RESEARCH ARTICLE

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Developmental stability of the golden jackal (*Canis aureus moreoticus*) populations in its expansion range in Bulgaria

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ABSTRACT

The European golden jackal (*Canis aureus moreoticus*) is widespread in Bulgaria and it was assumed that the species had occupied almost the entire country with less manifested presence in highlands. The general aim of this investigation was to carry out comparative analysis at a population level of developmental stability of the jackal inhabiting in different habitats (POP_1, inhabiting Sakar-Strandzha region, POP_2 inhabiting the Upper Thracian Plain and POP_3 inhabiting the western part of the Sub-Balkan valleys), located along the presumable route of the species movement northwards and westwards in the country. The population levels of developmental stability were assessed through fluctuating asymmetry determined as minor non-directional deviations from bilateral symmetry in 10 morphological non-metric skull characters. Looking at the fluctuating asymmetry as an indicator of environmental and genetic stress, there is no evidence of genetic depression by reproductive isolation in the studied populations. Moreover, there is no indication of relatively reduced developmental stability in any of the analyzed populations. In general, the results from the comparative population analysis of the craniological epigenetic fluctuating asymmetry in Bulgarian golden jackal with regard to the population developmental stability provided a substantial biological basis for their interpretation as biomonitor characteristics in a future biological monitoring of this species in the country.

Key words: *Canis aureus moreoticus,* European golden jackal, developmental stability, fluctuating asymmetry, non-metric characters

Introduction

The high ecological plasticity of the golden jackal (*Canis aureus*) allows it to settle a large geographic range covering the territories of South-eastern Europe and South Asia (Jhala & Moehlman, 2008). In Europe, over the past years, the species range expanded from the Balkans northwards and westwards. In particular, the presence of the golden jackal was recorded in Hungary, Slovakia, Austria, Italy, Ukraine, Belarus, Poland, Estonia and Lithuania (Arnold et al., 2012; Trouwborst et al., 2015).

In Bulgaria, the European golden jackal (*Canis aureus moreoticus*) is resident in the mammal fauna, as until the early 1960s it occurred only in the region of Strandzha Mountain, in the Southeast part of the country (Botev et al., 1979; Spassov, 1989). A significant increase in its population in Bulgaria was observed after 1985, together with extended distribution westwards and north-westwards from the original habitats in Strandzha-Sakar area, while the golden jackal occupied lowland and hilly habitats in the country (Spassov, 2007; Markov, 2012). Nowadays, it was assumed that the species had occupied almost the entire country with less manifested presence in highlands and the total number of jackals recently reached about 40 000 animals. It seems that the largest jackal population in Europe occurs in Bulgaria (Markov, 2011).

As a result of the intense process of spreading in the West-North part of the country the species resides in regions where the ecological conditions and the food base are quite different, and they determine the distribution, local abundance and population density of golden jackals in Bulgaria (Pomakov, 1981; Spassov, 2007; Markov, 2012; Stoyanov, 2012).

Considering the lack of knowledge on the developmental stability of the golden jackal at a population level in Bulgaria, our general aim was to carry out a comparative analysis of its diversity in different habitats, populated at the time of its recent expanding spread northwards and westwards in the country. The established values of the instability of the population of the jackal are interpreted as an indicator of the potential negative impact of genetic or ecological factors on its development in the country.

The assessment of the fluctuating asymmetry (FA), determined as small random deviations from bilateral symmetry in a morphological trait, normally distributed around a mean of 0 (for details see Palmer, 1994; Van Valen, 1962), is a frequently used method for measuring developmental stability or instability of animal populations. Embracing the idea that fitness will be reflected by the morphology of individuals suggested that fluctuating asymmetry (FA) may be a suitable morphological characteristic to score in developmental stability (e.g. Mitton 1978). In this respect a number of studies (Hartl et al., 1995; Zachos et al., 2007; White & Searle, 2007; Lovatt & Hoelzel, 2011) concerning on mammalian populations have generally found, though not always strongly expressed, negative correlations between genetic variability and FA and the results obtained by them support the use of FA as an indicator of fitness by mammalian species.

Bearing in mind the general biological relevance of the mammalian skull analysis to resolve several issues in mammals' microevolution and adaptations, in the present study we used the non-metric skull characters as a morphological approach to explore the population levels of developmental stability of the golden jackals following the direction of the presumptive movement of these animals from the Southeast to Northwest part of Bulgaria.

Materials and Methods

Study area and collecting of specimens:

The specimens (104 individuals – 56 males and 48 females), included into the comparative analysis of the levels of developmental stability, belong to three Bulgarian populations (POP_1, inhabiting Sakar-Strandzha region – 38 individuals – 19 males and 19 females; POP_2 inhabiting the Upper Thracian Plain – 31 individuals – 16 males and 15 females and POP_3 inhabiting the western part of the Sub-Balkan valleys – 35 individuals – 20 males and 15 females), located along the direction of the presumptive movement of the golden jackal from southeastern part to the northeastern part of the country (Figure 1).

