Molecular Markers for Genetic Diversity Studies of European Hare (*Lepus europaeus* Pallas, 1778) Populations

Noémi Soós, Szilvia Kusza*

Institute of Animal Science, Biotechnology and Nature Conservation, University of Debrecen, 4032 Debrecen, Böszörményi út 138., Hungary

Abstract

The purpose of this article is to give an overview of different molecular techniques which have been used in studies concerning population genetic issues of Lepus species and specifically of L. europaeus. The importance of these researches is ever-growing as the European populations of the brown hare have suffered several falloffs as a consequent upon both natural and anthropogenic effects. With developing tools and techniques molecular genetics have become the centrepiece of population genetics and conservation biology. Nucleic acid methods based on both bi- and uniparentally inherited DNA (allozymes, microsatellites, Y chromosome, mtDNA) are often used to study genetic structure, diversity and phylogeography of different species' populations due to their effectiveness in identifying genetic variability.

Keywords: brown hare, genetic methods, review

1. Methods

Allozyme methods

Developing the method of protein electrophoresis have provided a rather large set of marker genes thus making possible for researchers to identify homo- or heterozygosity at a particular nuclear DNA locus. At the beginning of the history of allozym surveys they were used to describe genetic variations in human and fruit-fly populations [1-3]. A considerable amount of these genetic variations have been described during times hereby there is rich literature available on allozyme data concerning for example populations' structures or broad scale variations across species' ranges [e.g. 4-6]. The method clearly has its advantages such as the samples can be processed in large quantities and there are many statistical procedures available for data assessment so the routine requires less time and training as other DNA methods [7].

On the other hand there are disadvantages of using allozyme techniques as well. Endemic species and populations which have gone through genetic bottlenecks commonly lack polymorphic loci [8]. Furthermore it has been described that one can find to be monomorphic all or most of the allozyme loci even in species with large geographic range [9]. Allozymes in addition can have different metabolic functions [10, 11] and several studies have shown that selection can act on allozyme frequencies [12, 13]. Therefore it can be determined that noncoding DNA sequences may be better genetic markers than gene products directly exposed to natural selection.

Multiple studies have been carried out on Lepus europeaus populations of Central and South-Eastern Europe from Poland to Greece as well as Anatolian and British ones [14 – 21] to describe genetic diversity within and among them. Along with morphological characteristics and mitochondrial DNA markers Hartl et al. [16] studied allozymes, and this method turned out to

* Corresponding author: Szilvia Kusza
Fax: 0036-52-486-285, Phone: 0036-52-508-444
Email: kusza@agr.unideb.hu

be the most informative in that particular case. However they found that neither of the methods considered separately is representative for overall gene pool diversity within populations. They found that the average heterozigosity and polymorphism was significantly different among populations and higher than values reported by several other studies of the species (Poland, [15]; Austria and Bulgaria, [19]) and those of Lepus timidus populations of Europe [22]. They have not found correlation between age or gender and heterozigosity. Vapa [23] and his colleagues surveyed the allozyme variability in brown hare populations of the region of Vojvodina (Serbia). Genetic variability have been found within the range described for other Central European populations [14-15, 17] as well as for the Bulgarian and the Greek [21] populations.

Microsatellite DNA (SSRs, STRs)

Microsatellite DNA can effectively be used in population genetic studies because of the very high amount of alleles (30–50) on single loci [24]. These fragments of the DNA are composed of tandem-repeat units of a few bases. The number of alleles is the consequence of the balance between the mutation-driven formation of new alleles and the elimination of existing ones by natural selection or genetic drift [25]. The high heterozigosity of microsatellite alleles suggest a considerably high mutation pressure along with a low value of fitness differences between the alleles [25]. These markers are eligible for describe allele frequencies in population genetic studies [7]. They show high levels of gene diversity therefore are used in phylogeographic surveys [26]. Furthermore they are not any less easy to use than other PCR – gel electrophoresis techniques once the primers are identified. Finding the usable primers for new species can be expressly time-consuming, however there has been described that microsatellite regions are often flanked by highly conserved sequences at the priming sites [27-29]. This phenomenon provides an easement for beginning researches on species which have not been previously studied if there is information on their close relatives. Researches on *L. europeaus* also have used microsatellite makers to describe genetic structure [e.g. 30, 31], diversity [e.g. 32, 33] or introgression by hibridization among Leporids of Europe [e. g. 34, 35].

