



Epigenetic Variation and Distinctness of Red Fox *Vulpes vulpes* (L., 1758) (Mammalia: Canidae) Populations in its European Range

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Abstract: This study describes the cranial epigenetic pattern of the red fox *Vulpes vulpes* (L.). The appearance of 14 cranial non-metric traits was recorded in specimens belonging to 25 red fox local populations inhabiting European continent: five from North Europe, eight from Central Europe, eight from Southwestern Europe and four from Southeast Europe. The results revealed the presence of well-expressed polymorphism in all studied cranial traits. The analysis of epigenetic variability, epigenetic distances and uniqueness of studied populations show that the red foxes from Central Europe and Balkan Peninsula are similar. By its craniological epigenetic characteristic, the red fox of the Balkan Peninsula belongs to the Central European subspecies *Vulpes vulpes crucigera* (Bechstein, 1789). The established degree of similarity of the investigated local populations of the red foxes in Europe is supported by the classical description of the subspecies variability and by the recent results of the biochemical genetic studies. The obtained results of the evaluation of epigenetic differentiation of red fox populations through non-metric skull characters have shown that epigenetic distances are a valid source of information on diversity of the species in its European geographical range. Further studies of the non-metric skull characters of red fox populations may be combined with molecular genetic studies on suitable genetic markers in order to reveal the genetic structure and phylogenetic relationships among the European populations.

Key words: Red fox, population diversity, epigenetic differentiation, Europe

Introduction

The red fox *Vulpes vulpes* (L., 1758), as a medium-sized canid, is the largest fox of the genus *Vulpes* Frisch, 1775. Due to its extremely adaptability, it is the most widely distributed carnivore in the world. The natural distribution of the red fox extends throughout the Northern Hemisphere, from the Arctic Circle to North Africa, Central America and the Asiatic steppes. In Europe, its natural habitats are mostly dry landscapes, including all types of forests, woodlands and open landscapes with abundant

scrubs. Red foxes are also abundant in moorlands, mountains, sand dunes and farmlands, from the sea level up to an altitude of 4,500 m (LARIVIÈRE & PA-SITSCHNIAK 1996). Although urbanization occurs at present, which is usually harmful to biodiversity, the red foxes flourish within human-modified landscapes (SCOTT et al. 2014) and find suitable development conditions in urban areas (STUBBE 1999). There, the red fox is most common in residential suburbs consisting of low-density houses; it is less common where industry, commerce or council-rented housing predominates (HARRIS & SMITH 1987).

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Red fox density is highly variable. Although an increase in fox numbers following successful rabies control by vaccination was widely reported in Europe, no direct measures of population density have been taken and it is assumed that the current population trend of this species is “Stable” (HOFFMANN & SILLERO-ZUBIRI 2016).

Modern ideas about the intra-species taxonomic structure of *Vulpes vulpes* recognise 45 subspecies as valid (WOZENCRAFT 2005). However, the conducted studies are still not sufficient and the number of subspecies deserves further attention. Recently, a number of new subspecies has been described and 13 recognised subspecies inhabit the territory of the Old World (CASTELLO 2018).

The epigenetic methods have been recognised as suitable tools to assess intraspecific variation and divergence among canid populations (SJØVOLD 1977). Besides the molecular genetic techniques, the evaluation of the polymorphism of the epigenetic traits by checking non-metric skull characters may be used to obtain information about the genetic variability within populations and between populations (ANSORGE 2001). The epigenetic variability and differentiation of the red fox populations in its European range are still insufficiently studied, although the analysis of epigenetic polymorphism among its populations may provide a useful tool for the examination of the intra-species biodiversity on the continent. Herein, we used epigenetic methods to explore variation of non-metric cranial features in red fox populations in Europe, including populations inhabiting the Scandinavian, Iberian and Balkan Peninsula as well as the territory of Central Europe.

This study aims to report on the comparative analysis of the variation of non-metric skull characters of the red fox in Europe in order to determine the population epigenetic variation and to analyse the geographical epigenetic relations and the epigenetic divergence.

