ORIGINAL ARTICLE

Study of non-metric characters of the skull to determine the epigenetic variability in populations of the European wildcat (*Felis silvestris silvestris*) and domestic cats (*Felis catus*)

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Abstract

We studied the variability of non-metric cranial traits, mainly foramina, of European wildcats (*Felis silvestris silvestris*) and domestic cats (*Felis catus*) from Germany based on 28 non-metric traits in 211 skulls. The domestic cats were grouped together as a statistical population. The wildcats were divided into two populations: Harz and Hesse, which were further subdivided, based on traffic infrastructure, natural landscape, and in the Harz, on time period. Epigenetic variability, epigenetic distance and the fluctuating asymmetry were calculated to assess genetic variability, possible depressions and population stability. The epigenetic variability I_{ev} of the wildcat groups ranged from 0.27 (Hesse II) to 0.40 (Harz I). The difference in I_{ev} between all specimens from Harz and Hesse respectively was less ($I_{ev} = 0.37$ Harz and 0.31 Hesse). Compared to other studies these values are not assumed to indicate genetic depression. The epigenetic distance between the wildcat samples is 0.0774 overall, and in each case higher between sub-groups of the Harz and Hesse than between groups within these regions, respectively. The significant epigenetic distance between Harz and Hesse might indicate—at least past formerly—restricted connectivity between these regions. The fluctuating asymmetry for wildcats in total is 11.74% and in the sub-groups it ranges from 8.47 to 16.14%. These values are below 20% are at the lower range known from populations of other mammal species. The use of fluctuating asymmetry had also been discussed critically in its usefulness to assess viability of populations.

Keywords Epigenetic distance · Fluctuating asymmetry · Craniological foramina · Germany · Harz · Hesse

Introduction

Morphological studies of the skull and skeleton yield information about the characteristics of a species, show postnatal ontogenetic changes, as well as variability in adults differing between populations within and across species representing phenotypic biodiversity. Often morphometric and increasingly geometric morphology methods are used to describe and potentially distinguish populations and/or closely related species. But, also minor non-metric skeletal features, often called 'epigenetic' traits (Corruccini 1976), are variable and are used to assess diversity of populations and species. The basic principles to study the variability in non-metric characters as indicators of genetic variability in mammals have been reviewed by Ansorge (2001). This research was mainly developed and established in the middle of the last century. At this time the heritability of non-metric traits in men and rodents were studied, for example, by Grünberg (1952), Berry (1963), Berry and Berry (1967) and Hilborn (1974). Even prior to these studies research dealt with the inheritance of dental characters in human twins, like Korkhaus (1930). Particularly Berry (1963) and Berry and Searle (1963) laid the basis for the current use and terminology in this field. They coined the term 'epigenetic polymorphism' to distinguish the variation in non-metric traits from true genetic polymorphism (Berry and Searle 1963). Thus, epigenetic variability refers to non-metric differences in the expression of morphological characters.

Differences in non-metric characters are a type of discontinuous variation. These characters are under basic genetic control but the discontinuity is due to developmental

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influences (Berry and Searle 1963). The basic genetic control and the general independence of growth render them suitable to assess the genetic variability behind them (Bauchau 1988). Sjøvold (1977) developed some basic methods in this field of non-metric variability to determine epigenetic variability and distance.

The use of non-metric traits and particularly the diversity observed in the specificity or expression of the traits, the 'epigenetic variability' was established as indicator for population genetics. Similarly, the analysis of fluctuating asymmetry of non-metric traits was used as measure of developmental stability (Ansorge 2001). As non-metric or epigenetic traits are easy to scan in museum collections, are generally assumed to be neither dependent on sex and age nor intercorrelated (Corruccini 1976) their use became attractive to assess the population status of species (Ansorge and Stubbe 1995; Ansorge et al. 2009, 2012; Lkhagvasuren et al. 2015; Ranyuk and Monakhov 2016). Specifically endangered species were examined to identify genetic impoverishment in populations (Pertoldi et al. 1998; Usukhjargal et al. 2020; Wiig and Bachmann 2014).

The wildcat (*Felis silvestris* Schreber, 1777) has a palaearctic distribution from Scotland to South Africa and from Morocco to South China (Driscoll et al. 2007), when the three groups *silvestris* (European wildcat), *lybica* (African wildcat) and *ornata* group (Asian or Indian wildcat) are understood to be subspecies (Kitchener 1991; Wozencraft 1993). More recently these are considered as separate species, which leaves a smaller geographic range for *Felis silvestris* with the subspecies *F. s. silvestris* from (Central) Europe, but now includes Scotland, Sicily and Crete, *F. s. grampia* originally from Scotland but probably extinct and *F. s. caucasica* from the Caucasus and Turkey (Kitchener et al. 2017). The domestic cat (*Felis catus* Linnaeus 1758) probably originated from the African wildcat and was distributed around the world by humans (Serpell 1988; Clutton-Brok 1999; Driscoll et al. 2009). The European wildcat (*F. s. silvestris*) is under special protection in Europe due to the Habitat Directive (Council Directive 92/43/EEC) adopted in 1992 by the European Union. For the reports to the EU on protected species under the Habitat Directive, studies on occurrence, conservation and management are necessary, but also the assessment of the diversity (morphological and genetic) and genetic viability of populations of the species are required. This diversity is mainly addressed using molecular tools (e. g., Steyer et al. 2016). However, so far non-metric traits have not been used to assess the population viability.