Mitochondrial DNA

Mitochondrial DNA has often been used in gene flow studies over the past few decades. Its popularity is resulting from several attributes which make it easy to use, such as being strongly conserved, having no introns and very few duplications as well as short intergenic regions in the sequence. It is easy to be amplified due to the small size and the abundance in animal tissues of the molecule. The strict orthology of encoded genes make it a reliable phylogenetic marker [36]. Though it has been established that mtDNA is not by any means as perfect test subject as it was thought to be [37, 38] its usage in molecular ecology and conservation genetics has not been decreased due to the above-mentioned characteristics. The most frequently used mitochondrial markers are the control region [32, 39 – 41] along with the cytB region [41, 42]. Although there are exceptions [43, 44] mtDNA is typically inherited maternally in eukaryote species. Sperm-derived mitochondria do enter the oocyte but they degrade by autophagy almost immediately after fertilization in *Caenorhabditis elegans* [45], and it is believed that in mammals the method of avoiding heteroplasmy caused by paternal mtDNA inheritance could be the same [46]. Albeit information can be provided only in connection with the female germ line it is important that the molecule is transmitted consistently across generations. This nature of the transmission provides an important easement for describing the origin and kinship of a biological specimen since large amounts of reference samples of closely or distantly related individuals may be available for comparison [44]. Mitochondrial DNA regions show polymorphism in different species thus providing a valuable method for determining genetic identity or diversity among a species' populations [47]

Based on main morphological parameters there are nine subspecies of the *Lepus europaeus* [48] however genetic surveys do not confirm these taxonomic results, which probably have originated from the well-known intra- and interspecific morphological variability of the genus [e.g. 49]. MtDNA-based evolutionary hypotheses are inconsistent with those deduced from data of proteins or morphology hence practically representing the nuclear genome [21, 48, 50]. Transmission of the two genomes differ remarkably [51] This along with the sex specific

natal dispersal of these species [52, 53] possibly cause the incongruence.

Researches using only mtDNA markers have shown genetic divergence of some degree between European brown hare populations. Hartl et al. [16] found the haplotype diversity value to be h=0.158 in Austria and Central-Europe. In Vojvodina region of Serbia and Montenegro the research of Djan et al. [47] showed an average value of h = 0.34. Mamuris et al. [54] described a high level of haplotype diversity (h=0.853) and a large number of haplotypes in South-Eastern-Europe. This is expressly higher than the values described in Scandinavia (0.38%; [55]) and Italy [1.3%; 56] and three times lower than the average in the brown hare populations of the Iberian Peninsula (6.2%; 57).

Screening of single nucleotide polymorphisms (SNP) makes possible to use low quality template DNA in researches by not needing long molecule fragments, and could reduce the surveys' costs [58]. This can lead to the complete replacement of microsatellite techniques [35], thus they have recently been used as genetic markers in population genetics researches [59]. They can be used to identify alleles within the nuclear genome or haplotypes in mitochondrial DNA. The method is based on detecting polymorphic nucleotide positions in particular DNA sequences. Testing the scale of polymorphism and the prevalence of different alleles or haplotypes requires DNA sequence data and a reference population. Every position can provide four polymorphisms at the maximum (by the four nucleotides). Thulin et al. [35] identified single nucleotide polymorphisms in *Lepus europaeus* and *L. timidus* mtDNA researches.

Y chromosome

As mentioned above most of times mtDNA surveys have been used in population genetics and conservation biology researches [e.g. 39; 60]. Although plenty of very important data have been provided by those, one can say, that neither of the methods are enough for getting an adequate panorama on the subject since no information about the male lineage is added to the results [61, 62]. This cannot be satisfactory in relation to species with females characteristically philopatric and among them the European brown hare [31, 63, 64]. This is the reason why researchers tend to use biparentally inherited genetic markers such as microsatellites [65 - 67]. These methods seem to resolve the problem of lacking paternal data but the recombining loci and the mostly uncharted mutation model [68] present obstacles to the comprehensive analysis. Though the stepwise mutation model might explain the allele size distribution in satellites with short repeat units [69]. A viable solution to the problem of getting adequately synthetic image on the population genetics of species like the *Lepus europeaus* is using mtDNA and Y chromosome markers in comparison.