Materials and Methods

The study of the epigenetic polymorphism of the red fox in Europe was carried out based on the study of 25 local populations from Europe. The characteristics of the epigenetic polymorphism in 20 local populations were investigated on the basis of 938 individuals. For the characterisation of other five northern European populations (2320 individuals), we used published data (SJØVOLD 1977) for the frequency distributions of the status of the examined epigenetic traits. Thus, the epigenetic cranial poly-



Fig. 1. Local populations of the red fox in Europe investigated by epigenetic traits: North Europe – Pop SW1 (province Skåne; 196 individuals), Pop SW2 – (province Östergötland; 448 ind.), Pop SW3 (province Uppsala; 456 ind.), Pop SW4 (province Dalarna; 448 ind.), Pop SW5 (province Jämtland; 772 ind.); Northeast Central Europe - Pop CZ1 (Moravia; 13 ind.), Pop CZ2 (Bohemia; 29 ind.), Central Europe - Pop AU1 (province Burgenland; 38 ind.), Pop AU2 (province Niederösterreich; 59 ind.), Pop AU3 (province Oberösterreich; 33 ind.), Pop AU4 (province Steiermark; 12 ind.), Southeastern Central Europe - Pop HU1 (province Hortobágy; 20 ind.), Pop HU2 (the plains in central Hungary; 23 ind.); Southwest Europe - Pop SP1 (province Cantabria; 57 ind.), Pop SP2 (province Pyrenees; 73 ind.), Pop SP3 (province Toledo; 110 ind.), Pop SP4 (province Granada; 71 ind.), Pop SP5 (province Valladolid; 43 ind.), Pop SP6 (province Burgos; 62 ind.), Pop SP7 (province Tarragona; 15 ind.), Pop SP8 (province West Galicia; 28 ind.); Southeastern Europe - Pop BG1 (mountain Stara planina; 16 ind.), Pop BG2 (mountain Vitosha; 95 ind.), Pop BG3 (mountains the Rila-Rhodope; 94 ind.) and Pop BG4 (plains in central and northeastern Bulgaria; 18 ind.).

morphism was explored by the examination of 3258 red foxes. The geographical location of the studied 25 local populations covers a significant part of Europe: five local populations (2320 individuals) from North Europe, eight from Central Europe (229 individuals), eight from Southwest Europe (486 individuals) and four from Southeastern Europe (223 individuals) (Fig. 1).

Only adult specimens were used in the present study. The age of the animals was determined by the growth, replacement and worn of the teeth (HARRIS 1978) and by the condition of the sutures between the skull bones (CHURCHER 1960) when the teeth were missing. All red fox skulls were analysed using

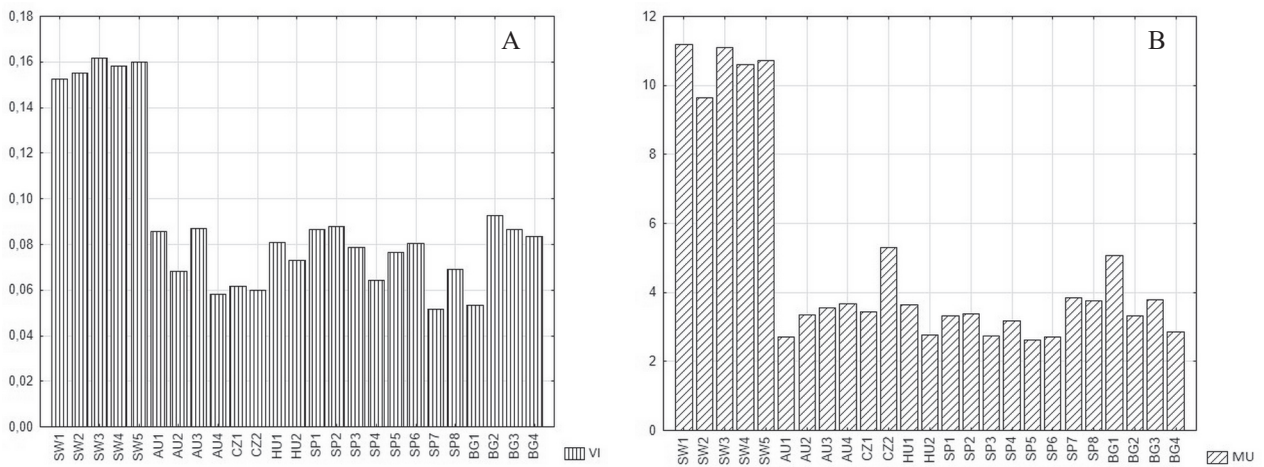


Fig. 2. Epigenetic variability (Vi) (Fig. 2A) and epigenetic uniqueness (MU) (Fig. 2B) of the investigated local populations of the red fox in Europe: North Europe (Pop SW1: Skåne; Pop SW2: Östergötland; Pop SW3: Uppsala; Pop SW4: Dalarna; Pop SW5: Jämtland); Northeast Central Europe (Pop CZ1: Moravia; Pop CZ2: Bohemia); Central Europe (Pop AU1: Burgenland; Pop AU2: Niederösterreich; Pop AU3: Oberösterreich; Pop AU4: Steiermark), Southeastern Central Europe (Pop HU1: Hortobágy; Pop HU2: the plains in central Hungary); Southwest Europe (Pop SP1: Cantabria; Pop SP2: Pyrenees; Pop SP3: Toledo; Pop SP4: Granada; Pop SP5: Valladolid; Pop SP6: Burgos; Pop SP7: Tarragona; Pop SP8: West Galicia); Southeastern Europe (Pop BG1: mountain Stara planina; Pop BG2: mountain Vitosha; Pop BG3: mountains the Rila-Rhodope; and Pop BG4: plains in central and northeastern Bulgaria).

