikus cosmidjaink voltak a 3, 8, 9, 11 és Y nyérc kromoszómákra. Egy további, NOR szekvenciákat tartalmazó cosmid a 2. és 5. kromoszómákra hibridizált. Öt próbát használtunk egyenként normális nyércek spermiumain a diszómiás frekvenciák értékelésére.

Az állatok: a nyércek vad-, vagy pasztellszínűek voltak és átlagos farm körülmények között tartott normális termelő vonalakból származtak. A spermát a mellékheréből gyűjtöttük a párzási időszak végén, március 24. és április 01. között.

Sperma preparáció: az összegyűjtött spermát 3 x PBS-ben mostuk, majd 70%-os etanolban 5 °C fokon tartottuk. A spermát fixálóban (metanol/ecetsav 3:1) nedves tárgylemezre cseppentettük és további, a megdöntött lemezre ejtett két csepp fixálóval mostuk. A lemez széléről a fixálót leszívtuk és a maradékot levegőn szárítottuk. A lemezeket egy hétig szobahőn tartottuk. A lemezeket dithiotreitol-lal (DTT) (200 mM KOH, 50 mM DTT) kezeltük fedőlemez alatt, melyet úgy húztunk át a lemezen, hogy 0,5 és 2 perc közötti különböző expozíciókat biztosítsunk. A fedőlemezeket desztillált vízzel leöblítettük, majd a tárgylemezeket neutralizáló pufferben (900 mM Tris-HCl, pH 8,3, 300 mM KCl, 200 mM HCl), végül desztillált vízben mostuk és levegőn szárítottuk.

DNS próbák: az öt próbát (AG25,chrY, AG32,chr11, AG34,chr2 és 5, AG63,chr9 és AG64,chr8) nick transzláció segítségével biotin-14dCTP-vel és biotin-14-dATP-vel jelöltük.

FISH: a spermiumokban lévő DNS-t 70% formamid/ 2xSSC-ben, pH 7,0-nél, 65 °C fokon 2 percig denaturáltuk, majd jégen tartott 70, 90 és 100%-os etanol-sorban 2-2 percig mostuk. Egy íg biotinizált próbát 30 il hibridizáló oldatban (45% formamid, 2xSSc, 10% dextránszulfát) használtunk. A hibridizáló keveréket 6 íg sheared genomic nyérc DNS-sel egészítettük ki; denaturálás 70 °C fokon 5 percig, majd 20 perc inkubálás 37 °C fokon. Az *in situ* hibridizáció, a denaturált spermiumokat hordozó lemezek és a hibridizáló mix inkubálása, 42 °C fokon 20 óráig tartott. Hibridizáció után a lemezeket kétszer, 45%-os formamiddal, majd háromszor, 2xSSC-ben mosták 39–42 °C fokon. A láthatóságot avidinhez konjugált fluoreszcens izotiocianáttal (FITC) érték el és a jelet biotinált anti-avidin antitesttel (Vector Laboratories) erősítették.

Mikroszkópos vizsgálatok: csak a jól körülhatárolt ondósejteket számoltuk és csak azokat a dupla foltokat számítottuk be, ahol az extra folt a normálissal azonos méretű volt és a két folt jól elkülönült. Több mint 10.000 ondósejtet számoltunk, ezek kromoszómánként 0,2–0,5 diszómiás arányokat mutattak.

AZ X- ÉS Y-KROMOSZÓMÁT HORDOZÓ BIKAONDÓSEJTEK EGYIDEJŰ FELISMERÉSE KÉTSZÍNŰ FLUORESZCENS *IN SITU* HIBRIDIZÁCIÓVAL

HASSANANE, M. — KOVÁCS, A. — LAURENT, P. — LINDBLAD, K. — GUSTAVSSON, I.

Az ivari kromoszómák kétszínű fluoreszcens *in situ* hibridizációját öt svéd holstein-fríz bika ondósejtjein alkalmaztuk. Bebizonyosodott, hogy erős fluoreszcens jelzésű cosmidok és levakart kromoszómák sikeresen használhatók markerként az ilyen típusú vizsgálatokban.

Az X és az Y, a várakozásnak megfelelően, 1:1 arányban oszlott meg, az egyedek között nem volt eltérés. A több Y-, mint X- spermium tendenciát az eltérő markerekkel magyarázzuk. Diszómiás spermiumok több, mint 0,1%-ban (0,067% XX, 0,029% YY és 0,029% XY) fordultak elő, ami az emberben közölteknél lényegesen kevesebb. A diploid ondósejtek aránya 0,5% volt.

A BIKAONDÓSEJTEK FISH-SZEXÁLÁSÁNAK ELSŐ EREDMÉNYEI MAGYARORSZÁGON

TARDY, E.P. — SZALAI, G. — GUSTAVSSON, I. — HASSANANE, M. —
LINDBLAD, K. — KOVÁCS, A. — HÁZAS, G. — TÓTH, A. — DOHY, J.

A szerzők sikeresen végeztek el fluoreszcens *in situ* hibridizációt mélyhűtött bikaspermakeneteken, egy C. Jörgensen-től ajándékba kapott Y-specifikus próbával. Kimagasló minőségű preparátumok érdekében előnyös volt a spermiumok dekondenzálása. Kísérleteket folytattak szarvasmarha X- és Y-kromoszómák levakart darabjaival, melyeket PCR-ral és coli-baktériumokban sokszorosítottak és biotinnal jelöltek

A LÓ (*EQUUS CABALLUS*), MINT A ROSSZINDULATÚ BŐRMELANÓMA GENETIKAI MODELLJE?

RIEDER, S. — CHECA-CORTES, M.L. — STRICKER, CH. — JOERG, H. — MEIJERINK, E. — STRANZINGER, G.

A melanociták növekedésében és differenciálódásában számos gén játszik szerepet. Némelyikük nagyobb hatású a pigmentációra. Különböző fajták és populációk szürke lovait kiemelten érinti a bőrmelanoma és belső rák. A szürke lovak speciális hajlama a melanomára valóban szembetűnő és régóta ismert, de ugyanakkor eltérő ivarú, eltérő családokba és fajtákba tartozó érintetlen öreg lovakat is találtak. Emberben és különböző állatfajokban is kimutatták ezen daganat növekedésének genetikai alapú befolyásoltságát.

