Use of mitochondrial DNA as a genetic marker in domesticated mammals

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Molecular genetic data have greatly improved our ability to study the evolution of domesticated mammals in the environment controlled by man. Mitochondrial DNA has proven to be especially helpful in studying the roots of present day populations. Studies have been done, e. g., in cattle, sheep, pig, goat and dog. These have indicated multiple maternal sources and varying levels of phylogeographic subdivision. More detailed studies are in progress to provide a more comprehensive picture of the historical processes of domestic breeds.

Key words: mtDNA, sequence data, phylogeography, population history, domestication

People have long been interested in the origin of their domesticated animals. A lot of archaeological work has been done to find out where and when did the domestications happen. Studies based, for example, on bone morphologies and investigating abundances of animal remains associated with early human civilization and distribution of different age classes in the deposits have been able to reveal signs of domestication, *e. g.*, in Near East, Mexico and China. The first domesticated forms of most important mammals used for production in Europe today (cattle, pig, sheep and goat) emerged about 10,000 years ago in different parts of Fertile Crescent (Smith, 1998). Archaeological studies have their limitations, for example, there cannot be findings on locations where no excavation has been done and it is not always possible to recognise the species, *e. g.*, sheep bone from goat bone.

Instead of digging the old remains, we have the possibility to mine information about domestication using the present day populations. Although for many questions protein polymorphism and especially microsatellites are invaluable tools, there is also need for a phylogenetic marker where quantifying the degree of relationship between different alleles is possible, DNA sequences. Until very recent times it was very difficult to obtain DNA sequence data. In intraspecies studies the need for studied animals is ten or hundred times larger than in interspecies studies and only the dramatic progress in our ability to study sequences in the last decade made also intraspecies studies feasible.

Mitochondrion is a membrane-bound cytoplasmic organelle that has its own DNA. There are several reasons why mitochondrial DNA (mtDNA) sequences are favoured over nuclear DNA sequences. For example, in mitochondrial studies the haplotype information comes directly, while in nuclear sequences determining the haplotype demands cloning or statistical inference with additional data. Anyway, in rare cases obtaining haplotypic information might be complicated for mtDNA, also as an individual might be heteroplasmic, having more than one type of mitochondria, and also nuclear pseudogenes might cause problems (Bensasson et al., 2001).

It has been estimated that the nuclear mutation rate is approximately 10^{-7} to 10^{-8} per nucleotide per generation (Nei, 1987). This is good for resolving ancient history, but as the domestication history is short in evolutionary scale a more rapidly evolving phylogenetic marker would be appropriate. The mutation rate of mtDNA has been estimated to be 2 to 3×10^{-7} (Horai et al., 1995), but there is a great heterogeneity in the substitution rate even in the mitochondrial control region (Meyer et al., 1999). In the mitochondrial genome it is possible to find regions where already a sequence of moderate length is informative about domestication.

An other beneficial property of mtDNA is clonal inheritance. It is transmitted to the progeny only from the mother (Giles et al., 1980; Hayashi et al., 1978; Hutchison et al., 1974). Therefore, introduced males or artificial insemination has no effect on the pool of mitochondria in the population. This has been observed in some African taurine cattle popu-

lations, where the mitochondrial types were typical of taurine, while autosomal and Y-chromosomal data indicated a male-mediated geneflow from zebu cattle (MacHugh et al., 1997). So mtDNA is especially informative about the primary spreading of domestic species and less disturbed by the secondary geneflow.

The evolution of mitochondrial DNA seems to be unaffected by recombination (Ballard, 2000), although there was also debate about the possibility of recombination (Awadalla et al., 1999). In absence of recombination the sequences maintain the phylogenetic signal and all differences among the sequences are due to mutations. It is possible to sample individuals and then, based on the differences, work backward to an ancestor that originates all sampled present-day variation. For example in sheep there are two main groups of mitochondria, and Hiendleder et al. (1998) estimated that the most recent common ancestor for the two groups dates between 375,000 to 750,000 years ago which exceeds the range of estimates for man 2–7 times. As mtDNA is inherited maternally, this suggests that there were live females of at least two separate subspecies of wild sheep taken to the human-controlled environment. The mitochondrial sequences cluster to distinct groups also in cattle, goat, pig and water buffalo. These groups of mitochondria in each of the species differ so greatly that the most recent common ancestors had to exist long before domestications (reviewed in MacHugh and Bradley, 2001).