Therefore, here, we study non-metric traits of the skull to evaluate epigenetic variability as an indicator of genetic diversity and thus population viability and health of wildcats in Germany, particularly in the Harz and in Hesse. Fluctuating asymmetry is used as measure of developmental stability according to Ansorge (2001). We also shed light on the question if wild and domestic cats might be differentiated by the use of non-metric traits, i.e., differences in the frequency of the specificity of some traits.

Materials and methods

Materials and study areas

In total, skulls of 71 domestic cats and 140 wildcats were examined (full list see Supplementary Material Table S1). The cats are housed in the following collections: the Senckenberg Museum für Tierkunde Dresden, the Central Repository for Natural Science Collections Department at Martin-Luther-Universität Halle-Wittenberg, the Senckenberg Museum of Natural History Görlitz, the Vonderau Museum in Fulda. The loan from the latter included two domestic cats from the Phylogenetische Museum in Jena. The animals date from 1878 to 2018. The determination of the cats was based on cranial volume and other morphological features and several are included in Stefen and Heidecke (2011). Those from the Vorderau Museum were determined by F. Müller.

The domestic cats come from all over Germany and were grouped together as one statistical population. The wildcats were divided into two populations: from the Harz and Hesse with foothills to Lower Saxony, Rhineland–Palatinate and Bavaria (Fig. 1). In addition, these two wildcat populations were subdivided each into two groups to identify possible differences within the populations. The division was made (a) on regional level using large traffic infrastructure which might be transversed by migrating wildcats and (b) on temporal scale using time periods. The A7 motorway was chosen to separate the Hesse population and the federal highway B81 for the Harz population. Additionally, the populations could be divided into two sub-samples based on the classification of natural landscapes. In the Harz, one group is limited to the 'östliche Harzabdachung' (Harz nl_1) and the second group includes all individuals northwest of it (Harz nl_2). In the Hesse population, one group occurs on the Hessian Franconian highlands and the East Hessian highlands (Hesse nl_1), while the other group covers the natural areas west of it (Hesse nl_2). In Hesse, however, this division corresponds to the grouping using the A7, except for few individuals, so that this subgrouping was not treated separately. A subdivision on the temporal scale was only made for the Harz population in the periods 1942–1980 and 1981–2018 (Harz t_1 and t_2 respectively). The specimens from Hesse date from a period too short for a comparable subdivision (1993–2018).

Fig. 1 Overview map of the localities of the examined wildcat skulls. The major German motorways are given (after https://de.wikipedia.org/wiki/Datei:Deutschland_Autobahnen_Master.svg, the list of motorways can be found at https://de.wikipedia.org/wiki/Liste_der_Bundesautobahnen_in_Deutschland), A7 and A5 are labeled. For the groups Harz and Hesse the sub-samples are indicated. According to traffic structures: Hesse I (black) and Hesse II (grey), east and west of A7; Harz I (black) and Harz II (grey) separated by the federal highway B81 (not shown) (**a**). The Harz group was also separated according to natural landscape (**b**): Harz nl$_1$ of the western Harz, and Harz nl$_2$ (black) of the 'östliche Harzabdachung' (grey)
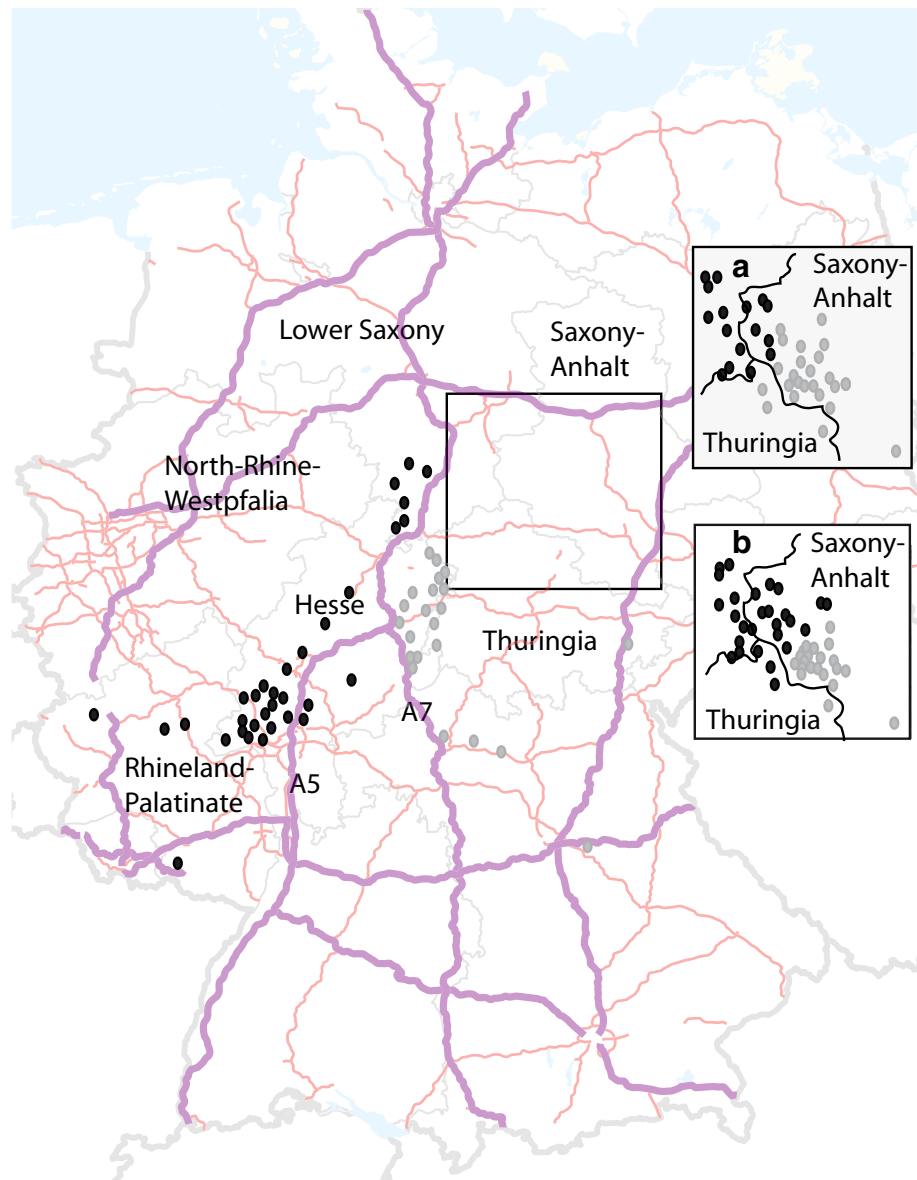
Methods