A melanocita-sorozat különböző génjeit analizáltuk camargue, spanyol és arab lócsaládokban fajok közötti PCR-ral. Ezek egyike a c-KIT proto oncogene (KIT), a tirozin kináze és az azt kötő törzssejt faktor (SCF) receptora. A KIT szerkezetileg a platelet-eredetű növekedési faktor receptorával rokon. A KIT mutációi összefüggnek az emberi tarkasággal, egy pigmentáltsági rendellenességgel, valamint az egér fehér pettyezettségével és a sertések domináns fehér színével. Az SCF erős hatású a vérképző sejtek, ősivarsejtek és melanociták osztódására és differenciálódására. A szerzők a humán, az egér és a szarvasmarha KIT homológját, a humán, a szarvasmarha és a sertés SCF-et, a ló cDNS-en genomiális DNS szinten részben klónozták és szekvenálták.

A néhány 100 és csaknem 3.000 bp közötti változatos méretű fragmentek próbaként szolgáltak. Ezekkel a próbákkal egy lambda-fág könyvtárat pásztázttak ismételten mindaddig, míg elérték a specifikus pozitív klónok izolálásra alkalmas kolóniáit. A KIT specifikus inzerteket fluoreszcens *in situ* hibridizációra (FISH) is felhasználták. A humán, az egér és a szarvasmarha c-KIT proto oncogént előzetesen a ló 3. kromoszómájának q13–21 régiójára térképeztük.

Emellett klónoztak és szekvenáltak a humán melanoma növekedés stimuláló aktivitás (Mgsa) ló-homológját. Az Mgsa terméke önálló növekedési faktorként működik, melyet először humán melanoma sejtekben találtak meg és a fehérjéknek abba a főcsaládjába tartozik, mely magában foglalja az interleukin-8-at és a platelet factor 4-et. Emberben és egérben az Mgsa a c-KIT proto onkogénnel azonos régióhoz térképeződik.

Így a ló 3. kromoszómája hosszú karjának a homológ régiója, a pigmentáció variánsok és az azokkal rokon komplex vonások érdekes jelölt régiója.

HETEROGÉN CITOGENETIKAI LELETEK MACSKA FIBROSZARKÓMÁKBAN

MAYR, B. — REIFINGER, M. — ALTON, K. — JUGL, M. — BREM, G.

A szerzők hat daganatos macska fibroszarkómáit elemezték citogenetikai módszerekkel. A fibroszarkómák macskákban más háziállatokhoz és az emberhez képest igen gyakoriak. Ezekben a daganatokban gyakran találtak citogenetikai elváltozásokat.

Az 1. sz. macskában az A1, B3 és C2 kromoszómák hosszú karjain deléciókat találtak. Emellett a daganat a D4 kromoszóma veszteségeit mutatta.

A 2. sz. macskában az A1 kromoszóma hosszú karjának töréseit fedezték fel. Emellett F2 és/vagy B1 monoszómiákat találtak.

A 3. sz. macskában az F1 kromoszóma triszómiái és elváltozásai voltak.

A 4. sz. macskában deléciók voltak az A2 kromoszóma hosszú karján és/vagy az A1 kromoszóma hosszú karján. Emellett néhány sejtben A3 és E2 triszómiával hiperdiploiditást figyeltek meg.

Az 5. sz. macskában, sok sejtben, az E1 kromoszóma rövid karjának nagy része elveszett. Emellett ezen sejtek közül néhányban C2 triszómia is volt.

A 6. sz. macskában néhány sejtben reciprok transzlokációt (D3q;E1q) találtak.

Tehát a házimacskák különböző fibroszarkómáinak citogenetikai képe meglehetősen változatosnak tűnik.

SZÁMBELI KROMOSZÓMA RENDELLENESSÉGEK ANALÍZISE SERTÉS-ONDÓSEJTBEN FISH TECHNIKÁVAL

RUBESS, J. — VOZDOVÁ, M. — KUBÍCKOVÁ, S.

Emberben az aneuploidiák a legsúlyosabb és leggyakrabban előforduló kromoszóma rendellenességek közé tartoznak, meddőséggel, spontán abortuszokkal és mentális retardációval, gazdasági állatokban embrionális és magzatelhalással kapcsolatosak.

A molekuláris citogenetika és különösen a fluoreszcens *in situ* hibridizaciós technika (FISH) legújabb fejlődése nagyszámú új megközelítést kínált az ivarsejtek kromoszóma rendellenességeinek vizsgálatára. A FISH a humán spermium aneuploidák vizsgálatának igen hatékony eszköze. A spermiumok kromoszóma vizsgálatára régóta csak az emberi spermium x hörcsög petesejt keresztezés állt rendelkezésre. Az eljárás azonban nagyon bonyolult és rendkívül munkaigényes. Öt ország, hét laboratóriumában végezték és csaknem 20.000 humán spermiumot vizsgáltak meg az elmúlt 15 év folyamán. A FISH alkalmazása nagy számú spermaminta számbeli kromoszóma rendellenességeinek igen gyors tesztelését teszi lehetővé. Spermadonorok tucatjainak vizsgálati eredményeiről beszámoló nagyszámú cikk áll rendelkezésünkre és jelenleg donoronként 10.000 spermiumot értékelnek.

Sok szerző kifejtett véleménye szerint az aneuploidiák gyakorisága emberben sokkal nagyobb, mint a többi vizsgált fajban. Mindamellett az emberen és laboratóriumi állatokon kívül más emlősök spermiumairól úgyszólván semmilyen adatunk sincs. Habár az állatspermium x hörcsög petesejt keresztezést gazdasági állatokon is használták, a legtöbb cikk a módszertannal foglalkozik és az értékelt spermiumok teljes száma nem haladta meg a néhány százat. Mégis, sertésben az aneuploid spermiumok aránya hasonló az ezzel a módszerrel emberben kapott adatokhoz.

Kawarasaki és mtsai (1995; 1996) módszertani cikkeitől eltekintve, a FISH módszer előnyeit a gazdasági állatok spermium-aneuploidiáinak vizsgálatában még nem aknázták ki.

A szerzők jelenlegi kutatásainak tárgya:

a) olyan technika kifejlesztése az aneuploidiák felismerésére állati spermiumokban, mely lehetővé teszi kettő, vagy három kromoszómára specifikus DNS próbák egyidejű használatát,

b) a kanspermium aneuploidiák alapvető gyakoriságainak megállapítása.