The Y chromosome in mammalian species is inherited strictly paternally, is characterized by a slow mutation rate in proportion to the mtDNA [70] and is almost entirely, approximately in 95%, nonrecombining (NRY). However, it has been described that the NRY can form palindromes by self-recombination and gene conversion [71, 72] at least in primates. This discovery has changed the terminology from NRY to MSY (male-specific region on Y). Mammalian Y chromosomes have lost most of their genes (in humans, more than 95%; [73]) and for this reason have become far smaller than their allosomal counterparts. They are believed to evolve by gene loss by certain theories [74, 75] and eventually settle in stasis. The sexdetermining region of Y (SRY) whose expression is the basis of the male sex development [76, 77], is the most conspicuous locus on the MSY. It has probably evolved by the truncation of the SOX3 gene on X [78]. About 5% of the Y chromosome's sequence recombines with the X chromosome. These recombining regions are termed as pseudo-autosomal due to their essentially diploidic nature. They code genes like the zink finger protein region (ZFY) or the amelogenin gene [79, 80]. Likewise autosomes MSY contains microsatellites [81], but there is little known regarding their evolution.

Y connected markers have been used by several studies in population genetics with the aim of shedding light on issues like male-driven evolution [e.g. 82, 83], demographic history of certain populations [84, 85] or the origin of male lineages [86, 87].

Hughes et al. [88, 89] have carried out a research to compare the conservation of Y-linked genes in humans and chimpanzees which revealed that there is excessive divergence between the two species' sequence structure. As a result of this study the MSY of the chimpanzee is now

sequenced as accurately as that of the human. However there is relatively few information on the Y chromosome of other mammalian species. On species of Lagomorpha a few studies have been carried out. There have been mapped Y chromosomal markers for *Lepus europaeus* including the complete coding sequence of the LeSRY locus, microsatellite loci (LeMS-Y) and introns of the zinc finger protein (ZFY) [62, 90]. Information on the Y chromosome sequence of *Oryctolagus cuniculus* have been published as well [91, 92].

2. Summary

This study have been given forth with the aim of making an attempt at providing an overview of what we have known of the nucleic acid markers used in brown hare (*Lepus europaeus* Pallas, 1778) studies concerning distribution, phylogeography, population structure and taxonomic status to this day. This Leporid is an important game species with an extended geographic range from Western-Europe to Mongolia. Being a species of economic value it has been introduced to various countries such as Argentina, Australia, Canada and Sweden [49]. These circumstances have motivated several researchers in carrying out studies on brown hare populations all over the European continent. Data have been provided on population structure, hibridization and introgression among species, however there are unclarified questions about the taxonomic status or the phylogeography of the brown hare. The molecular genetic techniques and large amount of markers identified so far could lead to a rapid progress in gaining population genetic data during conservation biology surveys. In studies concerning the populations of L. europaeus mtDNA markers have been used most frequently [e.g. 16, 56]. There are however valuable results of microsatellite and Y chromosome [63, 91] studies as well as provided by allozyme [e.g. 19, 23] researches. Though all of the above mentioned methods are useful and necessary they all have their disadvantages. For this reason one must consider carefully which technique is the best option to answer their particular questions having regard to both financial and scientific aspects.

Acknowledgements

This research was supported by the European Union and the State of Hungary, co-financed by the European Social Fund in the framework of TÁMOP-4.2.4.A/ 2-11/1-2012-0001 'National Excellence Program' and Hungarian State Eötvös fellowship. This paper was also supported by the János Bolyai Research Scholarship of the Hungarian Academy of Sciences. Authors thank the editor and anonymous reviewers for their helpful comments.

