

## GENETIC DIVERSITY AND PHYLOGENETIC RELATIONSHIPS IN GROUPS OF ASIAN GUARDIAN, SIBERIAN HUNTING AND EUROPEAN SHEPHERD DOG BREEDS

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### SUMMARY

*Motivation:* Analysis of Asian dog breeds has an important role in understanding phylogenetics of many dog breeds, because an East Asian origin of domestic dogs has been established.

*Results:* It was shown extremely close phylogenetic relationships in group of Asian guardian dog breeds and close relation to this group breed German Shepherd dog and group “Laika”. One of Portuguese breeds (Serra da Estrella Mountain Dog) reveals more relation to Asian guardian dogs than to other Portuguese breeds. Breed groups Central Asian Sheepdog, Northern Caucasian Volkodav and Laika characterized by relatively high level of genetic diversity in contrast with Caucasian Ovtcharka.

### INTRODUCTION

The origin of domestic dog and phylogenetics of dog breeds is very intriguing and complicated problem. Late years it was shown that dog has an origin from several wolf lines, most of all were distributed in East Asia (Savolainen *et al.*, 2002). Modern dogs have mtDNA haplotypes belonging to five mtDNA haplogroups (A, B, C, D, E). Three of them (A, B, C) include > 95 % of studied sequences. Haplogroups B and C in contrast with haplogroup A have star-like form of networks with central nodes. Many authors have shown that there is no breed – specific haplogroups of mtDNA (Vila *et al.*, Okumura *et al.*, and others).

In order to evaluate more detailed picture of some breed phylogenetic relationships we have chosen several Asian guardian dogs and combined them to groups according to their geographical distribution and origin. As a result we have following breed groups: a) Turkish dogs (akbash and kangal), b) Central Asian Sheepdog (CAS), c) Northern Caucasian Volkodav (NCV), d) Caucasian Ovtcharka (CO). By the same approach we have chosen group “Laika”.

For comparison we selected Portuguese breeds Portuguese Sheepdog (PS), Serra da Estrella Mountain Dog (SEMD), Azores Cattle Dog (ACD) and Dutch breed German Shepherd Dog (GSD). Different morphological type, employment, areas of origin and distribution characterize these breeds. By these reasons we analyzed them each as separate group.

## MATERIAL AND METHODS

Blood and hairs from “CAS”, “NCV”, “CO”, “GSD” and “Laika” were collected during veterinary practice and at canine moves in Caucasian and Moscow regions. Left variable segment of mtDNA was amplified with primers L15910 and H16498 (DeSalle *et al.*, 1993). Sequencing of gel-purified PCR products was performed using BigDye Terminator Kit v. 3.1 on ABI-Prism 3100-Avant instrument (Applied Biosystems, USA).

Nucleotide sequences and frequencies of haplotypes in breeds PS, SEMD, ACD, “Turkish dogs”, some German shepherds and “Laika” were taken from published papers (Savolainen *et al.*, 2002, van Ash *et al.*, 2005) and GenBank.

Nucleotide diversity, gene diversity,  $F_{st}$ , Tamura-Nei genetic distances and AMOVA were calculated using Arlequin v.3.0 (Excoffier *et al.*, 2005). Haplotype network was constructed using TCS v.1.21 (Clement *et al.*, 2000).

## RESULTS AND DISCUSSION

In group “CAS” we identified 14 haplotypes belonging to A (9), B (2), C (1), W/E (2) clades, in “CV” – 12 belonging to clades A (7), B (2), C (2), D (1), in “CO” – 5 haplotypes, belongs to clades A (3) and B (2). We identified 10 novel haplotypes, 6 of them belongs to clade A, 1 – to clade B, 1 – to clade C and 2 of them belong to clade W/E. It is interesting, that haplotypes from clade E closely related to wolf clade W were found before only in Japanese and Korean breeds.

As was previous shown (Savolainen *et al.*, 2002) haplogroup D has maximum diversity and high frequencies in Scandinavia and occasionally observed in Spain, Portugal and Turkey. Occurrence of early-identified haplotype D6 (RH20) in “CV” and D1-4 (RH25) in “Laika” may indicate a gene flow from Scandinavian breeds to breeds of these regions through Europe and Asia Minor.

CAS, CV and Turkish breeds share 3 common haplotypes, furthermore one haplotype RH1 (A11-14, 54) that occurs with high frequencies in “CAS”, “CV”, “CO”, “Laika” and “Turkish breeds” is absent in Portuguese breeds. As we show in Fig. 1 haplogroup D may originate from this haplotype.

Pairwise differences between groups measured as  $F_{st}$  are zero and therefore indicate absence of differentiation between “CAS”, “NCV”, “CO”, “Turkish dogs”, “Laika” and “GSD”. One of Portuguese breed - SEMD - shows  $F_{st}$  about 0,075 with these groups in contrast with 0,25 and 0,30 with other Portuguese breeds ACD and PS respectively. This fact reflects high level of differentiation between Portuguese breeds and less differentiation with groups specified above.