The basic principles in use and statistical analysis of non-metric traits were developed in the 1960s as is briefly outlined above. In this study, an epigenetic and thus non-metric analysis of collected skulls from museums was performed. This qualitative analysis is often used to detect and assess diversity and potential differentiations between populations or taxa. Usually characters of the skeleton are selected for study that are not directly conditioned by environmental influences and are assumed to be independent of ontogeny. Often skulls are used as they are under many constraints, are well studied and usually available in collections. Foramina are openings in the bone for nerves and/or blood vessels. The question generally posed is whether a certain foramen is present or not, or whether it is expressed single, double or even multiple. The presence of the non-metric characters traits is under basic genetic control and, therefore, the determination of epigenetic differences between groups enables an assessment of the basic genetic variability of the groups. (Berry 1975; Sjøvold 1977). The traits are assumed to be independent of age and sex.

A total of 28 non-metric characters of skull and mandible were selected for this study (Table 1; Fig. 2). Five characteristics were examined bilaterally on the mandible, twenty-two, again bilaterally, on the cranium, and one unilaterally on the cranium. The non-metric characteristics were selected partly on the basis of previous epigenetic studies of other carnivores (Sjøvold 1977; Wiig and Lie 1979; Wiig and Andersen 1988; Ansorge and Stubbe 1992,

Table 1 Non-metric traits on the skull of the wild and domestic cat

No	Trait
1	Additional small foramen anterior to *Foramen incisivum*
2	*Foramen maxillare I* dorsal to the first premolar
3	*Foramen palatinum minus* present
4	*Foramen nasale I* present
5	*Foramen nasale II* present
6	*Foramen maxillare II* above the *Foramen infraorbitale* present
7	*Foramen maxillare III* present along the suture between *Maxilla* and *Os incisivum*
8	*Foramen praemaxillare* present
9	*Foramen maxillare posterior* present
10	*Foramen postorbitale posterior* present
11	*Foramen zygomaticum* present
12	*Fenestra occipitalis* present as a non-ossified area above the *Foramen magnum*
13	*Canalis condylaris* double
14	additional small foramen on the *Canalis nervi hypoglossi*
15	*Foramen postglenoidalis present*
16	*Foramen alisphenoidalis ventralis* double
17	Additional small foramen dorsal to the *Foramen opticum*
18	Additional small foramen dorsal to the *Foramen rotundum* I
19	Additional small foramen next to the *Foramen rotundum* II
20	Additional small foramen posterior to behind the *Foramen ovale*
21	*Foramen postorbitale* present
22	First upper premolar missing
23	*Foramen mentale posterior* double
24	*Foramen mentale anterior* single
25	*Foramen angularis* present
26	*Foramen mandibulare anterior* multiple
27	*Foramen coronoidale* present
28	Additional foramen ventral to or next to the *Foramen infraorbitale*

1995; Ansorge et al. 2009) and our own considerations. First, foramina that were well visible or studied in other Carnivora like *Lynx* (Wiig and Andersen 1988) or *Lutra* (Ansorge and Stubbe 1992; Ranyuk and Ansorge 2015) were searched in *Felis* and the expression was checked in ten domestic and ten wildcats. Second, in case the expression was the same in all 20 specimens, the foramen was not considered further. Only if there were some differences, the foramen was checked in all examined skulls for being single, double, multiple or lacking.