All examined specimens were legally hunted in the course of population control programs organized by the local hunting associations and the analyzed skulls consisted partly of specimens kept in the science museum collections of public institutes, such as the Institute of biodiversity and ecosystem research (Sofia) and National Museum of Natural History (Sofia), but most of them belonged to private collections. Only adult specimens were used in the present study. Determination of the specimens' age was based on the degree of wear of the upper incisors (Lombaard, 1971).

Analysis of Developmental stability

Developmental stability was investigated using a set of quantitative non-metric characters presented as a number of paired foramina in the golden jackal skull. For the purposes of this study the appearance of 10 cranial bilateral non-metric characteristics, selected from the ones designed and used by Markov et al. (2017) in the assessment of epigenetic variation and distinctness of the golden jackal populations in the Southeast European range of the species, was recorded and registered separately on both sides of the skull. These skull traits and their examined status in the European golden jackal were as follows: T1 – Accessory anterior mandibular foramen – present; T2 – Accessory mental foramen – present; T3 – Nasal foramen – present; T4 – Accessory frontal foramen – present; T5 – Frontal canal – present; T6 – Ethmoidal foramen – separated; T7 – Accessory foramen ovale – present; T8 – Median sphenoidal foramen – present; T9 – Hypoglossal canal – double; T10 – Condylar canal – double.

As measure of developmental stability, we used the fluctuating asymmetry, a non-directional difference in the right and left sides (Ri – Li) of bilateral non-metric characters. The fluctuating asymmetry analysis was performed in accordance with the algorithm proposed by Ansorge et al. (2012) for estimation of the genetic variability of the mammals' population, considering that: (i) the degree of fluctuating asymmetry is defined as a ratio of the number of asymmetric occurrences of a single character to the sample

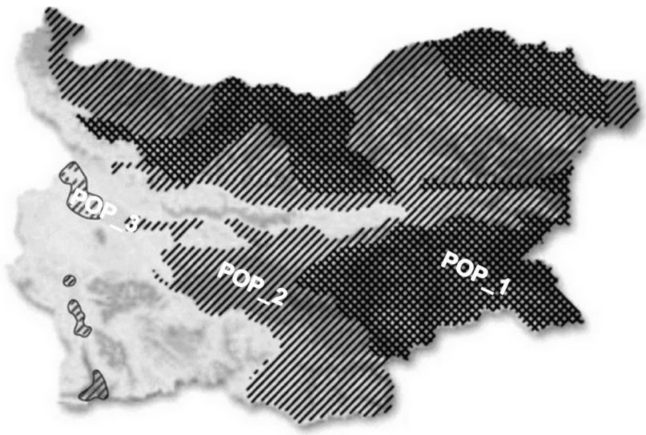

Figure 1. *Topographical location of the studied populations (POP_1, inhabiting Sakar-Strandzha region, POP_2 inhabiting the Upper Thracian Plain and POP_3 inhabiting the western part of the Sub-Balkan valleys) on the background of contemporary distribution and density of golden jackal (Canis aureus) in Bulgaria (following graphic information by Spassov (2007) and numerical information by AEFA (2014). Shaded areas denote moderate to low density; crossed areas indicate high density, patches to the west show isolated population clusters.*

size; (ii) the unscaled mean of fluctuating asymmetry of all characters results in the degree of asymmetry of the population. Before proceeding with the asymmetry analysis, statistical tests (Palmer, 1994) were performed to prove the presence of fluctuating asymmetry and to reject the possibility of the presence of directional asymmetry or antisymmetry in the expression of each analyzed traits. The significant differences ($p < 0.05$) between the mean values of the fluctuating asymmetry at the population level were tested by the analysis of variance (ANOVA) and the Bonferroni adjustment test was applied to obtain an enhanced estimation of their statistical significance. All calculations were performed using the statistical package STATISTICA (2011).

The proved in previous studies (Markov et al., 2017) absence of sexual dependence in the expression of the 10 investigated characters allowed us to pool the specimens of both sexes into one sample for each population in further analyses of the examined populations.

Results

The performed statistical tests according to the algorithm proposed by Palmer (1994) for examination of the asymmetry relations of all selected bilateral characters showed that the presence of the fluctuating asymmetry can be assumed and to reject the possibility of the presence of directional asymmetry or antisymmetry in the expression of each analyzed traits.

The results of analyses of the fluctuating asymmetry carried out separately for each investigated morphometric character in the three studied populations and the generalized degree of asymmetry of each population are presented on Figure 2 (A and B).

The individual values of the fluctuating asymmetry of the investigated non-metric skull characters (T1 – T10) are specific for each of the three golden jackal populations (Figure 2 A). The highest degree of asymmetry was found in POP_2 (FA = 0.2113), and the lowest – in POP_1 (FA = 0.1783); the population POP_3 has a medium degree of FA = 0.1945 (Figure 2 B). At the same time, at the local population level, the investigated golden jackal populations in Bulgaria demonstrate an absence of a statistically significant degree ($p<0.05$) of divergence in regard to the fluctuating asymmetry.