For phylogeny inference we calculated Tamura-Nei genetic distances taking into account different transition/transversion rates, differences in base frequencies and transition rates between purines and between pyrimidines. As shown in Fig. 2 “Laika”, “CO” and “GSD” slightly stand out from pointed out nondifferentiated group.

AMOVA indicates the following: 1) combining all breeds in one group 16,36 % was among breeds component and 83,64 % – within breed, 2) combining “CAS”, “NCV” and “Turkish dog” in one group, “CO” and “PS” in another, and others breeds each in separate group the level of within-groups component was the minimal and among-group’s was maximal values in comparison with other possible combinations based on genetic distances. 3) In case of groups of breeds were combined according to geographical regions of their distribution, among group component was always negative and nonsignificant.

Gene diversity was high in groups “CAS”, “NCV”, “Laika” (0,83–0,88) middle in “GSD” and “Turkish dogs” (0,70–0,76) and low in “PS”, “ACD” and “CO” (0,48; 0,64; 0,65). Low gene diversity in breeds subjected to cultural breeding may be due by prevalent inbreeding.

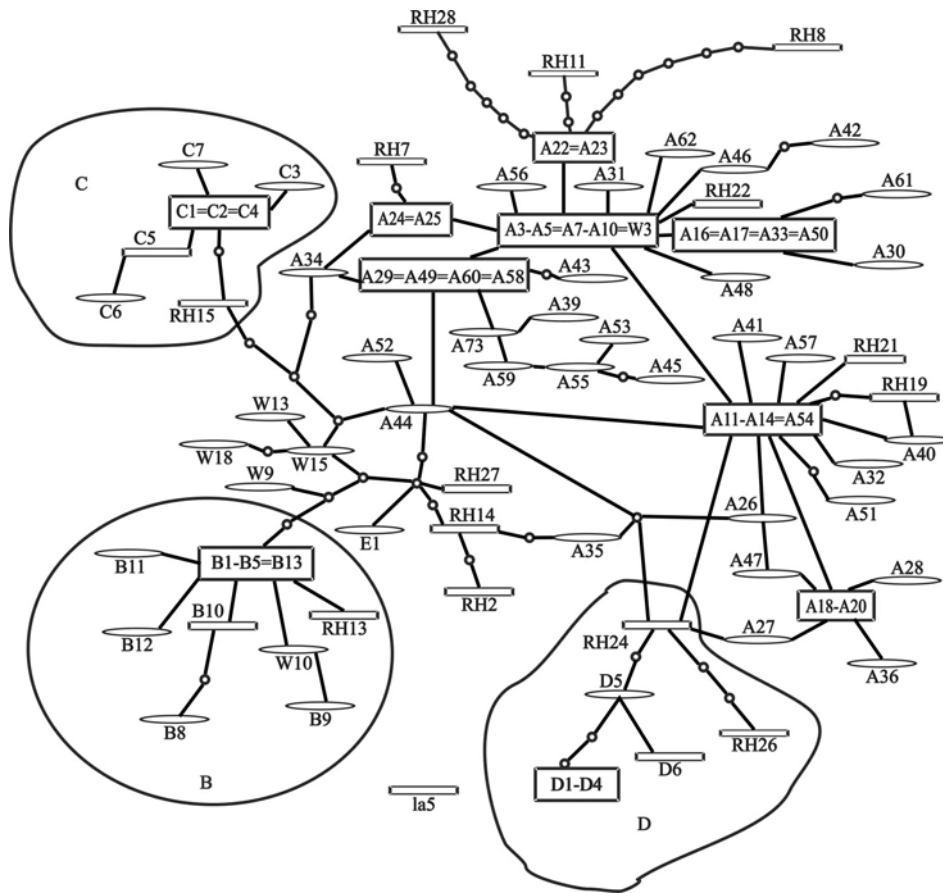


Figure 1. Haplotype network of dog mtDNA D-loop haplotypes. Haplotypes typed in rectangles were detected in “CAS”, “CV”, “CO” and “Laika”. Haplogroups B, C, D are whirlpooled.

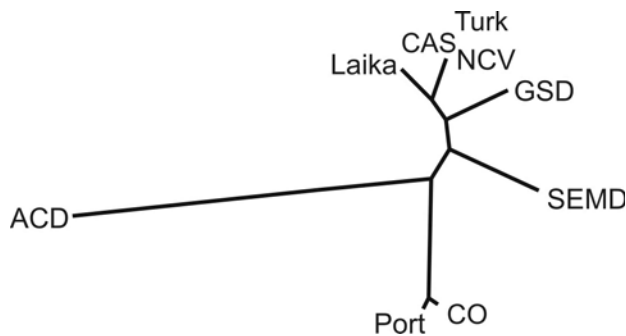


Figure 2. UPGMA tree based on Tamura-Nei distances.

Our results indicate major role of gene flow between regions during forming of dog breeds. We have shown a possibility of Asian origin of European breed German Shepherd Dog and high involvement “Asian” guardian dog lines in genesis of Portuguese breed Serra da Estrela Mountain Dog.

Genetic data presented in this work have historical and archeological confirmations.

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