The χ^2-test at a significance level of $p < 0.05$ (Weber 1980) was used to check for differences in the frequency of the characteristic expression. It also served to ensure homogeneity in the distribution by age and sex. After the age- and sex-dependent foramina were eliminated, the epigenetic variability (I_{ev}), the epigenetic distance (MMD) and the fluctuating asymmetry (FA) could be calculated based on the remaining foramina.

According to Smith (1981), the degree of epigenetic variability I_{ev} for the entire population is calculated as follows:

$$I_{ev} = I - \frac{\sum_{i=1}^{n} |50\% - F_i|}{r * 50\%},$$

where $r =$ number of traits; $F_i =$ frequency of the respective feature.

The degree of epigenetic distance between the groups was determined using the mean measure of divergence (MMD) and a formula proposed by Sjøvold (1977). Finally, 20 independent characters were used.

$$MMD = \frac{1}{r} \sum_{i=1}^{r} \left(\Theta_{1i} - \Theta_{2i} \right)^2 - v_i,$$

where $r =$ number of traits; $n =$ sample size; $p =$ frequency of traits; $\Theta = \arccos (1 - 2p)$; $v_i = n_1^{-1} n_2^{-1}$.

The statistical significance of MMD is calculated on the basis of the standard deviation of MMD, the SMMD:

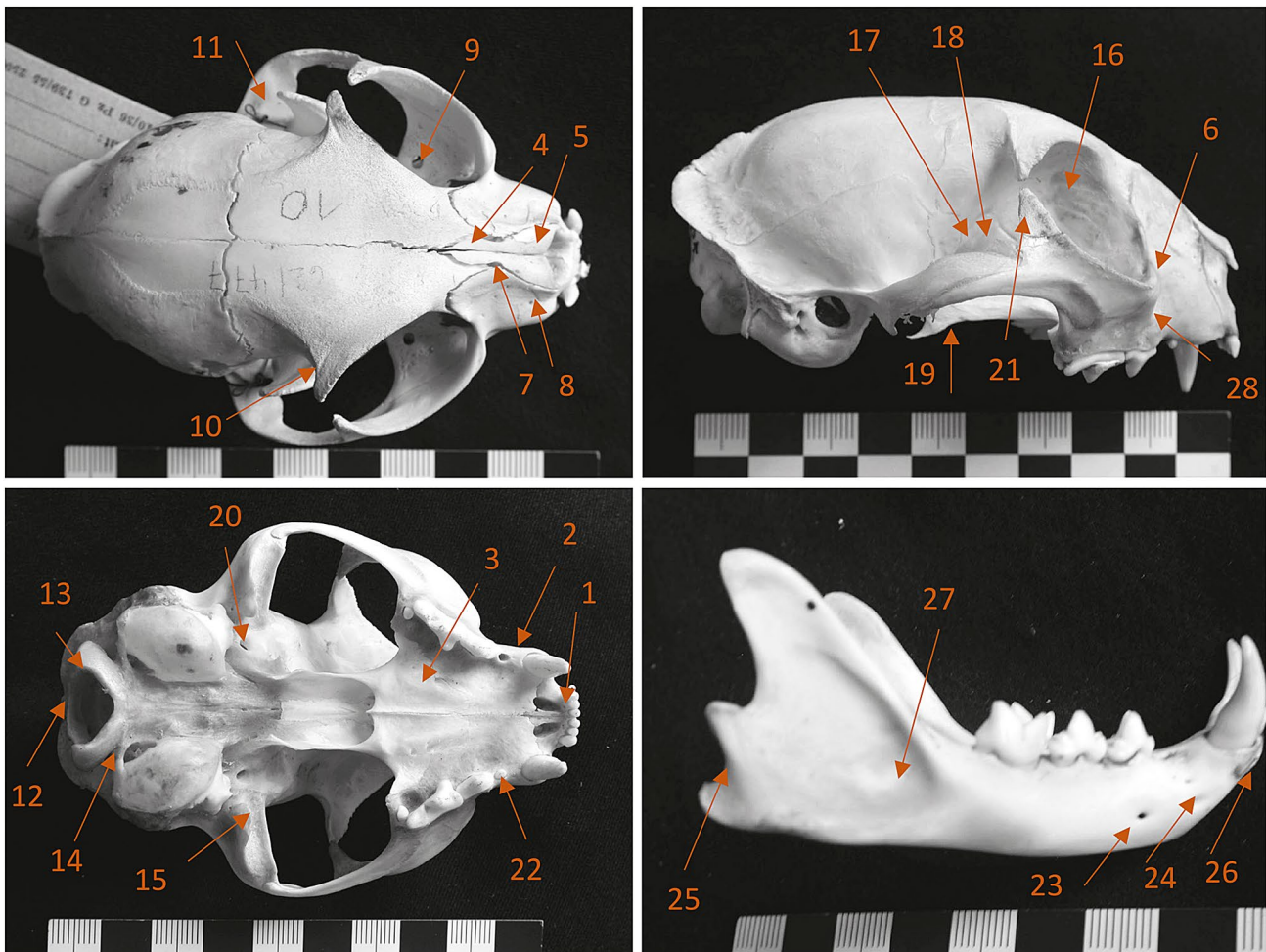$$s_{MMD} = \sqrt{\frac{2 \sum_{i=1}^{r} v_i^2}{r^2}}.$$

