

Raccoon dog, *Nyctereutes procyonoides*, populations in the area of origin and in colonised regions — the epigenetic variability of an immigrant

Hermann Ansorge^{1,*}, Maryana Ranyuk², Kaarina Kauhala³, Rafał Kowalczyk⁴ & Norman Stier⁵

¹ State Museum of Natural History Görlitz, PF 300154, D-02806 Görlitz, Germany (*corresponding author's e-mail: hermann.ansorge@smng.smwk.sachsen.de)

² Institute of Plant and Animal Ecology, RAS, 8 Marta str., 202, Ekaterinburg, 620144, Russia

³ Finnish Game and Fisheries Research Institute, Itäinen Pitkätatu 3 A, FI-20520 Turku, Finland

⁴ Mammal Research Institute, PL-17-230 Białowieża, Poland

⁵ Dresden University of Technology, Chair of Forest Zoology, Pienner Str. 7, D-01737 Tharandt, Germany

Received 4 Feb. 2008, revised version received 29 May 2008, accepted 9 June 2008

Ansorge, H., Ranyuk, M., Kauhala, K., Kowalczyk, R. & Stier, N. 2009: Raccoon dog, *Nyctereutes procyonoides*, populations in the area of origin and in colonised regions — the epigenetic variability of an immigrant. — *Ann. Zool. Fennici* 46: 51–62.

We studied the epigenetic variability and epigenetic distance of raccoon dogs from seven European localities and the species' Amursk area of origin. The studies were based on 24 non-metric traits in 1046 skulls. Native raccoon dogs from the Amursk region showed the same low level of epigenetic variability as the European populations, giving no indication of a founder effect or inbreeding. Epigenetic distances between raccoon-dog populations were generally high. The German regions formed a separate cluster with a rather high epigenetic distance to the Finnish-Polish group. This indicates different migration lines of the species. The native raccoon dogs of the Amursk region were completely separate from the European populations as a consequence of the distinct reproductive isolation of about 60 years, as well as an effect of the colonisation and migration history of the species.

Introduction

Lastingly established invasive species often have large effects on the native ecological systems. They can cause a lot of economical expense, being the reason for enormous difficulties in the management of native ecosystems (Strayer *et al.* 2006). Therefore every effort is made to understand the mechanism of the species to become invasive, to assess the invasion risk or to pre-

dict determinants of invasion success. Although only factors directly related to humans, like the propagule pressure, are ascertained by extensive studies as being responsible for the invasion course of vertebrates, it is generally accepted that evolutionary processes should adjust the effect of invaders over time (Jeschke & Strayer 2006). Even clear proof could be shown for the importance of the genotype–environment interaction in the effectiveness of invasive insects

(Boman *et al.* 2008), and in plants, genetic polymorphism and alterations are widely accepted as a main factor for the establishment of invasive species (Jahodová *et al.* 2007). On the other hand, investigations of the genetic variability of invasive mammal species are of particular interest for assessing the phylogenetic base of founder populations, the course and history of immigrations or the success of a permanent colonisation. However, genetic variability and relationship of European invasive mammals have hardly been investigated (Hollmann & Schröpfer 1999, Kruska & Schreiber 1999, Suchentrunk *et al.* 2002, Zachos *et al.* 2007).

Therefore, we used the chance given by the availability of huge collections of skulls of the invasive raccoon dog to estimate the epigenetic variation in non-metric characters as a result of genetic relationship. For the evaluation of genetic relations, not only new and modern molecular methods are taken into consideration nowadays. Morphological investigations of non-metric skeletal characters also reflect the actual genetic background of the phenotypic appearance. For this phenomenon the term “epigenetic” has been established at least since the middle of the last century (Berry & Berry 1967, Ansorge 2001). The non-metric characters offer the opportunity of utilising mammalian skulls kept in museum collections for genetic studies. This method is used here for the investigation of epigenetic variability in the raccoon dog as a very successful invasive species.