14 non-metric traits whose expression is genetically controlled (BERRY 1975) and is independent from growth and sex (BERRY 1975, ANSORGE 2001). According to ANSORGE et al. (2009), non-metric traits are exposed to a minimum of selective pressure, which makes them as epigenetic markers reflecting the genetic circumstances of the relevant phenotype; therefore, they are suitable for the assessment of the genetic variability and divergence among populations red fox populations (SJØVOLD 1977).

The examined 14 characters were selected according to the previous studies on cranial epigenetic variability and documented in the literature for epigenetic non-metrical investigation of medium-sized canid species, including for the red fox (SJØVOLD 1977). These characters were: No. 1 Third lower molar – *missing*; No. 2 Accessory anterior mandibular foramen – *present*; No. 3 Accessory mental foramen – *present*; No. 4 Premaxillary-frontal contact – *present*; No. 5 Accessory frontal foramen – *present*; No. 6 Frontal canal – *present*; No. 7 Ethmoidal foramen – *separated*; No. 8 Accessory interior foramen palatine – *present*; No. 9 Accessory minor foramen palatine – *present*; No. 10 Emissary foramen – *present*; No. 11 Hypoglossal canal – *double*; No. 12 Condylar canal – *double*; No. 13 Condylar canal – *open ventrally*; No. 14 Ventral condylar canal – *present*.

For uniform results, all data have been investigated on the left side of the skull.

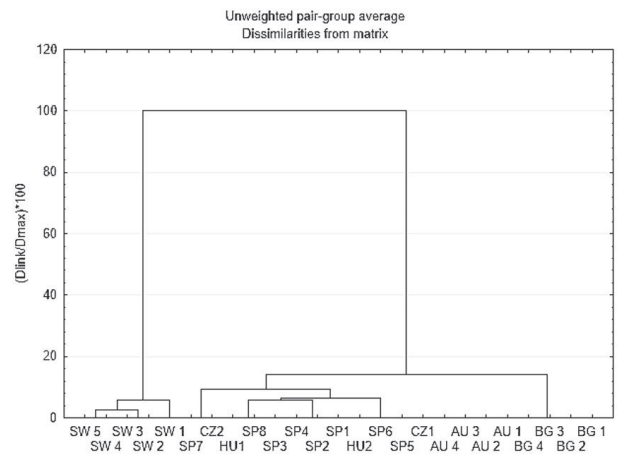


Fig. 3. Degree of the epigenetic cranial similarity of the local populations of the red fox in Europe: North Europe (Pop SW1: Skåne; Pop SW2: Östergötland; Pop SW3: Uppsala; Pop SW4: Dalarna; Pop SW5: Jämtland); Northeast Central Europe (Pop CZ1: Moravia; Pop CZ2: Bohemia); Central Europe (Pop AU1: Burgenland; Pop AU2: Niederösterreich; Pop AU3: Oberösterreich; Pop AU4: Steiermark), Southeastern Central Europe (Pop HU1: Hortobágy; Pop HU2: the plains in central Hungary); Southwest Europe (Pop SP1: Cantabria; Pop SP2: Pyrenees; Pop SP3: Toledo; Pop SP4: Granada; Pop SP5: Valladolid; Pop SP6: Burgos; Pop SP7: Tarragona; Pop SP8: West Galicia); Southeastern Europe (Pop BG1: mountain Stara planina; Pop BG2: mountain Vitosha; Pop BG3: mountains the Rila-Rhodope; and Pop BG4: plains in central and northeastern Bulgaria)

The Epigenetic variability (V_i) after SMITH (1981) and the Mean measure of divergence (MMD) after SJØVOLD (1973) between each single sample and all the others were computed according to the established frequency distribution of the examined epigenetic characters. Measure of uniqueness (MU) was calculated after BERRY (1963) for each sample as a sum of its statistically significant ($P < 0.05$) epigenetic distances from the other samples.

The cluster analysis was performed using the algorithm of the unweighted pair-group average analysis (UPGMA); calculations were computed using the statistical package STATISTICA 2008, v. 8.0.