Fig. 2 Non-metric traits selected for the study on the skull and mandible of the wild and domestic cat in dorsal, lateral and ventral view and lateral view of the mandible. The corresponding legend is found in Table 1. The basic figure of skulls is from Stefen and Heidecke (2012)

Statistical significance is given if MMD > 2*SMMD ($p = 0.05$) according to Sjøvold (1977).

The relation of the number of asymmetric occurrences of a single character to the overall sample size defines the fluctuating asymmetry (FA). The unscaled mean for all characters gives the degree of asymmetry in the population (Palmer and Strobeck 1986, 2003; Ansorge et al. 2012). A low degree of asymmetry indicates high fitness and stability and vice versa a high degree of asymmetry a low stability (e.g., Swaddle et al. 1994). This allows conclusions to be drawn about the developmental stability of a population.

Microsoft Office Excel 2007 and the software package AnthropMMD from the program R (version 3.6.1) developed by Santos (2018) were used for the calculation.

Results

Of the 28 characteristics examined, four foramina showed a statistically significant correlation to sex (trait number 6, 9, 25, 27) and another four to age (# 3, 14, 24, 26; see Table 1). Thus, these eight were eliminated from further analyses.

Epigenetic variability

The epigenetic variability of the two wildcat populations Harz and Hesse differ with 0.06 ($I_{ev} = 0.37$ and 0.31,

Table 2 Epigenetic variability I_{ev} of the studied wildcat populations from the Harz and Hesse as well as their sub-samples according to traffic infrastructure, time periods and natural landscapes and domestic cats

Population	Subdivision	Number of individuals	I_{ev}
Harz		76	0.37
Hesse		64	0.31
Total wildcats		140	0.36
Domestic cats		71	0.48
Harz I	West of B81	26	0.40
Harz II	East of B81	50	0.35
Hesse I	West of A7	41	0.32
Hesse II	East of A7	23	0.27
Harz t_1	1942–1980	32	0.37
Harz t_2	1981–2018	44	0.36
Harz nl_1	östliche Harzabdachung	34	0.34
Harz nl_2	Western Harz	42	0.40

B81 federal highway, *A7* federal motorway

Table 3 The degree of epigenetic distance between the mentioned grouped indicated by the mean measure of divergence (MMD); the MMD values of non-metric skull features of the given wildcat populations to each other and to the studied domestic cats calculated with AnthropMMD

	Hesse	domestic cats
Harz	0.0774*	0.1513*
Hesse		0.2194*
Total wildcats		0.1597*

*Statistically significant

respectively), and in both as well as combined ($I_{ev} = 0.36$) is lower than in the studied domestic cats ($I_{ev} = 0.48$). (Table 2) The epigenetic variability of the sub-samples Hesse I and II, as well as Harz I and II differ by 0.05. All determined I_{ev} are in a similar range from 0.31 to 0.40, but one, of the group Hesse II east of the A7, is lower ($I_{ev} = 0.27$). The sub-groups of the Harz divided on the basis of natural landscape show a slight difference in the epigenetic variability, whereas the samples based on collection date have a very similar I_{ev} (Table 2).

Epigenetic distance

The epigenetic distance between the groups of wildcats from Hesse and Harz is statistically significant. But between the sub-groups divided by traffic infrastructure (Harz I and II and Hesse I and II, respectively) and between Harz nl_1 and Harz nl_2 there are no significant differences (Tables 3, 4, 5). There is a significant difference in MMD between the wildcats from Harz and Hesse, as well as between the sub-groups

Table 4 The degree of epigenetic distance between the mentioned grouped indicated by the mean measure of divergence (MMD); MMD values of non-metric skull features of the named wildcat subpopulations grouped by traffic arteries and domestic cats, calculated with AnthropMMD

	Harz II	Hesse I	Hesse II	Domestic cats
Harz I	−0.0032	0.1034*	0.1144*	0.0938*
Harz II		0.0543*	0.0788*	0.1778*
Hesse I			0.0243	0.1772*
Hesse II				0.2905*

*Significant

Table 5 The degree of epigenetic distance between the mentioned grouped indicated by the mean measure of divergence (MMD); MMD values of non-metric skull features of wildcat populations grouped by the classification of natural landscape, calculated with AnthropMMD

	Harz nl2	Hesse I	Hesse II	Domestic cats
Harz nl1	−0.0139	0.0557*	0.0699*	0.1648*
Harz nl2		0.0843*	0.1078*	0.1169*

*Significant

Hesse I and II to Harz I and II. In addition, there is a significant difference between all examined wildcat samples and the domestic cats (Fig. 3). The MMD between the groups Harz t_1 and Harz t_2 is 0.0106 and, therefore, not significant.