A vizsgálatok első eredményeként a kanspermiumokban az 1, 10, 8 és az Y kromoszómák aneuploidiáinak gyakoriságát tárgyaljuk meg.

AZ ANEUPLOIDIA GYAKORISÁGA *IN VITRO* ÉRLELT SZARVASMARHA PETESEJTEKBEN

LECHNIAK, D — SWITONSKI, M.

Az aneuploidia az emlős ivarsejtekben gyakran észlelt jelenség. Ez elsősorban emberben jól dokumentált, ahol az aneuploid ivarsejtek aránya a petesejtekben 1,5–51,9%, az ondósejtekben pedig 3,9–10%. Már kimutatták, hogy a háziállatok petesejtjeinek általános nondisjunkciós rátája 2 és 7% között váltakozik, de az analizált sejtek száma viszonylag kicsi. Az aneuploid petesejtekből — amennyiben termékenyülnek — aneuploid embriók lesznek, melyek rendszerint a vemhesség korai szakaszában elhalnak. Háziállatokban az aneuploid embriók gyakorisága nem haladja meg a 2%-ot (*King*, 1990). Ennek a vizsgálatnak a célja az aneupoidia arányának megállapítása *in vitro* érlelt szarvasmarha petesejtekben.

A petesejtek gyűjtése és érlelése a *Lechniak és mtsai* (1996) által leírt módon történt. Röviden: a petefészkeket a helyi vágóhídon gyűjtötik, a cumulus-petesejt komplexeket kiszippantotják, mossák, majd ivarzó tehén szérumával és hormonokkal kiegészített TCM 199 tápfolyadékban 39 °C fokon, párás, 5% széndioxid-tartalmú atmoszférában 24 óráig érlelik. Érlelés után a petesejteket övező cumulus sejteket 0,25% tripszin + 0,02% EDTA-oldatban inkubálva és pipettázással távolítják el. A levegőn szárított kromoszóma lemezeket (*Tarkowski*, 1966) 5%-os Giemsával festetik.

A citogenetikai analízist 226 szekunder szarvasmarha petesejten végezték el. 186 sejtben haploid kromoszóma készletet azonosítottak. Az aneuploid petesejtek csoportja hipo- és hiperhaploid sejtekből állt, az előbbiek voltak többségben. A 29-nél kevesebb kromoszómát tartalmazó metafázisokat nem vették számításba és technikai műterméknek tekintették. A hiperhaploid petesejtekből számított aneuploidia aránya elérte a 7,1%-ot. Az X kromoszómát valamennyi analizált petesejtben azonosítottuk, tehát csak autoszómális aneuploidiákat találtak.

A DIPLOID MÁSODLAGOS SERTÉS PETESEJTEK GYAKORISÁGA *IN VITRO* ÉRLELÉS UTÁN

SOSNOWSKI, J. — WAROCZYK, M. — LECHNIAK, D. — SWITONSKI, M.

Sertésben gyakran számolnak be a korai fejlődési szakaszokban lévő embriók elhalásáról. A poliploidia, mint a polispermia, vagy a nem-redukált diploid petesejtek termékenyülésének hatása az elhalást okozó faktorok egyikének tűnik. Ennek a vizsgálatnak az volt a célja, hogy megállapítsuk a különböző kocákból egyedileg gyűjtött és in vitro érlelt (IVM) petesejtek között a diploidok arányát.

A vágás után 15–20. percen belül begyűjtött ötven pár petefészek szolgált az elsődleges petefészkek forrásául. Alacsony nyomást (kb. –0,1 bar) használva cumulus-petesejt komplexumokat aspiráltak a látható (2–5 mm átmérőjű) follikulusokból. Csak egyenletesen granulált ooplazmájú és kompakt, érintetlen cumulus sejtekkel övezett petesejteket válogattak ki az érlelésre.

Az egyes donorok petesejt-párjából kiválasztott petesejtek csoportját 100 mikroliteres tápfolyadék (TCM-199+20% ivarzó tehén szérum + 75 NE/ml HCG + 1 µg/ml 17-béta esztradiol + 50 µg/ml gentamicin + 100 NE/ml penicillin) cseppekben 38,5 °C-fokon, párás, 5% széndioxidos atmoszférában, 40–44 óráig érlelték. A kromoszóma lemezeket a *Tarkowski* (1966) által leírt eljárás szerint végezték.

Összesen 2275 petesejtet nyertek az 50 pár petefészekből (páronként 45,5 petesejt, 12 és 191 között váltakozva). Ezek közül 949 petesejtet választottak ki *in vitro* érlelésre (41,7%, 949/2275). Az egy pár petefészekből IVM-re kiválasztott petesejtek száma 7 és 87 között változott, 19-es átlaggal). Kromoszóma lemezeket 870 petesejtből preparáltak (97,1%, 870/949), de csak 540 petesejt volt alkalmas a citogenetikai értékelésre (62,1%, 540/870) és ezek közül 529 (98,0%, 529/540) jutott el a telofázis I. (9 petesejt), vagy a metafázis II. (520 petesejt) stádiumig. Összesen 168 osztódás (32%, 168/520) mutatott diploid kromoszóma számot. Diploid petesejteket 36 donor kocában figyeltek meg. A diploid metafázis II. egyedi aránya 7,1 és 76,9% között változott.

NÖVEKEDÉSI HORMON POLIMORFIZMUS (RFLP) 10. NAPOS SERTÉS EMBRIÓKBAN

LECHNIAK, D. — LONG, S.E. — NISSEN, A.K.

Ennek a vizsgálatnak a célja annak vizsgálata volt, hogy van-e összefüggés az embriók genotípusa (a pGH lokuszon) és az alomszám, valamint a 10. napos embriók körmérete között.