References

1. Harris H., Enzyme polymorphisms in man, Proceedings of the Royal Society B 1966, 164, 298–310
2. Hubby, J.L., Lewontin, R.C., A molecular approach to the study of genic heterozygosity in natural populations I. The number of alleles at different loci in *Drosophila pseudoobscura*, Genetics, 1966, 54, 577–594
3. Johnson, F.M., Kanapi, C.G., Richardson, R.H., Wheeler, M.R., Stone, W.S., An analysis of polymorphisms among isozyme loci in dark and light *Drosophila ananassae* strains from America and Western Samoa, Proceedings of the National Academy of Sciences of the United States of America, 1966, 56, 119–125
4. Nei, M., Genetic distance between populations. American Naturalist, 1972, 106, 283–292.
5. Nei, M., Analysis of gene diversity in subdivided populations. Proceedings of the National Academy of Sciences of the United States of America, 1973, 70, 3321–3323
6. Selander, R.K., Johnson, W.E., Genetic variation among vertebrate species, Annual Review of Ecology and Systematics, 1973, 4, 75–91
7. Parker, P.G., Snow, A.A., Schug, M.D., Booton, G.C., Fuerst, P.A., What molecules can tell us about populations: choosing and using a molecular marker. Ecology, 1998, 79(2), 361–382
8. Barrett, S.C.H., Kohn, J.R., Genetic and evolutionary consequences of small population size in plants: implications for conservation. In: Genetics and conservation of rare plants, Falk, D.A., Holsinger, K.E. Eds. Oxford University Press, New York, New York, USA, 1991, pp. 3–30
9. Mashburn, S.J., Sharitz, R.R., Smith, M.H., Genetic variation among *Typha* populations of the southeastern United States, Evolution, 1978, 32, 681–685
10. Mitton, J.B., Physiological and demographic variation associated with allozyme variation. In: Isozymes in plant biology, Soltis, D.E., Soltis, P.S. Eds. Dioscorides Press, Portland, Oregon, USA, 1989, pp. 87–105
11. Watt, W.B., Cassin, R.C., Swan, M.S., Adaptation at specific loci. III. Field behavior and survivorship