Fluctuating asymmetry

The FA of all the studied wildcats, 11.74%, is clearly below the FA of the domestic cats with 19.04% (Table 6). In the wildcats from Hesse in total as well as in the sub-samples, the FA is smaller than in the Harz wildcats again in all sub-groupings.

Discussion

The relatively simple method to study the variability in non-metric skeletal characteristics—often focused on cranial foramina—has been established as indicator of genetic variability (Ansorge 2001) and thus ultimately of viability of populations or samples (temporal or geographic). Specially endangered species were examined to identify genetic depletion such as inbreeding or bottleneck effects in the populations (Ansorge and Stubbe 1995; Pertoldi et al. 1998; Wiig and Bachmann 2014; Lkhagvasuren et al. 2015). Additionally, it can be used to assess the divergence and distance between populations. (Ansorge 2001) The prerequisite for such studies is a high number of non-metric characters. This was given here by the use of 28 foramina, which is in the

Fig. 3 Dendrogram of the epigenetic distances of the subpopulations grouped by traffic infrastructure based on the MMD values (*x*-axis) in Table 4
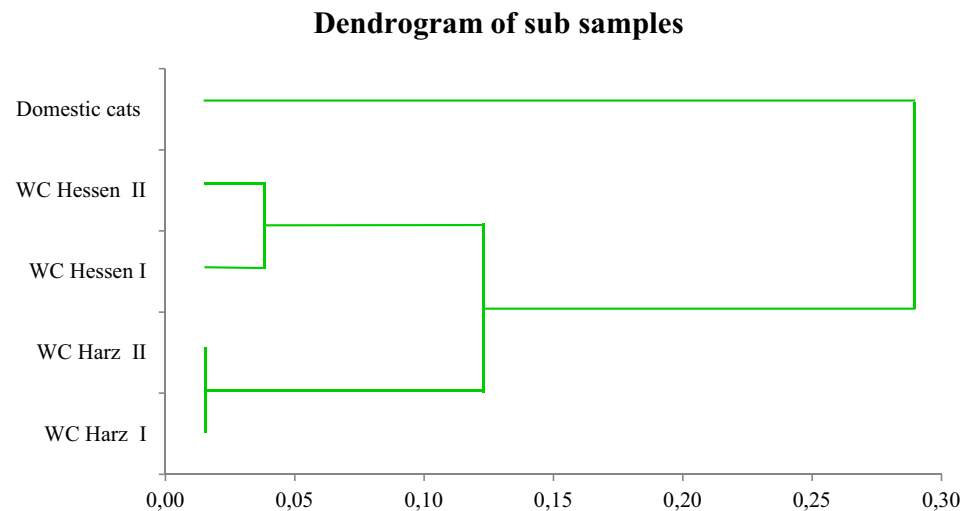

Dendrogram of sub samples

Table 6 Fluctuating asymmetry (FA) of the wildcats from Harz and Hesse as well as sub-groupings according to traffic infrastructure, time period and natural landscape and domestic cats

Population	Subdivision	Number of individuals	FA [%]
Harz		76	13.32
Hesse		64	9.82
Domestic cats		71	19.04
Total wildcats		140	11.74
Harz I	West of B81	26	13.25
Harz II	East of B81	50	13.39
Hesse I	West of A7	41	10.52
Hesse II	East of A7	23	8.47
Harz t_1	1942–1980	32	12.01
Harz t_2	1981–2018	44	14.21
Harz nl_1	östliche Harzabdachung	34	15.29
Harz nl_2	Western Harz	42	16.14

lower range but comparative to other studies in this field (e.g., 11 characters for water shrews (Thier et al. 2019); 24 characters for *Nyctereutes procyonoides* (Ansorge et al. 2009), 40 for harbour porpoise, *Phocoena phocoena* (Gao and Gaskin 1996) and 70 characters in Przewalski's horses (Usukhjargal et al. 2020)). These examples clearly show that more foramina are considered in species when they have larger skulls. With the found correlation to sex and age in some traits, the number of actually analyzed ones was limited to 20, still suitable for the size of cat skulls.

Non-metric trait variation is considered to be age and sex independent and traits are considered not inter-correlated (Corruccini 1976). This, however, has been addressed in several studies, for example, by Markowski (1995) and, therefore, traits with a statistically significant correlation to sex and age were eliminated here also. But in skulls under several developmental constraints every structure, including

the foramina, are probably inter-correlated to some degree. However, no statistically significant inter-correlations were found using the χ^2 test.