The natural range of the raccoon dog (*Nyctereutes procyonoides*) is the Far East, i.e. the Amur–Ussuri region in Russia as well as parts of China, northern Vietnam, Korea and Japan (Kauhala & Saeki 2004). From 1928 to 1955 the species was introduced into several locations in Siberia and the European part of the former Soviet Union, from where the raccoon dog has spread to central and northern Europe (Nowak & Pielowski 1964, Lavrov 1971, Heptner & Naumov 1974, Judin 1977, Nasimovich 1984, Nowak 1984), also colonizing western countries to different extents. Recently the raccoon dog has reached Switzerland and Macedonia (Weber *et al.* 2004, Cirovic 2006). Unfortunately, the precise origin of specific introduced populations is quite unknown. Nevertheless, they most likely

originally descended from populations from the most eastern part of the former Soviet Union because any import of raccoon dogs from foreign countries seems to be very unlikely.

This rapid expansion of a medium-sized carnivore is considered not to be only a phenomenon of the special population dynamics of the species (Ansorge & Stiebling 2001), but also represents a unique genetic field experiment, whereby repeated founder effects as well as secondary gene introgressions are assumed. Although the genetic variability and relationship have hardly been investigated, a first account of allozymic variability of German raccoon dogs was given by Suchentrunk *et al.* (2002). However, nothing is known about the genetic features of their populations of origin. Furthermore, this represents the first report on non-metric skull characters for this species.

The general aim of the study is on the one hand to compare the degree of epigenetic variability of the earlier or recently established populations with that of the native population to detect bottleneck effects after different periods. On the other hand, the epigenetic distances between the raccoon dog populations should provide information on their genetic relationship and the reproductive isolation as well as on the putative migration courses of the species.

Material and study areas

The study is based on a total of 1046 raccoon dog skulls from seven European regions (Fig. 1) and the Amursk area of origin.

Only 58 specimens, trapped in 1968–1975, are from the Amursk district (region 1). The skulls are kept in the mammal collection of the Zoological Museum of the Moscow State University. The Amursk district is probably the region from where the first introduced populations descended (Nowak 1993). Raccoon dog habitats there are characterized by bogs, riverbanks or floodplains. Limiting factors of the population are food availability and other natural habitat conditions, but not human influence (Judin 1977). The abundance of the Amursk raccoon dogs is estimated to be 0.3–0.4 individuals per 1 km², but information is sparse (Heptner & Naumov 1974).

Raccoon dogs had immigrated into southern Finland by the middle of the last century and became established between the 1960s and 1980s in southern and central Finland. Soon after this phase of increase, the Finnish material of 317 skulls was collected during a slight period of decline to about 0.5 individuals per 1 km² (Helle & Kauhala 1995, Kauhala 1996). The collection material is kept in the Finnish Game and Fisheries Research Institute Turku. The skulls originated from the large provinces of Häme (region 2, $n = 163$) and Kymi (region 3, $n = 154$), typical southern Finnish landscapes partly composed of forest, bogs and numerous lakes, but also partly of agricultural landscapes. The raccoon dog is the most abundant carnivore species there.

Ninety skulls were collected in 1987–2005 from the comparatively small area (600 km²) of the Białowieża Primeval Forest (region 4) in eastern Poland. They are now kept in the collection of the Mammal Research Institute Białowieża. Raccoon dogs colonised the area in the early 1950s (Dehnel 1956), living there in deciduous and mixed forests of one of the best preserved woodlands in Europe. They reached a density of about 0.5–0.7 individuals per 1 km² (Jędrzejewska & Jędrzejewski 1998). Raccoon dogs in Białowieża Primeval Forest became more numerous than two native carnivores — red fox and badger (Jędrzejewska & Jędrzejewski 1998, Kowalczyk *et al.* 2003).