It is also possible to estimate the time of the domestication of a species. The number of captured animals and the initial genetic variation are in most scenarios small at the beginning, but after domestication the number of animals increases, and there is room for more diversity. If we again start from the present day sample and work backwards in an expanding population, we notice that the genealogy lines to the most recent common ancestor form a star-like pattern where most branches unite right before the expansion started. If we compare two random individuals, the most common number of differences between them corresponds to the number of mutations happened in the lines since the most recent common ancestor who lived just before the expansion. This produces a "peak" in the mismatch distribution, and this can be used to estimate the time of domestications. For example, in goat there are at least three different domestications. Based on mismatch distributions, Luikart et al. (2001) estimated that if the first domestication happened 10,000 years ago, the second happened 6000 and the last only 2000 years ago.

Population history leaves tracks also in the geographical distribution of variation. In cattle, sheep and pig the European and Asian breeds have different predominant mitochondrial types (reviewed in MacHugh and Bradley, 2001). In sheep, mitochondria belonging to one group were observed only in breeds of European origin, while the other was rare in these breeds, but predominant in sole form in Asian native breeds (Hiendleder et al., 1998). In this first study the European sheep were presented by populations in New Zealand and they could have gotten the Asian types through hybridisation. Our new data (Tapio et al., 2002) from Northern Europe anyway confirm that the Asian type mitochondria are present in many European breeds as a rare form. In goat, the mitochondrial type descending from the last domestication is restricted to Southern and Eastern Asia (Luikart et al., 2001). The geographical structuring is clearest in cattle, and three separate starting points for radiations have been inferred: one close to India for zebu cattle, one in Africa for African taurine cattle, and one in Near-East for European taurine cattle (Troy et al., 2001).

For some domestic species presumptive wild ancestors still exist and it is feasible to test the phylogenetic relationships. Domestic dog has the highest number of possible ancestor species as all species in the genus *Canis* and dhole (Asian wild dog) and African wild dog are known to hybridise. Would the spectacular range of size and conformation of domestic dog result from hybrid origin? Especially grey wolves and golden jackals and sometimes also coyotes and Ethiopian wolves have been suggested as possible ancestors. Anyway, mitochondrial and other molecular studies support the Eurasian grey wolf as the sole ancestor (Vilá et al., 1999) as the maternal roots of dog and wolf are tightly intertwined. As there is almost as much mitochondrial diversity in dogs as in wolves, the great diversity in domesticated dog is not due to the contribution of several canid species, but due to selection on well-represented wolf heritage.

Mitochondrial studies in domesticated mammals have concentrated on resolving the phylogenetic roots of domesticated species. The emerging picture is complex and emphasises the distinction of gene tree and species tree and also the vagueness of the concept of species. The research is shifting more clearly from the origins of agriculture to the details of spreading of domestic animals, and this needs more comprehensive sampling than previously done. The molecular studies have needed archaeological results for calibration and hypothesis building, but now also the archaeological research benefit from the molecular studies, *e. g.*, mtDNA can be used to distinguish goat bone from sheep bone (Loreille et al., 1997). Interaction of different branches of science will continue to be fruitful. More de-

tailed work is being done, for example, in Northern Europe and this will continue to provide information not only on the domesticated animals, but also on human culture at the same time.

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MITOCHONDRINĖS DNR, KAIP GENETINIO ŽYMEKLIO, PANAUDOJIMAS NAMINIŲ GYVŪNŲ TYRIMUOSE

S a n t r a u k a

Žmonės nuo seno domisi naminių gyvūnų kilme. Archeologinių kasinėjimų metu surinkti kaulai rodo, kad galvijai, avys, ožkos ir kiaulės buvo prijaukintos anksčiau nei prieš 10000 metų. Pastaruoju metu, tobulėjant molekuliniams genetiniams metodams, atsirado galimybė nustatyti ne tik branduolinės, bet ir mitochondrinės DNR seką. Šių dienų populiacijų tarprūšiniams tyrimams taikant šį metodą, siekiama išsiaiškinti naminių gyvūnų kilmę. Mitochondrinė DNR tyrimui pasirinkta neatsitiktinai. Mitochondrijos genomas yra haploidinis ir paveldimas tik iš motinos, jame nevyksta rekombinacija, o mutacijų dažnis daug didesnis nei branduolinėje DNR. Gauti rezultatai rodo, kad galvijų, avių, ožkų ir kiaulių domestikavimo procesas istorijos eigoje vyko keliais etapais skirtingose geografinėse vietovėse. Visos šios rūšys turėjo keletą skirtingų laukinių protėvių, kurie davė pradžią dabartiniams naminiams gyvūnams. Priešingai, tiriant šunų veisles buvo pastebėta, kad didelė fenotipinė įvairovė atsirado ne dėl skirtingų laukinių protėvių hibridizacijos, o selekcijos pagalba atrenkant Eurazijos pilkuosius vilkus. Šiuo metu atliekami išsamesni tyrimai turėtų suteikti daugiau informacijos apie naminių gyvūnų domestikavimo istoriją.

Raktažodžiai: mtDNR, sekos duomenys, filogeografija, populiacijos istorija, domestikacija