among Colias PGI genotypes are predictable from in vitro biochemistry, Genetics 1983, 103, 725–739
12. Bergmann, F., The allelic distribution at an acid phosphatase locus in Norway spruce (*Picea abies*) along similar climatic gradients, Theoretical and Applied Genetics, 1978, 52, 57–64
13. Powers, D.A., Ropson, I., Brown, D.C., Van Benedon, R., Cashon, R., Gonzalez-Villansenes, L.I., DiMichele, J.A., Genetic variation in *Fundulus heteroclitus*: geographic distribution, American Zoologist 1986, 26, 131–144
14. Hartl, G.B., Markowski, J., Kovacs, G., Grillitsch, M., Willing, R., Biochemical variation and differentiation in the brown hare (*Lepus europaeus*) of Central Europe. Zeitschrift für Säugetierkunde, 1990, 55, 186–193
15. Hartl, G.B., Markowski, J., Świątecki, J., Janiszewski, T., Willing, R., Genetic diversity in the Polish brown hare *Lepus europaeus* Pallas, 1778: implications for conservation and management, Acta Theriologica, 1992, 37, 15 – 25
16. Hartl, G.B., Suchentrunk, F., Nadlinger, K., Willing, R., An integrative analysis of genetic differentiation in the brown hare *Lepus europaeus* based on morphology, allozymes, and mitochondrial DNA, Acta Theriologica, 1993, 38(2), 33–57
17. Hartl, G.B., Nadlinger, K., Willing, R. Allozymes in mammalian population genetics and systematics: Indicative function of a marker system reconsidered, In: Molecular Ecology and Evolution: Approaches and Applications, Schierwater et al. Eds. Birkhäuser Verlag, Basel, Switzerland, 1994, pp. 299–310
18. Sert, H., Suchentrunk, F., Erdoğan, A., Genetic diversity within Anatolian brown hares (*Lepus europaeus* Pallas, 1778) and differentiation among Anatolian and European populations, Mammalian Biology, 2005, 70, 171–186
19. Suchentrunk, F., Michailov, C., Markov, G., Haiden, A., Population genetics of Bulgarian brown hares *Lepus europaeus*: allozymic diversity at zoogeographical crossroads, Acta Theriologica, 2000, 45(1), 1-12
20. Suchentrunk, F., Jaschke, C., and Haiden, A., Little allozyme and mtDNA variability in brown hares (*Lepus europaeus*) from New Zealand and Britain - A legacy of bottlenecks? Mammalian Biology, 2001, 66, 48–59
21. Suchentrunk, F., Mamuris, Z., Sfougaris, A.I., Stamatis, C., Biochemical genetic variability in brown hares (*Lepus europaeus*) from Greece, Biochemical Genetics, 2003, 41, 127–140
22. Suchentrunk, F., Polster, K., Giacometti, M., Ratti, P., Thulin, C.-G., Ruhlé, C., Vasiľev, A.G., Slotta-Bachmayr, L. Spatial partitioning of allozyme variability in European mountain hares (*Lepus timidus*): gene pool divergence across a disjunct distributional range? Zeitschrift für Säugetierkunde 1999, 64, 1-11
23. Vapa, M., Selmic, V., Obreht, D., Vapa, L., Allozyme variability in the natural population of hares. Proc. Nat. Sci., Matica Srpska 1999, 97, 85-91
24. Amos, B., Schlötterer, C., Tautz, D., Social structure of pilot whales revealed by analytical DNA profiling, Science, 1993, 260, 670–672
25. King, D.G., Morris, Soller, M., Kashi, Y., Evolutionary tuning knobs, Endeavour 1997, 21(l), 36 – 40
26. Mengoni C. Phylogeny and genetic diversity of Italian species of hares (genus *Lepus*). *PhD Thesis - Università di Bologna*, Italy, 2011
27. Deka, R., Shriver, M. D., Yu, L. M., Conservation of human chromosome 13 polymorphic microsatellite (CA)n repeats in chimpanzees, Genomics 1994, 22, 226–230
28. Ellegren, H., Polymerase-Chain-Reaction (PCR) analysis of microsatellites - a new approach to studies of genetic relationships in birds, Auk, 1992, 109, 886–895
29. Primmer, C.R., Møller, A.P., Ellegren, H., A widerange survey of cross-species microsatellite amplification in birds, Molecular Ecology, 1996, 5, 365–378
30. Ben Slimen, H., Suchentrunk, F., Stamatis, C., Mamuris, Z., Sert, H., Alves, P.C., Kryger, U., Shahin, A.B., Ben Ammar Elgaaied, A., Population genetics of cape and brown hares (*Lepus capensis* and *L. europaeus*): A test of Petter's hypothesis of conspecificity, Biochemical Systematics and Ecology 2008, 36, 22-39
31. Fickel, J., Schmidt, A., Putze, M., Spittler, H., Ludwig, A., Streich, W.J., Pitra C., Genetic structure of populations of European brown hare: Implications for management, Journal of Wildlife Management 2005, 69(2), 760-770
32. Canu, A., Scandura, M., Luchetti, S., Cossu, A., Iacolina, L., Bazzanti, M., Apollonio, M., Influence of management regime and population history on genetic diversity and population structure of brown hares (*Lepus europaeus*) in an Italian province, European Journal of Wildlife Research 2013, 59, 783–793
33. Thulin, C.-G., Malmsten, J., Laurila, A., Differences in body mass, health status and genetic variation between insular and mainland brown hares (*Lepus europaeus*) in Sweden, European Journal of Wildlife Research 2012, 58, 897 – 907
34. Antoniou, A., Magoulas, A., Platis, P., Kotoulas, G., Assessing the genetic landscape of a contact zone: the case of European hare in northeastern Greece, Genetica, 2013, 141, 23–40
35. Thulin, C.-G., Fang, M., Averianov, A. O., Introgression from *Lepus europaeus* to *L. timidus* in Russia revealed by mitochondrial single nucleotide polymorphisms and nuclear microsatellites, Hereditas, 2006, 143, 68-76