Most of the material from Germany ($n = 581$) was collected between 1994 and 2003, and is kept in the collections of the State Museum of Natural History Görlitz and the Institute for Forest Botany and Zoology Tharandt. The first raccoon dogs invaded Germany in the middle of the 1960s, but did not become truly established until 1990 (Stubbe 1989, Ansorge 1998, Drygala *et al.* 2000). After a long period of sporadic occurrence and low population densities, the population has rapidly increased and regular reproduction has been noticed since about 1990 (Ansorge & Stiebling 2001). In Mecklenburg (region 5, $n = 181$) and Brandenburg (region 6, $n = 241$), abundant populations have been established within the last ten years. Their habitats extend from agricultural landscapes to large-scale woodland interspersed by semi-natural postglacial structures such as bogs, morainic

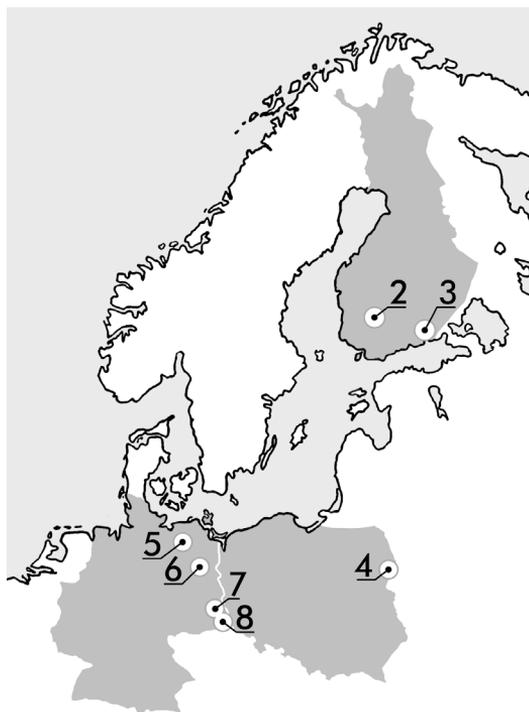


Fig. 1. European raccoon dog sample areas: region 2 (Häme, Finland), region 3 (Kymi, Finland), region 4 (Białowieża, Poland), region 5 (Mecklenburg, Germany), region 6 (Brandenburg, Germany), region 7 (Upper Lusatian Pondland, Germany), region 8 (Upper Lusatian Hilly Country, Germany).

lakes and kettle holes. The smaller raccoon dog population of Upper Lusatia live in the Pondland district (region 7, $n = 110$) under very good feeding conditions, as well as in the Hilly Country, where they are influenced by high hunting pressure and road mortality (region 8, $n = 49$). Raccoon dog densities of the four German regions were hard to estimate due to rapid population increase during the study period.

Methods

Age determination

The data of all samples were divided in the two age classes: juvenile (up to one year old) and adult (older than one year) to evaluate the potential dependence of non-metric character expression on normal growth. We estimated the ages of