A szerzők hét, duroc spermával inszeminált lapály x nagy fehér koca 10. napos embrióit vizsgálták az anyák levágása után (0. nap=a termékenyítés napja). Az embriókat lefényképezték, és egyenként lefagyasztották (*Nissen és mtsai,* 1997). Ezután DNS-t vontak ki és az embriókat PCR-ral, SRY primerek használatával szexálták. Összesen 93, tíznapos embrió genotípusát határozták meg a pGH lokuszon PCR-RFLP analízissel. Ebben a vizsgálatban a *Kirkpatrick* (1992) által meghatározott szekvenciájú pGH génnek megfelelő primer-párt, valamint két enzimet: az MspI-et és a Hae II-t használtuk. A PCR eljárás a *Kirkpatrick* (1992) által leírt volt. A PCR-t 30 mikroliteres végső térfogatban végezték. Minden PCR-termék felét Msp I-gyel (a PCR-termék 4U/15T1 része), a másik felét (2,5U/15T1) pedig Hae II-vel emésztették. A pGH genotípusokat 2%-os agaróz gélen tették láthatóvá. A PCR-termék Msp I-es emésztése után, az előállított DNS-fragmentek szerint, három különböző genotípust lehetett azonosítani: (++) genotípus –222bp, 147bp és 137bp; (+–) genotípus

— 284 bp, 222 bp, 147bp és 137bp; (– –) genotípus — 284bp és 222bp. A Hae II-vel emésztett sokszorosított DNS három lehetséges fragment kombinációt nyújtott: (++) genotípus — 333bp és 173bp; (+–) genotípus — 506bp, 333bp és 173bp; (– –) genotípus — csak 506bp fragment.

Az elemzett tíznapos sertésembriók adatait az *1. és 2. táblázatok* mutatják. Msp I (– –) genotípust nem találtak.

A pGH genotípusok lehetséges kapcsolatait az egyes kocák embrióin belül, illetve a különböző kocák embriói között (körméret, ivar) és az embriók száma között később mutatják be a szerzők.

1. táblázat

A pGH genotípus alléi változatainak megoszlása a vizsgált tíznapos sertés embriókban

Koca	összes	Msp I genotípusok			Hae II genotípusok		
		(++)	(+–)	(– –)	(++)	(+–)	(– –)
I	24	21	3	—	2	10	12
II	26	26	—	—	—	13	13
III	15	7	8	—	—	15	—
IV	13	13	—	—	5	8	—
V	7	—	7	—	—	7	—
VI	5	5	—	—	4	1	—
VII	3	3	—	—	—	—	3
összes	93	75	18	—	11	54	28
genotípus frekvenciák		0,81	0,19	0,00	0,12	0,58	0,30
alléi frekvenciák	0,9 (+)		0,1 (–)	0,41 (+)	0,59 (—)		

2. táblázat

AZ Msp I/Hae II genotípus változatok frekvenciái

Koca	Msp I/Hae II genotípus kombinációk					
	++/++	++/+–	++/– –	+-/++	+–/+–	+-/– –
I	1	8	12	1	2	—
II	—	13	13	—	—	—
III	—	7	—	—	8	—
IV	5	8	—	—	—	—
V	—	—	—	—	7	—
VI	4	1	—	—	—	—
VII	—	—	3	—	—	—
össz.	10	37	28	1	17	—
frekv.	0,10	0,40	0,30	0,01	0,19	0,00

LEHETŐSÉG A KRÓNIKUS BESUGÁRZÁS CITOGENETIKAI HATÁSAINAK CSÖKKENTÉSÉRE, EGÉRBEN

MOSSE, I.B. — PLOTNIKOVA, S.I. — KOSTROVA, L.N. — DUBOVIC, B.V. — MOLOPHEI, V.P.

A bioszféra radioaktív szennyezettsége alacsony dózisú ionizáló sugárzás tartós hatásának teszi ki az élő szervezetek nagy csoportjait, beleértve a humán populációkat. A szerzők már korábban tanulmányozták a melanin befolyását az ionizáló sugárzás genetikai hatásaira; kimutatták, hogy a melanin injekciók, illetve ennek a pigmentnek az etetése az elváltozott állati csírasejtek (ecetmuslica, egér) és tenyésztett emberi sejtek gyakoriságát 2–4x csökkentette. A melanin a több generáció besugárzásából eredő, a populációkban feldúsult mutációs terhet ugyancsak csökkentette.

A krónikus besugárzás hatásainak melaninnal való befolyásolásának vizsgálata igen fontos és sürgős volt.

Az állati szőrökből izolált melanin befolyását az akut és krónikus besugárzás hatásaira egérben és humán limfocitákban vizsgálták. 2,5 hónapos, 22 g súlyú, hím egereket használtak. A keményítőgélt, illetve a melanin szuszpenziót egy speciális tűvel naponta a gyomorba injektálták. A melanint 0,3 és 30 mg/ kg közötti koncentrációkban adagolták. Az egerek 1–3 Gy Cs137 gammasugárzást kaptak 0,007 Gy/h (krónikus besugárzás) és 420 Gy/h (akut besugárzás) adagban. A behatás befejezése után, 2,5–3 hónappal később az állatokat leölték. Erre az időközre a besugárzott spermatogónia kijavítására volt szükség. A reciprok transzlokációk szintjét a spermatociták metafázisaiban *Iven* citogenetikai módszerével elemezték. A kísérleti adatok statisztikai szignifikanciáját a t-kritériummal erősítették meg, ahol az „n" a vizsgált egerek számának felelt meg.

A melanin spontán mutációs szintre gyakorolt hatásának vizsgálatai azt bizonyították, hogy magának a melaninnak egyik koncentrációban sem volt mutagén hatása, még akkor sem, ha 30 napig adagolták.

A melanin valamennyi vizsgált koncentrációban mutatta az akut gamma sugárzás mutagén hatásának csökkentését, befolyása a krónikus besugárzás genetikai hatására még kifejezettebb volt. Az adatok azt mutatják, hogy a pigment az összes használt koncentrációban jelentősen csökkentette a különböző adagú krónikus besugárzás által indukált mutációk arányát.

Nagyon nehéz a melanin antimutagén hatásának összehasonlítása az akut, illetve a krónikus besugárzás esetén, mert az első esetben egyetlen melanin injekciót használtak, míg a másodikban a melanint sokszor injektálták (naponta 1x 10–20 napig). Mégis le lehet vonni a következtetést, hogy a melanin nem kevésbé, hanem inkább még hatékonyabb a krónikus, mint az akut besugárzás esetében.