36. Gissi, C., Iannelli, F., Pesole, G., Evolution of the mitochondrial genome of Metazoa as exemplified by comparison of congeneric species, Heredity, 2008, 101, 301-320
37. Galtier, N., Nabholz, B., Glémin, S., Hurst, G. D. D., Mitochondrial DNA as a marker of molecular diversity: a reappraisal. Molecular Ecology 2009, 18, 4541–4550
38. Hurst, G.D.D., Jiggins, F.M., Problems with mitochondrial DNA as a marker in population, phylogeographic and phylogenetic studies: the effects of inherited symbionts, Proceedings of the Royal Society B, 2005, 272, 1525–1534
39. Jansen, T., Forster, P., Levine, M.A., Oelke, H., Hurles, M., Renfrew, C., Weber, J., Olek, K., Mitochondrial DNA and the origins of the domestic horse, Proceedings of the National Academy of Sciences of the United States of America 2002, 99, 10905–10910
40. Kasapidis, P., Suchentrunk, F., Magoulas, A., Kotoulas, G., The shaping of mitochondrial DNA phylogeographic patterns of the brown hare (*Lepus europaeus*) under the combined influence of Late Pleistocene climatic fluctuations and anthropogenic translocations, Molecular Phylogenetics and Evolution, 2005, 34, 55–66
41. Stamatis, C., Suchentrunk, F., Moutou, K. A., Giacometti, M., Haerer, G., Djan, M., Vapa, L., Vukovic, M., Tvrtković, N., Sert, H., Alves, P. C., Mamuris, Z., Phylogeography of the brown hare (*Lepus europaeus*) in Europe: a legacy of south-eastern Mediterranean refugia? Journal of Biogeography 2009, 36, 515–528
42. Alves, P.C., Ferrand, N., Suchentrunk, F., Harris, D.J., Ancient introgression of *Lepus timidus* mtDNA into *L. granatensis* and *L. europaeus* in the Iberian Peninsula, Molecular Phylogenetics and Evolution 2003, 27, 70–80
43. Breton, S., Beaupré, H.D., Stewart, D.T., Hoeh, W.R., Blier, P.U., The unusual system of doubly uniparental inheritance of mtDNA: isn't one enough? Trends in Genetics 2007, 23, 465–474
44. Pereira, F., Carneiro, J., van Asch, B., A Guide for Mitochondrial DNA Analysis in Non-Human Forensic Investigations, The Open Forensic Science Journal 2010, 3, 33-44
45. Sato, M. and Sato, K.: Degradation of paternal mitochondria by fertilization-triggered autophagy in *C. elegans* embryos, Science, 2011, 334(6059), 1141-1144
46. Al Rawi S., Louvet-Vallée, S., Djeddi, A., Sachse, M., Culetto, E., Hajjar, C., Boyd, L., Legouis, R., Galy, V., Postfertilization Autophagy of Sperm Organelles Prevents Paternal Mitochondrial DNA Transmission, Science, 2011, 334, 1144-1147
47. Djan, M., Obreht, D., Vapa, L., Polymorphism of mtDNA regions in brown hare (*Lepus europaeus*) populations from Vojvodina (Serbia and Montenegro), European Journal of Wildlife Research 2006, 52, 288–291
48. Mamuris, Z., Moutou, K.A., Stamatis, C., Sarafidou, Th., Suchentrunk F., Y DNA and mitochondrial lineages in European and Asian populations of the brown hare (*Lepus europaeus*), Mammalian Biology 2010, 75, 233–242
49. Flux, J.E.C., Angermann, R., The Hares and Jackrabbits. In: Rabbits, Hares and Pikas: Status Survey and Conservation Action Plan, Chapman, J.A., Flux, J.E.C. Eds. The World Conservation Union, Gland, Switzerland, 1990, pp. 61-94.
50. Koutsogiannouli, E. A., Moutou, K. A., Stamatis, C., Mamuris, Z., Analysis of MC1R genetic variation in Lepus species in Mediterranean refugia, Mammalian Biology, 2012, 77(6), 428–433.
51. Moore, W. S., Inferring phylogenies from mtDNA variation: mitochondrial – gene trees versus nuclear-gene trees, Evolution, 1995, 49(4), 718-726
52. Hulbert, I.A.R., Iason, G.R., Elston, D.A., Racey, P.A., Homerange sizes in a stratified upland landscape of two lagomorphs with different feeding strategies. Journal of Applied Ecology 1996, 33, 1479–1488
53. Reitz, F., Léonard, Y., Characteristics of European hare *Lepus europaeus* use of space in a French agricultural region of intensive farming, Acta Theriologica, 1994, 39(2), 143-157
54. Mamuris, Z., Sfougaris, A.I., Stamatis, C., Genetic structure of Greek brown hare (*Lepus europaeus*) populations as revealed by mtDBNA RFLP-PCR analysis: Implications for conserving genetic diversity, Biological Conservation 2001, 101(2), 187-196
55. Thulin, C.-G., Jaarola, M., Tegelström, H., The occurrence of mountain hare mitochondrial DNA in wild brown hares, Molecular Ecology, 1997, 6, 463–467
56. Pierpaoli, M., Riga, F., Trocchi, V., Randi, E., Species distinction and evolutionary relationships of the Italian hare (*Lepus corsicanus*) as described by mitochondrial DNA sequencing, Molecular Ecology 1999, 8, 1805–1817
57. Pérez-Suárez, G., Palacios, F., Boursot, P., Speciation and paraphyly in western Mediterranean Hares (*Lepus castroviejoi, L. europaeus, L. granatensis* and *L. capensis*) revealed by mitochondrial DNA phylogeny, Biochemical Genetics, 1994, 32, 423-436
58. Morin, P. A., Luikart, G., Wayne, R. K. et al., SNPs in ecology, evolution and conservation, Trends in Ecology & Evolution 2004, 19, 208-216
59. Brumfield, R.T., Beerli, P., Nickerson, D.A., Edwards, S.V., The utility of single nucleotide polymorphisms in inferences of population history, TRENDS in Ecology and Evolution 2003, 18(5), 249-256
60. Pitra, C., Lieckfeldt, D., Alonso, J.C., Population subdivision in Europe's great bustard inferred from