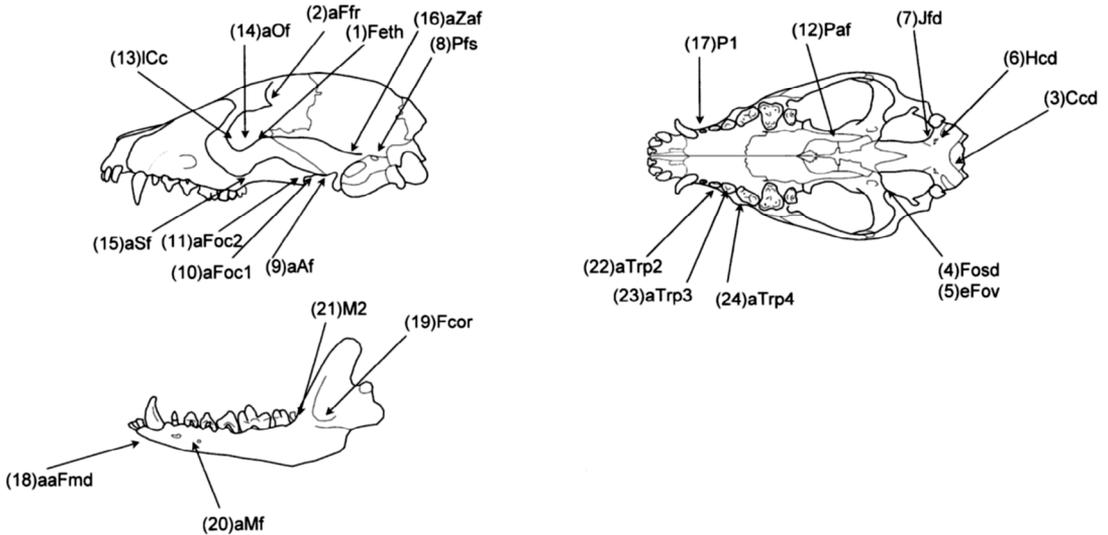


Fig. 2. Position of 24 non-metric characters in the raccoon dog skull.

the raccoon dogs by various skull-developmental criteria, such as teeth abrasion especially of incisors, obliteration of sutures, development of the postorbital constriction and the sagittal crest, as well as the roughness of the cranium surface. In addition, the age of all animals, except in the Amursk sample (region 1), was determined by longitudinal root sections of the canines and counting the incremental cementum lines (Driscoll *et al.* 1985, Kauhala & Helle 1990, Ansorge 1995). Sex determination was taken from the collection labels.

Epigenetic analysis using of non-metric characters

Non-metric skeletal characters are being increasingly used in epigenetic analyses. The use of non-metric traits for population genetics is based on the high heritability of non-metric characters (Berry 1978). Furthermore, the minor variants of non-metric skeletal characters are of lower importance for an organism than selectively more relevant traits. Therefore such traits are exposed to a minimum of selective pressure, qualifying them as epigenetic markers reflecting the genetic circumstances of the relevant phenotype.

To evaluate the epigenetic variability, a set of 24 non-metric characters were selected — 19

foraminal traits and five characters of the teeth (Fig. 2):

- | | | |
|----------------------|---|----------------------|
| 1 (Feth) | <i>foramina ethmoidalia</i> | completely separated |
| 2 (aFfr) | <i>accessory foramen frontale</i> | present |
| 3 (Ccd) | <i>canalis condylaris</i> | double |
| 4 (Fosd) | <i>foramen ovale</i> | subdivided |
| 5 (eFov) | emissary foramen beside the <i>foramen ovale</i> | present |
| 6 (Hcd) | <i>canalis hypoglossus</i> | double |
| 7 (Jfd) | <i>foramen ugulare</i> | double |
| 8 (Pfs) | <i>foramina postzygomata</i> | separated |
| 9 (aAf) | <i>accessory foramen alare</i> | present |
| 10 (aFoc1) | accessory foramen beside <i>canalis opticus</i> 1 | present |
| 11 (aFoc2) | accessory foramen beside <i>canalis opticus</i> 2 | present |
| 12 (Paf) | <i>accessory foramen pterygoidea</i> | present |
| 13 (ICc) | <i>canalis lacrimalis</i> | completely separated |
| 14 (aOf) | <i>accessory foramen orbitale</i> | present |
| 15 (aSf) | <i>accessory foramen sphenopalatinum</i> | present |
| 16 (aZaf) | <i>accessory foramen zygomaticum</i> | present |
| 17 (P ¹) | first upper premolar | missing |
| 18 (aaFmd) | <i>accessory anterior foramen mandibulare</i> | present |

- 19 (Fcor) *foramen coronoidale* present
 20 (aMf) accessory *foramen mentale* present
 21 (M_2) last lower molar missing
 22 (aTrP²) accessory tooth roots in upper P²
 23 (aTrP³) accessory tooth roots in upper P³
 24 (aTrP⁴) accessory tooth roots in upper P⁴

Because to date no study on non-metric skull characters in the raccoon dog has been carried out, selection of skull characters was based on their frequency and variability and results of earlier studies on carnivores (Sjøvold 1977, Wiig & Lie 1979, Ansorge 1992, Ansorge & Stubbe 1995, Pertoldi *et al.* 1997). All characters occur bilaterally, and the traits were therefore registered on both sides of the skull separately.