Kitűnt, hogy a melanin aktivitása független a használt koncentrációtól. Valamennyire bizonyított, hogy csak kis mennyiségű melanin tud bejutni a sejtekbe és a sejteken belüli melanin mennyisége nem növekszik az azokon kívüli melanin koncentrációval — ez a tény magyarázhatja az ilyen korreláció hiányát.

Ennek a pigmentnek a sugárzás-védő hatása erős elektron felvevő és leadó képességével és antiradikális aktivitásával kapcsolatos. Világos, hogy az alacsony dózisú sugárzás használata esetén a melaninnak jobb esélye van, hogy elkapja a szabad radikálisokat, vagy az elektronokat.

A szerzők az egér csírasejtek genetikai analízisének segítségével igazolták a krónikus besugárzás genetikai hatásai csökkentésének lehetőségét a melanin pigment alkalmazásával.

CANIN KROMOSZÓMA-SPECIFIKUS PAINTING PRÓBÁKKAL ANALIZÁLT KROMOSZÓMA RENDELLENESSÉGEK KUTYA EMLŐRÁK SEJTVONALBAN

TAP, O.T. — RUTTEMAN, G.R. — ZIJLSTRA, C. — DE HAAN, N.A. — BOSMA, A.A.

A szerzők egy korábbi vizsgálatában (*Mellink és mtsai*, 1989) egy 12 1/2 éves szuka emlőrákjának, két metasztázisából származó három sejtvonalát analizálták, hagyományos citogenetikai módszerekkel (Giemsa festés és GTG-sávozás). Ezeknek a sejteknek a jellemző kromoszóma száma 76 volt, míg a kutya normális kromoszóma száma 2n=78. A két kétkarú kromoszóma mellett, melyeket a két X kromoszómának tartottak, egy kis metacentrikus marker kromoszómát találtak. Szimmetrikus sávozottságából azt a következtetést vonták le, hogy izokromoszóma lehetett, de eredetét nem tudták megállapítani.

Most az egyik sejtvonalat canin kromoszóma-specifikus painting próbákkal, fluoreszcens *in situ* hibridizációval, újra megvizsgálták. Ezeket a próbákat a Sanger Centre-ből (Hinxton, Cambridgeshire, U.K.) kapták, ahol azokat normális áramlási citométerrel osztályozott kutya kromoszómákból állították elő (*Langford és mtsai*, 1996). Mind a 33 elérhető próbát (A-Z és aa-ff) alkalmaztak.

A sejtvonal jellemző kromoszóma száma a további átoltások után 77 volt. A sejtek 90%-ában három, 10%-ában négy kétkarú kromoszóma volt. A következő paint-ekkel észleltek eltéréseket: paint H (a 8. és a 11. kromoszómákhoz hibridizál; *Langford és mtsai*), paint L (a 13. és 15. kromoszómákhoz hibridizál; *Langford és mtsai*), paint dd (a 37. kromoszómához hibridizál; A Kutya, Canis familiaris Standarizált Kariotípusának Bizottmánya, ez a találkozó), paint ee (a 38. kromoszómához hibridizál; A Kutya, Canis familiaris Standarizált Kariotípusának Bizottmánya, ez a találkozó), és paint X (az X kromoszómához hibridizál; *Langford és mtsai*). Az összes eltérés teljes kromoszómákat tartalmazott, melyek a következők: — az egyik X kromoszóma hiánya, — 8., vagy 11. izokromoszóma kialakulása (a sejtek 10%-ában), — 13., vagy 15. izokromoszóma kialakulása, és — egy 37. és egy 38. kromoszóma centrikus fúziója (feltehetően *Mellink és mtsai* marker kromoszómája).

Három, az emberi mellrák fejlődésével kapcsolatos gén: BRCA1, ERBB2 és TP53 géntérképezési adatai vannak meg a kutyában. Ezen gének egyike sincs a vizsgált sejtvonalakban abnormálisnak talált kromoszómákon.

Ezen vizsgálat eredményei azt mutatják, hogy a kromoszóma painting a kutya daganatsejtek vizsgálatának leghasznosabb eszköze.

KROMOSZÓMA TÖRÉS ÉS GENETIKAI KÓR FELISMERÉSE MOLEKULÁRIS GENETIKÁVAL

MONTEAGUDO, L.V. — POSTIGLIONI, A. — LLAMBI, S. — ARRUGA, M.V.

Az emberi és állati kromoszómák törékeny helyei sokféle rendellenességgel, köztük onkológiai betegségekkel, valamint különféle metabolikus és funkcionális zavarokkal mutatnak összefüggést. Az ember fajban 1991. óta ismert a trinukleotid ismétlődések szerepe az ilyen törési helyek létrejöttében.

Az egyenlőtlen meiótikus crossing over kiterjeszti ezeket az ismétlődéseket. Ha bizonyos mennyiségű ismétlődés halmozódik fel, kromoszóma instabilitás lép fel a fenotípus elváltozásaival együtt. A RED módszer használatával már nem csak az instabil ismétlődések, de a potenciálisan veszélyes mennyiségű ismétlődést hordozó "premutált" egyedek is felismerhetők.

A fő cél standard eljárások kidolgozása a tenyészállat jelöltek értékelésére. Ilyen módon nem csupán a kromoszómálisan instabil állatok, hanem a premutált egyedek is kitörölhetők. Az állatállományok jövőbeni genetikai leromlását úgy kell megelőzni, hogy nem használunk premutált állatokat a mesterséges termékenyítésben.

„100 ÉVES AZ ÁLLAMI GYAPJÚMINŐSÍTÉS"

AZ ORSZÁGOS MEZŐGAZDASÁGI MINŐSÍTŐ INTÉZET JUBILEUMI EMLÉKÜLÉSE

1998. szeptember 16., Budapest MTA Székház

A megnyitót dr. *Neszmélyi Károly*, az OMMI főigazgatója tartotta, majd a jubiláló intézetet és a megjelenteket köszöntötte dr. *Kovács Ferenc* akadémikus, osztályelnök, *Sándor István* osztályvezető (FVM) az ágazat jelenéről és jövőjéről tartott előadást.

Az Országos Magyar Királyi Gyapjúminősítő Intézet 1898. évi létrehozásáról, az állami gyapjúminősítés megszervezéséről, dr. *Baltay Mihály* állattenyésztési igazgató (OMMI), a gyapjúminősítés 1945. utáni történetéről dr. *Záhonyi József*, ny. osztályvezető emlékezett meg.