Results

The summary analysis of the epigenetic variability of 25 local populations in Europe (Fig. 2A) showed that the red foxes from the northern part of the continent had a pronounced higher epigenetic variability than those in the southern part of the continent. The epigenetic variability of the foxes from Central and Southern Europe was relatively equal. Highest epigenetic variability had the foxes of Vitosha Mountain ($V_i = 0.0928$), and lowest – the foxes from the plain area in the Province Tarragona ($V_i = 0.0514$).

The expressed epigenetic uniqueness of the studied 25 local populations in Europe (Fig. 2B) showed that it was highest in the northernmost part of the range of the species – in orderliness from $MU=9.6388$ in foxes from Östergötland to $MU=11.1916$ in local population from Skåne. The values of the epigenetic uniqueness of the local fox populations in Central and Southern Europe was relative low. The highest epigenetic population uniqueness was revealed in the fox population in Northeast Central Europe, i.e. in Province Bohemia ($MU=5.2995$), and lower – in the fox population from the Iberian Peninsula (West Spain, Province Valladolid, $MU=2.6382$).

The established degree of similarity between all 25 local fox populations based on the epigenetic distances between them (Fig. 3) confirmed the intra-regional epigenetic similarity of local populations inhabiting the Scandinavian Peninsula, Central Europe, the Iberian Peninsula and the Balkan Peninsula. At the same time, the epigenetic cranial characteristics of fox populations from the Scandinavian Peninsula were clearly outlined by their epigenetic characteristics from those inhabiting Central Europe, the Iberian and the Balkan Peninsula.

Discussion

Epigenetic polymorphism has been manifested in the expression of the craniological characteristics in the studied populations of the red fox from Europe. Analysis of the epigenetic variability of the red fox in Europe has shown that it is highest in local populations of foxes inhabiting the Scandinavian Peninsula. There has been a tendency for decreasing of the epigenetic variability in the south direction, as the foxes from the plains of the Iberian Peninsula have the lowest epigenetic variability. The foxes from the mountain habitats of the Balkan Peninsula have high epigenetic variability. Despite the slight variations, it remains similar in all studied local populations from Southern Europe. This confirms that, despite the different ecological conditions in the habitats of the species, there are homogeneous populations by their non-metric craniological characteristics in the central and southern parts of the continent.

The analysis of the epigenetic variability, epigenetic distances and the uniqueness of the studied fox populations supports that the foxes from the territory of Northern Europe have a well-defined craniological distinction from those inhabiting Central, Southwestern and Southeastern Europe. The lack of statistically significant epigenetic distances between most of the compared local fox populations in Central Europe and the Balkan Peninsula demonstrates their craniological epigenetic homogeneity in this part of the continent. Based on these results, it can be assumed that the red fox from the Balkan Peninsula, based on its craniological characteristics, belongs to the Central European subspecies *Vulpes vulpes crucigera* (Bechstein, 1789).

The established degree of similarity of the studied local fox populations in Europe, based on epigenetic distances between them, is supported by the classical description of the subspecies diversity of the red fox (*Vulpes vulpes*) on the continent. There are four subspecies (MILLER 1912) in Europe: *Vulpes vulpes vulpes* L. occurring in the Scandinavian Peninsula; *Vulpes vulpes crucigera* distributed in Central and South Europe; *Vulpes vulpes silacea* Miller in the Iberian Peninsula and *Vulpes vulpes ichnusae* Miller from the Mediterranean islands Corsica and Sardinia. The established degree of similarity is synchronous with current genetic investigations of the fox in Europe indicating the nominotypical *Vulpes vulpes vulpes* as distributed in the Scandinavian Peninsula and the northernmost parts of European Russia (MACDONALDS & REYNOLDS 2004, STATHAM 2014, 2018); the subspecies *V. v. silaceus* in the Iberian Peninsula (STATHAM 2018); *V. v. crucigera* in

Central Europe (MACDONALDS & REYNOLDS 2004, THEACHER et al. 2011, STATHAM 2018) and the Balkan Peninsula (STATHAM 2018). This also explains the difficulties in the determination of the systematic status of the red fox from Central and South Europe, further complicated by the geographical isolation and the high genetic variability of the species in Europe (GALOV et al. 2014).

The obtained results of the evaluation of epigenetic differentiation of red fox populations through non-metric skull characters show that epigenetic distances are a valid source of information on biodiversity of this species in its European range. Further research of the non-metric skull characteristics of red fox populations has to be combined with molecular genetic studies on suitable genetic markers in order to reveal the genetic structure and phylogenetic relationships among European populations.

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