Data analyses

We analysed all non-metric characters for their homogeneity in age and sex. The frequencies of the character expressions of the subsamples were compared using a χ^2 -test at a significance level of $p = 0.05$ (Weber 1980), and we excluded sex- or age-dependent characters from further investigation.

The epigenetic variability of every single character resulted from the numerical difference from a frequency of 50%, and the epigenetic variability of the respective population is presented by the mean variability of all single characters of this population. According to Smith (1981), we calculated the degree of epigenetic variability I_{ev} for a population sample as follows:

$$I_{ev} = 1 - \frac{\sum_{i=1}^n |50\% - F_i|}{n50\%} \quad (1)$$

where n = number of characters and F_i = frequency of the i th character.

We used two methods for the evaluation of epigenetic divergence between the populations: Smith's "Mean Measure of Divergence" (MMD) derived from the Mahalanobis distances (Sjøvold 1977) and a standard discriminant analysis (Multrus & Lucyga 1996).

After eliminating age- and sex-dependent non-metric characters, all remaining traits were used to calculate the MMD using a formula (Eq. 2) proposed by Sjøvold (1977).

$$\text{MMD} = \frac{1}{r} \sum_{i=1}^r (\Theta_{1i} - \Theta_{2i})^2 - v_i \quad (2)$$

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

Variance and standard deviation (S_{MMD}) of the MMD being:

$$S_{\text{MMD}} = \sqrt{\frac{2 \sum_{i=1}^r v_i^2}{r^2}} \quad (3)$$

indicated the statistical significance at the level of $p = 0.05$ to be $\text{MMD} > 2S_{\text{MMD}}$.

We compared all population samples with each other with MMD calculations. For their abstraction by a cluster tree, we chose the "unweighted pair group method with arithmetic average" (UPGMA) because this tree reflects the phenotypic similarities originally developed for constructing taxonomic phenograms. The UPGMA employs a sequential clustering algorithm, in which the tree is built stepwise. Clustering of the MMD matrix was performed with NTSYS-pc (Numerical Taxonomy and Multivariate Analysis System) (Rohlf 1994).

Additionally, we carried out a "Standard Discriminant Analysis" (Multrus & Lucyga 1996) to determine which characters discriminate between the samples. The standard discriminant analysis results in statistically supported distances between the groups and means of canonical discriminant functions for samples. The separations between the groups are calculated as Squared Mahalanobis Distances (Lindeman *et al.* 1980). Another purpose of applying the discriminant analysis is the issue of predictive classification of cases. The classification matrix shows the number of cases that were correctly classified and those that were misclassified. For the standard discriminant analysis, we selected 640 specimens using only adult animals and skulls with the complete data set. For this standard model, left and right sides of the bilateral traits were pooled and geographical localities were used as the grouping variable.

Results

The analysis of the chosen non-metric characters

of homogeneity in age and sex supported age dependence in five characters (1 Feth, 2 aFfr, 3 Ccd, 18 aaFmd, 20 aMf) and sex-specific expression in one character (9 aAf). We excluded these traits from further analysis.

Epigenetic variability

The epigenetic variability (I_{ev}) of all raccoon-dog samples pooled together was 0.30 (Table 1). The lowest epigenetic variability was found in the Białowieża population (0.17), whereas three of the German samples as well as one of the Finnish samples showed slightly higher indices (0.31–0.35). Raccoon dogs from the area of origin (Amursk region) ranked within the range of the general epigenetic variability (0.28; Table 1). It indicates neither geographical nor temporal tendencies.

Table 1. Epigenetic variability I_{ev} of the raccoon dog samples based on 18 non-metric skull characters ($n = 1046$).

Regions	I_{ev}
1. Amur (Russia)	0.28
2. Häme (Finland)	0.27
3. Kymi (Finland)	0.35
4. Białowieża (Poland)	0.17
5. Mecklenburg (Germany)	0.26
6. Brandenburg (Germany)	0.35
7. Pondland (Germany)	0.31
8. Hilly Country (Germany)	0.32
Average	0.30

Table 2. Mean measures of divergence (MMD) between the raccoon dog populations based on 18 non-metric skull characters ($n = 1046$). Asterisks indicate statistical significance ($p < 0.05$).