Epigenetic variability

The epigenetic variability of wildcats in the different samples ranges from 0.27 (Hesse I) to 0.40 (Harz I, see Table 2). The I_{ev} of the domestic cats is higher (0.48). These epigenetic variabilities of wildcats are within the range known from populations of some other Carnivora ranging from 0.17 for some populations of *Nyctereutes procyonoides* (Ansorge et al. 2009), to 0.32 for *Mustela putorius*, or 0.35 for *Martes martes* both in Saxony also (Ansorge 1992, 1994) and up to 0.45 in some samples of *Meles meles* (Eichstädt et al. 1997).

Both wildcat populations show a high degree of polymorphism and, therefore, using this indicator, no genetic depletion could be assumed. Molecular studies showed that the genetic diversity of wildcat populations in Germany is quite high despite of a possible bottleneck history and only smaller populations showed reduced genetic diversity (Eckert et al. 2010). The fact that the the I_{ev} of the domestic cat is higher has probably two reasons: lumping all domestic cat skulls from across Germany to one group and domestication that lead to different breeds (even though many arose very recently). Williams (2007) emphasizes that domestic cats are a "walking gene pool". They were studied here for comparative reasons and not with the aim to assess the 'population status' of domestic cats particularly as free breeding and partner choice can be questioned.

The grouping according to the major traffic infrastructure shows differences in the I_{ev} between the populations in the Harz and in Hesse (Table 2). Although the findpoints of the cats in Hesse are much further apart, there is less variation, which would hint to lower genetic diversity. This is in accordance with the fact, that the Harz is

less fragmented as only country roads transect it and so the animals have the opportunity to walk further distances easier. Country roads are generally easier to cross than highways (Hötzel et al. 2007). In Hesse, however, the animals are separated by the A7 motorway and according to Hupe et al. (2004), there are too few safe crossing structures. In addition, several individuals avoid using the same crossing aid.

The epigenetic variability for the Harz sub-samples of the two time periods are very close to each other ($I_{ev} = 0.37$ and $I_{ev} = 0.36$) which does not indicate genetic depletion over time. However, a subdivision in finer scaled and shorter periods was not possible due to the resulting small sample sizes. Combining all skulls from a time period over 100 years to one overall sample might influence the result, but an unknown degree. The subdivision of the animals according to the natural landscapes shows differences in the I_{ev}: A slightly lower I_{ev} (0.34) was found in the area of the 'östliche Harzabdachung' than in the western Harz (Harz nl_1, I_{ev} 0.40) which cannot be clearly explained and might be a natural random effect. The specimens grouped as Harz nl_2 were found closer to each other and more likely represent a natural population with less migration than in Harz nl_1. Additionally, the long time interval during which the studied skulls were collected might pose a problem here, as different aspects but those we selected for sub-grouping might be relevant but go undetected.

Epigenetic distances

The MMD values between the groups for the Harz and Hesse are statistically significant, but not for their respective sub-groups Hesse I and II, Harz I and II, nor Harz nl_1 and Harz nl_2. The MMD values for wildcats range from -0.0032 to 0.1144; those for domestic cats are larger (Tables 3, 4, 5). As assumed, wild and domestic cats have larger genetic distances to each other than different wildcat populations to each other (Fig. 3). For raccoon dogs in Germany MMD values that range between -0.01 and 0.40 were determined with distances up to 1.13 when European populations were compared to the native one in the Amursk region. It was considered "generally high, being at levels hardly found in any other mammalian species" (Ansorge et al. 2009). The MMDs for Eurasian otters from Europe and Kamchatka varied from 0.018 to 0.509 (Ranyuk and Ansorge 2015). Thus, the MMDs found here for wildcats for the sub-groupings are comparable to the MMDs for other species—considering the relative short geographic distances.

In future studies, it would be interesting to compare more different regional populations of wildcats, different wildcat subspecies and domestic cats.

Fluctuating asymmetry

The FA of the wildcats ranged between 9.82% in Hesse and 13.32% in the Harz (all specimens from each region considered). These relatively low values indicate high fitness and developmental stability of the populations assuming the often-hypothesized negative correlation of FA with heterozygosity and fitness and genetic variability. The FAs of the sub-groups differ but are all relatively low (Table 6). The largest asymmetries show the sub-groups Harz nl_1 and nl_2 (15.29% and 16.14%). Zachos et al. (2007) showed a fluctuation from 4.8 up to 67% between the different traits in roe deer (sample sizes from 16 to 26). For small mammals' asymmetries in non-metric characters of 14–44% are assumed (Lazarová 1999), so that the observed values here are at the lower end to just below the known range.

The FA of metric and non-metric features is caused by different influences during ontogenesis (Pertoldi et al. 1997; Suchentrunk et al. 1998). Some authors assume it is due to features influencing the development during ontogenesis (Siegel and Doyle 1975). Others also assume genetic stress like inbreeding or hybridization as further cause (Parsons 1990) so that clear associations to individual factors are difficult. However, a study on an inbreed wolf population did not show an increased FA (Wiig and Bachmann 2014) so the assumption that inbreeding increases FA was not supported there.