Discussion

Looking at the fluctuating asymmetry of epigenetic cranial signs as an indicator of environmental as well as genetic stress (Ansorge, 2001) and assuming that a higher degree of asymmetry suggests lower developmental stability and lower fitness, there is no evidence of genetic depression by reproductive isolation in the studied populations, nor is there any indication of relatively reduced developmental stability in any of them. This conclusion was drawn using the morphological approach to assess the developmental stability of jackals through non-metric skull characters and was also confirmed by the intensive increase of the jackals' abundance in all territories of Bulgaria. It was based on the data obtained from the comparative analysis of the fluctuating asymmetry in the Bulgarian populations of the golden jackal, varying from FA = 0.18 to FA = 0.21, which is comparable with the fluctuating asymmetry of non-metric traits found in different mammal species (FA = 0.14 to FA = 0.44) developing under normal conditions (Markowski, 1993; Lazarová, 1999;

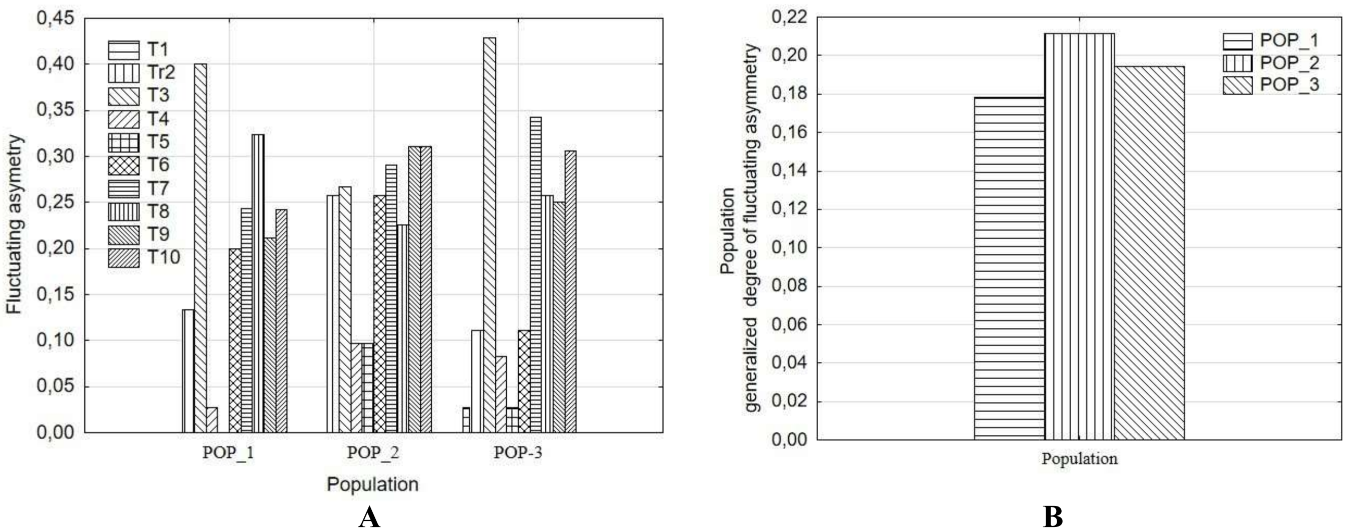

Figure 2. *Values of the fluctuating asymmetry: (A) investigated non-metric skull characters (T1÷T10) in the three studied populations; (B) mean values of the generalized degree of fluctuating asymmetry of each population expressed as an unscaled mean of fluctuating asymmetry of all investigated characters. POP_1, inhabiting Sakar-Strandzha region, POP_2 inhabiting the Upper Thracian Plain and POP_3 inhabiting the western part of the Sub-Balkan valleys.*

Vasilev, 2005). This conclusion was also corroborated by the official data from the Ministry of agriculture, food and forestry about the presence of jackals on the territory of Bulgaria (Yankulov & Russev, 2017), showing that the species numbers in 2017 had increased by 2046 individuals (4.1%), compared with 2016, and reached 49 584 individuals. At the same time, the golden jackal begins to settle on the mountainous part of the country, as the increase of species numbers is accompanied by the expansion of occupied territories.

Although the spreading of the golden jackal is causing today increased scientific and practical interest because of the expansion of this species on the European continent, the present study is the first to reveal its developmental stability in different populations across the expanded Bulgarian area. The obtained results allow drawing the conclusion that in the course of the expansion from the southeastern part towards the west-northern part of Bulgaria, the golden jackal inhabits favorable biotopes and its populations are not exposed to environmental/genetic stress. The population studies on jackals along the investigated transect in Bulgaria should be widened and should include new populations and combined morphological and molecular biochemical-genetic approach. In this way, the population developmental stability of the golden jackal in its Bulgarian range would be determined more precisely. Establishing this important indicator of population development of the species will help to take management measures for its new emerging populations.

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