A gyapjútermeléssel kapcsolatos új genetikai kutatások eredményeit és azok gyakorlati alkalmazásának lehetőségeit dr. *Dohy János* akadémikus, (GATE), a juhtenyésztési tenyész-célok változását *Székely Pál* osztályvezető (OMMI) ismertette. *Megyerné dr. Nagy Judit* laboratóriumvezető (OMMI) beszámolójából elénk rajzolódtak az OMMI Gyapjúminősítő Laboratóriumának jelenlegi feladatai és jövőbeli célkitűzései, valamint a gyapjúvizsgálatoknak a magyar juhtenyésztésben betöltött szerepe. Megismerkedtünk egy, a napjaink csúcstechnikáját képviselő, új korszerű, képanalizátoros szálfinomságmérő műszerrel, az „OEDA"-val, melyet *Tóth István* ügyvezető igazgató (*Ottó Kühnen*) mutatott be.

A laboratóriumi gyapjúminősítésnek a termelői gyapjútételek értékesítésében betöltött szerepéről beszélt *Balogh Zoltán* ügyvezető igazgató (Aramium Kft), majd dr. *Kukovics Sándor* tudományos osztályvezető (ÁTK) szemléletes előadásban vázolta fel a kutatás és a Gyapjúminősítő Laboratórium kapcsolatát.

A teljesség igénye nélkül, az alábbiak szerint ismertethető vázlatosan az a szakmai történeti folyamat, amelynek a jubileumi emlékülés igyekezett méltó emléket állítani.

A múlt század utolsó évtizedében kezdődött meg az addig gyakorlati állattenyésztés tudományos alapokra helyezése, amely a már korábban bevezetett törzskönyvezés adtainak objektív, műszeres vizsgálatokkal történő alátámasztását jelentette.

Idén éppen 100. esztendeje annak, hogy a századvégen, dr. *Rodiczky Jenő*, az Osztrák Magyar Monarchia méltatlanul elfelejtett tudósa, a páratlan szervezőképességgel megáldott mezőgazda, kutató, tanár, szakíró és polihisztor megalapította az Országos Magyar Királyi Gyapjúminősítő Intézetet, és ezzel laboratóriumi hátteret teremtett a gyapjú értékmérő tulajdonságainak pontos meghatározására.

Az Intézet alapításkori feladatai a következők voltak:

- juhnyájak bonitálása a szelekció és a kiegyenlítettebb nyájak kialakítása érdekében,
- módszerek kimunkálása és bevezetése az egyedi gyapjú és tejtermelő-képesség megállapítására,
- értékes vérvonalak felkutatása, ezek törzskönyvi nyilvántartása,
- a beküldött gyapjúminták szálátmérőjének, szakítószilárdságának, nyújthatóságának, szortimentumának és tisztagyapjú tartalmának meghatározása,
- a gyárilag mosott gyapjútételek kondicionálása,
- hivatalos aukciókra küldött gyapjútételek „rendement"-jának meghatározása a gyapjú pontos árának megállapítása céljából,
- kutatómunka a juhtenyésztés és a gyapjúminősítés egész területén,
- a kutatások eredményeinek és a témakörrel kapcsolatos, hasznosítható ismeretek publikálása, népszerűsítése,
- szakvélemények készítése a Földművelésügyi Minisztérium, a gazdasági egyesületek és a gyapjút termelő gazdák részére.

A gyapjúvizsgálatokhoz használt műszerek, eszközök, melyeknek egy részét maga *Rodiczky* konstruálta, az akkori idők legkorszerűbb technikáját képviselték.

Az Intézet a nyájak osztályozásán kívül törzsjuhászatok létesítésében is közreműködött: magas színvonalú fejőjuhászatok létrehozásához próbafejések végzésével járult hozzá. Kisebb juhállománnyal rendelkező gazdák részére kezdeményezte gyapjútermelésük egyesületi alapon történő fejlesztését.

Az Intézet vezetője 1910-ig *Rodiczky Jenő*, 1914-ig *Kovácsy Béla*, 1927-ig *Kovács Imre*, majd 1949-ig *Schandl József* egyetemi tanár volt. *Schandl* professzor vezetése alatt az Intézet vizsgáló módszerei tovább korszerűsödtek. A nagyszámú szálfinomság-vizsgáiat gyors elvégzését tette lehetővé az általa konstruált klasszifikátor. A rutinvizsgálatokon kívül kiterjedt kutatásokat folytattak a juhtenyésztés és a gyapjúismeret számos témakörében. *Schandl* professzor szakmailag személyes irányította az ország 20–22 legnagyobb juhtenyésztő üzemében a tenyésztő munkát, és az ő vezetése alatt folyt az Intézetben a juhok országos törzskönyvezése. A juhtenyésztők érdekeit képviselte a FUTURA Gyapjúosztálya mellett működő Gyapjúbecslő Bizottság elnökeként is.

Schandl professzor a szakirányú sajtóban igen nagyszámú publikációval járult hozzá a magyar fésűsmerinó fejésének bevezetéséhez, és sok-sok könyve, tanulmánya foglalkozott a juhtenyésztés és a gyapjúismeret aktuális kérdéseivel.

A harmincas évek második felében a selyem vizsgálatával bővült az Intézet vizsgálati spektruma.

Az Intézet alapjában véve átvészelte a II. Világháborút. A berendezések és a könyvtár viszonylag épen maradtak. 1949-ben, a Rákosi rendszer leváltotta *Schandl* professzort a Gyapjúminősítő Intézet éléről, az Intézet is megszűnt, illetve mint Gyapjúminősítő Osztály, az Országos Mezőgazdasági Minőségvizsgáló Intézethez került, sok más, addig önálló vizsgáló intézménnyel együtt.

Az osztály a Gyapjúminősítő Intézet tevékenységi körét szinte teljes egészében átvette, de ezek módosultak és kiegészültek az akkori gyakorlati igényeknek megfelelően.