In the common shrew, *Sorex araneus*, the level of developmental stability as estimated by FA highly correlates with population density—but only in cyclic populations. Also, a relation of FA and breeding success was found (Zakharov et al. 1991). These authors also noted difference in FA between the years. If this is a general issue, the cat samples studied here collected over many years might be influenced by such fluctuation. With species of which material in collections is limited, the forming of subpopulations on a yearly basis or at least of short time intervals is impossible.

FA in the sexual ornaments of males has also been used to indicate the quality of males, for example, by Møller (1990, 1991).

Due to the unclear causes of FA, for example, Lens et al. (2002) critically discuss FA. Zachos et al. (2007) have addressed the assumed negative correlation between FA and genetic variability and did not find statistically significant correlations between FA and genetic variability. However, they concluded, "the question of the genetic factors governing developmental stability is still far from being settled." (Zachos et al. 2007: p. 398). And more recently Beasley et al. (2013) consider the use of FA legitimate as marker of environmental stress. To discover for example, which genes play a role in the development of foramina would make it possible to untangle how much is

caused by genes and what is due to developmental, environmental and other epigenetic influences.

Individual foramina

The study showed that there is no foramen to differentiate wild and domestic cats, fostering the short list of really diagnostic morphological features (Piechocki 1990; Stefen and Görner 2009). However, some differences in the frequency of the specificity of some foramina were observed: for example, the *foramen alisphenoidalis ventralis* (#16) on the right side is double in 6.82% of wildcats but in 42.42% of domestic cats. This might be just a random effect, as the same foramen on the left side was double in 11.28% of wildcats and 36.36% of domestic cats which gives a less developed difference. The least differences in this respect between the wild and domestic cats were observed in the *foramen opticum* (#17) and *foramen incisivum* (#1) (see Table S2, S3).

It is known that wild and domestic cats hybridize to different degrees within the distribution range of wildcats in Europe (Pierpaoli et al. 2003). Despite of several studies on the distinction between wildcats, domestic cats and their hybrids, a morphological characterization of hybrids is not possible. This refers also to foramina, which do not even differ between wild and domestic cats. Whether—the relatively low—rate of hybridization in Central Germany (Krüger et al. 2009; Steyer et al. 2018) might affect epigenetic variability, distance or FA cannot be assessed (so far).

Polymorphism in non-metric traits: epigenetic variation and genetics

The study of non-metric characters like here is one approach to describe and understand variation in nature or biodiversity. For a long time mainly (morpho-) metric diversity was used and starting in about the 1930s and 1940s examples of genetic polymorphism were given (Berry and Searle 1963). These authors coined the term "epigenetic polymorphism" for "discontinuous variation, which is characterized by the fact that the discontinuity is not determined at fertilization but at some later stage in development". (Berry and Searle 1963: p. 578). There are, however, "genetic factors determining the main features of the 'epigenetic landscape'" (Berry and Searle 1963: p. 578). At that time not much was known about the connection of genotype and phenotype, and the interest to understand the underlying genetic causes for biodiversity manifested in different phenotypes of organisms and in particularly mammals closest to humans, is still of great interest. With the advances in genetics, moving to genomics in recent years, new methods arose to approach this question. Knowing, for example, which genes are playing a role in the development of foramina would make it possible to untangle how much is due to genes and what is

due to developmental and other epigenetic influences. Shedding more light into that would also help to understand and assess for which aspects epigenetic variability and/or FA are indicative and how reliable their current use in the assessment of populations is. The use of the *Forward Genomics* approach (Hiller et al. 2012) currently aims at identifying target genes which play a role determining specific mammalian phenotypes like for instance the vomeronasal organ (Hecker et al. 2019a), loss of teeth, development of body armor in pangolins and armadillos (Sharma et al. 2018) or feeding preferences (Hecker et al. 2019b). Even though variability on the population level cannot be addressed in this way, it might help to contribute to the understanding of the genetic basis of individual foramina.

In association with cranial foramina there is one known variable ossification defect in humans—enlarged parietal foramina—which is known to be a "hereditary condition and genes associated with it have been identified" (Griessenauer et al. 2013: p. 543). Nevertheless, "information on genotype–phenotype correlations is incomplete" (Mavrogiannis et al. 2006).

Conclusion

The epigenetic variability for all wildcat groups were within the range known for other Carnivora, but at the lower middle range. These values still do not indicate severe genetic depletion for wildcats in the Harz and Hesse. Similarly, the low FA values are within the range known from other species and do indicate developmental stability. Neither the form nor presence or absence of the studied foramina nor their FA values are useful to differentiate wild and domestic cats.

Overall future advances in genetics and genomics will hopefully shed more light on the genetic and epigenetic basis of non-metric traits so that it can be better understood what epigenetic distance and FA really indicate and if their use in population assessment can be further warranted and more specified.

Author contributions LL conducted study and drafted the manuscript, CS initiated the study and finalized the manuscript, co-author of application for funding.

Data availability List of material and results are in electronic supplementary data.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval Only material stored in collections was used.

Consent to participate Both authors agree on participation.

Consent for publication Both authors agree on publication.

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