mitochondrial and nuclear DNA sequence variation, Molecular Ecology, 2000, 9, 1165–1170
61. Lindgren, G., Backstrom, N., Swinburne, J., Hellborg, L., Einarsson, A., Sandberg, K., Cathran, G., Vila, C., Binns, M., Ellegren, H., Limited number of patrilines in horse domestication, Nature Genetics 2004, 36, 335–336
62. Putze, M., Nürnberg, S., Fickel, J., Y-chromosomal markers for the European brown hare (*Lepus europaeus*, Pallas 1778). European Journal of Wildlife Research 2007, 53, 257 – 264
63. Fickel, J.: Molekularbiologie und Phylogenie von *Lepus europaeus* Pallas, 1778 - und anderen europäischen Lepus-Arten (in German). In: Handbuch der Säugetiere Band 3/II: hasentiere Lagomorpha, Krapp, F., Ed. Aula-Verlag Wiebelsheim, Germany, 2003, pp. 27–34
64. Fickel, J., Lieckfeldt, D., Pitra, C., Analysis of genetic diversity and structure in neighbouring populations of the European brown hare (*Lepus europaeus*, Pallas 1778), Zeitschrift Für Jagdwissenschaft 1999, 45, 230–237
65. Burton, C., Krebs, C.J., Taylor, E.B., Population genetic structure of the cyclic snowshoe hare (*Lepus americanus*) in southwestern Yukon, Canada, Molecular Ecology, 2002, 11, 1689–1701
66. Fickel, J., Hohmann, U., A methodological approach for noninvasive sampling for population size estimates in wild boars (*Sus scrofa*), European Journal of Wildlife Research, 2006, 52, 28–33
67. Lünser, K., Fickel, J., Lehnen, A., Speck, S., Ludwig, A., Low level of genetic variability in European bisons (*Bison bonasus*) from the Bialowieza National Park in Poland, European Journal of Wildlife Research 2005, 51, 84 – 87
68. Li, Y-C., Korl, A.B., Fahima, T., Beiles, A., Nevo, E., Microsatellites: genomic distribution, putative functions and mutational mechanisms: a review. Molecular Ecology, 2002, 11, 2452–2465
69. Valdes, A.M., Slatkin, M., Freimer, N.B., Allele frequencies at microsatellite loci: the stepwise mutation model revisited, Genetics 1993, 133, 737–749
70. Schaffner, S.F., The X chromosome in population genetics. Nature Reviews Genetics, 2004, 5, 43–51.
71. Cavalli-Sforza, L.L., Feldman, M.W., The application of molecular genetic approaches to the study of human evolution, Nature Genetics, 2003, 33, 266–275
72. Rozen, S., Skaletsky, H., Marszalek, J.D., Minx, P.J., Cordum, H.S., Waterson, R.H., Wilson, R.K., Page, D.C., Abundant gene conversion between arms of palindromes inhuman and ape Y chromosomes, Nature 2003, 423, 873–876
73. Graves, J.A.M., The degenerate Y chromosome – can conversion save it?, Reproduction Fertility and Development, 2004, 16, 527–534
74. Bachtrog D. 2008: The temporal dynamics of processes underlying Y chromosome degeneration. Genetics 179, 1513–1525.
75. Charlesworth, B., Charlesworth, D., The degeneration of Y chromosomes, Philosophical Transactions of the Royal Society B, 2000, 355, 1563–1572
76. Knower, K.C., Kelly, S., Harley, V.R., Turning on the male – SRY, Sox9 and sex determination in mammals, Cytogenetic and Genome Research, 2003, 101, 185–198
77. Palsbøll, P.J., Vader, A., Bakke, I., El-Gewely, M.R., Determination of gender in cetaceans by the polymerase chain reaction, Canadian Journal of Zoology 1992, 70, 2166–2170
78. Graves, J.A.M., Sex chromosomes and sex determination in weird mammals, Cytogenetic and Genome Research 2002, 96, 161–168
79. Mardon, G., Page, D.C., The sex-determining region of the mouse Y chromosome encodes a protein with a highly acidic domain and 13 zinc fingers, Cell 1989, 56, 765–770
80. Mitchell, R.J., Kreskas, M., Baxter, E., Buffalino, L., Van Oorschot, R.A., An investigation of sequence deletions of amelogenin (AMELY), a Y-chromosome locus commonly used for gender determination, Annals of Human Biology, 2006, 33, 227–240
81. Kayser, M., Sajantila, A., Mutations at Y-STR loci: implications for paternity testing and forensic analysis, Forensic Science International, 2001, 118, 116–121.
82. Miyata, T., Hayashida, H., Kuma, K., Yasunaga, T., Male-driven molecular evolution demonstrated by different rates of silent substitutions between autosome- and sex chromosome-linked genes, Proceedings of the Japan Academy, 1987, 63, 327–331
83. Wyckoff, G. J., Wang, W., Wu, C. I., Rapid evolution of male reproductive genes in the descent of man, Nature, 2000, 403, 304–309
84. Shen, P., Wang, F., Underhill, P.A., Franco, C., Yang, W-H., Roxas, A., Sung, R., Lin, A.A., Hyman, R.W., Vollrath, D., Davis, R.W., Cavalli-Sforza, L.L., Oefner, P.J., Population genetic implications from sequence variation in four Y chromosome genes, Proceedings of the National Academy of Sciences of the United States of America 2000, 97, 7354–7359
85. Stumpf, M.P., Goldstein, D.B., Genealogical and evolutionary inference with the human Y chromosome, Science, 2001, 291(5509), 1738–1742
86. Thomson, R., Pritchard, J.K., Shen, P., Oefner, P.J., Feldman, M.W., Recent common ancestry of human Y chromosomes: evidence from DNA sequence data, Proceedings of the National Academy of Sciences of the United States of America, 2000, 97, 7360– 7365
87. Tosi, A.J., Morales, J.C., Melnick, D.J., Comparison of Y chromosome and mtDNA phylogenies leads to unique inferences of macaque