A háborúban nagyrészt elpusztult juhállományunk. A háború utáni „mennyiségi", majd „minőségi" felfuttatás időszakában, az FM által elrendelt országos bonitálási programban, és az ehhez kapcsolódó gyapjúvizsgálatokban, a Gyapjúminősítő Osztálynak oroszlánrésze volt. Új vizsgálati módszereket dolgoztunk ki a gyapjú fizikai károsodásának és szálfinomságának vizsgálatára. Az 1950-ben megalakult Mezőgazdasági Gyapjúminősítő Bizottság elnöki teendőit a Gyapjúminősítő Osztály vezetője látta el, a laboratóriumi hátteret pedig a Gyapjúminősítő Osztály adta. Az MGYB feladata a felvásárolt termelői gyapjútételek minősítése volt. Működését a MÉM, 1969-ben megszűntette, így a gyapjú felvásárláskor többé már nem tudta a gyapjútermelő gazdaságok érdekét képviselni.

Az osztály, mint szabványbázis aktívan közreműködött a gyapjúvizsgálati szabványsorozat kidolgozásában.

1962-től, a Tollfeldolgozó Vállalat kérésére, az osztályrészt vett a vitás, illetve a hamisított toll tételek vizsgálatában és a toll vizsgálati szabványsorozat kidolgozásában.

A nyolcvanas évek elejétől, több éven keresztül, vizsgáltuk a Várpalotai Juhtenyésztési Társulás taggazdaságaiban megtermelt és előválogatott, évi, kb. 400 t gyapjút. A laboratóriumi vizsgálatok segítségével a partnerek pontos képet kaptak gyapjújuk minőségéről és áráról.

Hazai fésűsmerinó állományunkat, az 50-es évektől kezdve, számos külföldi fajtával keresztezték a gyapjúminőség, a húsformák, a tejelőképesség és a szaporaság javítására. Fajtatiszta, illetve keresztezett egyedeken nagyszámú vizsgálatot végzett osztályunk és megállapíthattuk, hogy az import fajtákkal történt keresztezések hatására hogyan változik meg a merinógyapjú minősége. A becsléssel történő minősítés, a finomságvizsgálat meglehetősen megbízhatatlanná vált a fenotípusosan megváltozott gyapjúminták esetében, ezért 1988-ban osztályunkon üzembe helyeztük az akkori csúcstechnikát képviselő, ausztrál fejlesztésű, lézersugaras, számítógéppel egybekötött modern, automata szálfinomság-vizsgáló készüléket, ami igen pontos adatokat szolgáltat a tudományos igényű szelekcióhoz, a fajtaminősítés és a tenyészérték-becslés végrehajtásához.

Vizsgálati eredményeit a kereskedelmi gyapjútételek minőségi kontrolljában is hasznosítjuk, ugyanis a *gyapjú szálfinomság a világpiacon elsődleges ármeghatározó tényező.*

Osztályunk többszöri átszervezés után (OMFI, OTÁF, ÁTMI, MMI), jelenleg mint az Országos Mezőgazdasági Minősítő Intézet Sertés- és Juhtenyésztési Osztály keretében működő, Gyapjúminősítő Laboratórium tevékenykedik. Vizsgálatainkat a vonatkozó gyapjú és tollvizsgálati szabványok, a Juh Teljesítményvizsgálati Kódex és az IWTO (Nemzetközi Gyapjú- és Textil Szervezet) normatívái szerint végezzük, mint a Nemzeti Akkreditáló Testület által elismert vizsgáló laboratórium.

1998. január 1-ével bekapcsolódtunk a nemzetközi vérkeringésbe is: a brüsszeli székhelyű INTERWOOLLABS (Gyapjú és Textilvizsgáló Laboratóriumok Nemzetközi Szövetsége) akkreditált tagjai közé felvette laboratóriumunkat. A 117 taglaboratóriummal rendelkező szervezet célja a gyapjúvizsgálatok nemzetközi szintű összehangolása, a vizsgálati pontosság növelése nemzetközi körvizsgálatokkal és a taglaboratóriumok akkreditálása.

Az ezredforduló küszöbén összességében megállapítható, hogy a szakmai elődök gyakorlati és elméleti munkáján is alapuló korszerű gyapjúvizsgálati technológiánk — megfelelő fejlesztéssel — meg fog felelni a XXI. század kihívásainak is.

Megyerné dr. Nagy Judit

Rövidített útmutató a kéziratok elkészítéséhez

(Részletesen lásd Állattenyésztés és Takarmányozás, 1993. 42. 1.91–95.p.)

Az Állattenyésztés és Takarmányozás kéthavonta megjelenő tudományos folyóirat. Foglalkozik az állatitermék-előállítás valamennyi ágával, beleértve az összes állatfajt, azok tenyésztését, tartását, takarmányozását és az életfolyamatokkal kapcsolatos minden kérdéskört. Közöl, elsősorban eredeti tudományos közleményeket, de egyes esetekben a tárgykörhöz tartozó szakirodalmi áttekintéseket és szükség szerint aktuális termeléspolitikai koncepciókat. Ismertet disszertációkat, beszámolókat tudományos rendezvényekről, összefoglalókat az egyetemek és a kutatóintézetek kiadványaiból. A közleményeket magyar vagy angol nyelven jelenteti meg.

A kéziratok szöveges részét magyar VAGY angol nyelven, míg az összefoglalót, a táblázat- és ábraszövegeket magyar ÉS angol nyelven kell a szerkesztőségnek megküldeni: írógéppel vagy printerrel jól olvashatóan leírva (összesen legfeljebb 20 oldal, oldalanként 30 sor, soronként 58-60 betű), két példányban, vagy 3,5 v. 5,25"-es floppyn. A szöveges részt lehetőleg ASCII textfile-ban (esetleg Windows-ban vagy WP-ben), a táblázatokat (és ábrákat) QUATRO PRO-ban kérjük elkészíteni. Ez esetben beküldendő a biztonságosan csomagolt floppy és egy példány printelt anyag (a szerkesztőség hozzájárulásával a kéziratok a fent nem említett rendszerekben is beküldhetők). Az összefoglalókat, a táblázatokat és az ábrákat, valamint ezek jegyzékét külön-külön oldalon kell elkészíteni.

A dolgozat tartalmáért a szerző(k) felel(nek). A kézirat (ill. a floppy) az ÁLLATTENYÉSZTÉS és TAKARMÁNYOZÁS szerkesztőségének címére: Állattenyésztési és Takarmányozási Kutatóintézet, 2053 Herceghalom, küldhető be.

A beérkezett kéziratokat a szerkesztőség (anonim) lektoráltatja, és amennyiben szükséges (a bíráló nevének közlése nélkül), visszaküldi a végleges változat elkészítése érdekében.

A dolgozat címe legyen tömör, fejezze ki a munka tartalmát. Meg kell adni a szerző(k) teljes nevét, a közlemény elkészülési helyének (intézményének) pontos elnevezését magyar és angol nyelven, továbbá a szerzők postacímét. Az összefoglaló legyen tömör, tájékoztasson a közlemény célkitűzéséről, módszereiről, eredményeiről és következtetéseiről (maximum 1200 betűhely /nyelv).

A bevezetés és/vagy irodalmi áttekintés tartalmazza az elvégzett kutatómunka célkitűzését, valamint a kapcsolódó szakirodalmi referenciákat. Az anyag(ok) és módszer(ek) c. fejezet tartalmazza a kísérlet(ek)ben felhasznált valamennyi anyag és módszer leírását, valamint az alkalmazott biometriai eljárásokat. Az eredmények c. fejezetben kell leírni az elért eredményeket, a hozzátartozó táblázatokkal és ábrákkal együtt. A következtetések fejezet szükség szerint összevonható az „Eredmények"-kel, de tartalmaznia kell azok megvitatását a hazai és nemzetközi szakirodalom tükrében. Az irodalomjegyzék csak a közleményben hivatkozott műveket tartalmazhatja, az első szerzők neve szerinti ABC sorrendben és valamennyi szerző családnevének feltüntetésével. Kérjük az idegen nevek és szavak, továbbá a folyóiratok nemzetközileg elfogadott rövidítéseinek pontos használatát.

Minden táblázatot külön lapon kérünk beküldeni. A táblázat címe legyen rövid, sorszáma a jobb felső sarokba kerüljön, elhelyezése keresztirányú legyen, ne tartalmazzon több, mint „megnevezés+nyolc számoszlop"-ot. Elkerülendő ugyanazon adatok közlése táblázatban és ábrán. Az angol(magyar) nyelven nem érthető szöveget zárójelbe tett számmal kell jelölni, majd a táblázat alatt, a fordítást közölni. A táblázat legjobb beillesztési helyét a szövegbe, a kézirat bal margóján kell jelezni. Az ábrák elkészítésére, értelemszerűen mindazon előírások érvényesek, mint a táblázatokra. Beküldendő egy példányban az eredeti méretben (max. 12,5x18,5 cm, álló) és kivitelben vagy olyan (fekete-fehér) fényképen, ami megfelelően kontrasztos. A hátoldalon az ábra sorszámát és a szerző nevét fel kell tüntetni.

A disszertációk ismertetését magyar ÉS angol nyelven, nyelvenként maximum 2500 betűhely terjedelemben kell elkészíteni.

Kérjük szerzőinket, fogalmazzanak világosan és érthetően, segítsék elő, hogy szakmánk nyelvezete mind jobban megfeleljen a szép magyar beszéd és fogalmazás követelményeinek.

A szerkesztőség fenntartja magának a jogot arra, hogy szükség esetén, a kéziratban kisebb javításokat, módosításokat végezhessen el (pl. magyarítás, táblázat- vagy ábramódosítás).

A kéziratból készült hasáblevonatot az első szerző részére küldjük meg, hogy a szükséges javításokat kék színnel, a szabványos korrektúrajelekkel, az aktuális sorban, a lap jobb vagy bal margóján elvégezve, azt három napon belül visszaküldje.

ÁLLATTENYÉSZTÉS és TAKARMÁNYOZÁS

Főszerkesztő (Editor-in-chief): Prof. Gundel János, Ph.D.

Szerkesztő (Editor): Regiusné Mőcsényi Ágnes, Ph.D.

A szerkesztőség tanácsadó testülete (Editorial advisory board):

Prof. Bodó Imre, D.Sc., elnök (President)

Prof. G. Brem (Ausztria)
Prof. F. Habe (Szlovénia)
Prof. In K. Han (Korea)
Prof. J. Hodges (Ausztria)
Prof. A. Just, D.Sc. (Dánia)
Prof. H. Kräusslich (Németország)
Prof. T.G. Martin (USA)
Prof. M.W.A. Verstegen (Hollandia)

Dr. Baltay Mihály
Dr. Demeter János
Prof. Dohy János, akadémikus*
Fehér Károly, Ph.D.
Prof. Fésüs László, D.Sc.
Prof. Horn Artúr, akadémikus*
Prof. Horn Péter, akadémikus*
Incze Kálmán, Ph.D.
Kállay Béla, Ph.D.

Dr. Kárpáti József
Prof. Keserű János
Prof. Kovács József
Lengyel Lajos, Ph.D.
Dr. Merkei Attila
Prof. Rafai Pál
Prof. Schmidt János, D.Sc.
Prof. Szakály Sándor
Prof. Veress László, D.Sc.

* Member of Hung. Acad. of Sci.

Szerkesztőség, kiadóhivatal: (Address) Állattenyésztési és Takarmányozási Kutatóintézet
2053 Herceghalom, Gesztenyés út 1.
Telefon/Fax: (36) 23–319–133

Felelős kiadó: (Publisher) Prof. Fésüs László, D.Sc., főigazgató

HU ISSN: 0230 1814

A lap a Földművelésügyi és Vidékfejlesztési Minisztérium tudományos folyóirata
A kiadást támogatja: (Sponsored by) Földművelésügyi és Vidékfejlesztési Minisztérium, Bábolna RT.

Megjelenik évente hatszor
Előfizetési díj: 1 évre 2800 Ft ÁFA-val
Kiadja és terjeszti Állattenyésztési és Takarmányozási Kutatóintézet
Előfizethető a kiadónál, vagy átutalással az MNB 232–90174–0808 pénzforgalmi jelzőszámra
Külföldön terjeszti a Batthyany Kultur-Press KFT, 1011 Budapest, Szilágyi Dezső tér 6.
T/F: 1–201–8891; 1-212–5303 E-mail: batthyanya@kultur-press.hu.
Orders may be placed with Batthyany Kultur-Press Ltd., Szilágyi Dezső Square 6. 1011 Budapest, or with any of its representatives abroad

Készült az Állattenyésztési és Takarmányozási Kutatóintézetben, Herceghalom (5/99)
A nyomda felelős vezetője: Kurucz István