evolutionary history, Molecular Phylogenetics and Evolution 2000, 17, 190–192
88. Hughes, J.F., Skaletsky, H., Pyntikova, T., Minx, P.J., Graves, T., Rozen, S., Wilson, R.K., Page, D.C., Conservation of Y-linked genes during human evolution revealed by comparative sequencing in chimpanzee, Nature, 2005, 437, 101–104
89. Hughes, J.F., Skaletsky, H., Pyntikova, T., Graves, T.A., van Daalen, S.K.M., Minx, P.J., Fulton, R.S., McGrath, S.D., Locke, D.P., Friedman, C., Trask, B.J., Mardis, E.R., Warren, W.C., Repping, S., Rozen, S., Wilson, R.K., Page, D.C., Chimpanzee and human Y chromosomes are remarkably divergent in structure and gene content, Nature, 2010, 463, 536-9
90. Wallner, B., Huber, S., Achmann, R., Non-invasive PCR sexing of rabbits (*Oryctolagus cuniculus*) and hares (*Lepus europaeus*), Mammalian Biology, 2001, 66, 190–192
91. Geraldes, A., Rogel-Gaillard, C., Ferrand N., High levels of nucleotide diversity in the European rabbit (*Oryctolagus cuniculus*) SRY gene. Animal Genetics, 2005, 36, 349–351
92. Geraldes, A., Carneiro, M., Delibes-Mateos, M., Villafuerte, R., Nachman, M. W., Ferrand, N., Reduced introgression of the Y chromosome between subspecies of the European rabbit (*Oryctolagus cuniculus*) in the Iberian Peninsula, Molecular Ecology, 2008, 17, 4489–4499.