	2	3	4	5	6	7	8
1. Amur (Russia)	0.69*	0.70*	1.13*	0.82*	0.74*	0.87*	0.90*
2. Häme (Finland)		0.02*	0.19*	0.40*	0.39*	0.57*	0.58*
3. Kymi (Finland)			0.22*	0.39*	0.36*	0.55*	0.55*
4. Białowieża (Poland)				0.55*	0.63*	0.84*	0.82*
5. Mecklenburg (Germany)					0.27*	0.40*	0.37*
6. Brandenburg (Germany)						0.02*	0.03*
7. Pondland (Germany)							–0.01
8. Hilly Country (Germany)							

Epigenetic population divergence

Epigenetic divergence analysis carried out by pair-wise comparisons of the samples (MMD calculations) and based on the remaining 18 characters resulted in a very variable epigenetic differentiation of the raccoon dog populations (Table 2). All regions showed a generally high level of significant epigenetic differentiation (range: 0.19–1.13), except for two natural units within the geographical region of Upper Lusatia (not significant), and geographically directly neighbouring samples from the two Finnish and three of four German regions, which each showed at least tenfold lower MMDs than all other pair-wise comparisons (range: –0.01 to 0.03). The divergence between the putative original population from Amursk and the introduced populations gave the highest values (range: 0.69–1.13; Table 2).

The resulting cluster tree (Fig. 3) showed a very impressive pattern of generally highly distant groups except between the two neighbouring regions mentioned above. The most northern German population (region 5, Mecklenburg) was clearly separated from the other German regions. The German regions together formed a separate cluster with a rather high epigenetic distance to the Finnish-Polish group. The native raccoon dogs of the Amursk region were completely separated from the European populations (Fig. 3).

Supplementary calculations using a Standard Discriminant Analysis significantly discriminated raccoon-dog samples by all characters except for four traits (7 Jfd, 21 M2, 22 aTrP2, 24 aTrP4). Distances between the geographically neighbouring samples 6 (Brandenburg), 7

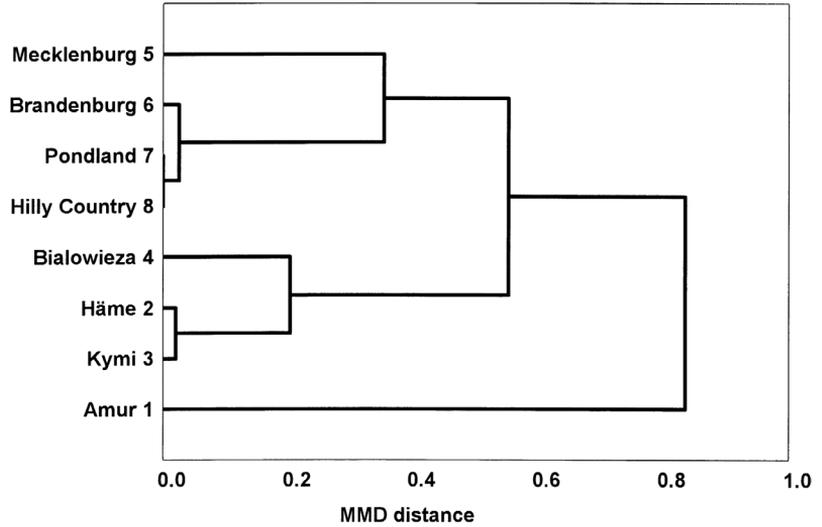


Fig. 3. Unweighted pair-group average (UPGMA) dendrogram of epigenetic distances based on the mean measure of divergence (MMD) derived from 18 non-metric skull characters of the raccoon dog samples ($n = 1046$).

(Upper Lusatia Pongland) and 8 (Upper Lusatia Hilly Country) were not significant (range: 0.67–1.37), whereas all other regions were significantly separate from one another (range: 1.99–68.77; Table 3). The distances between the Amursk population and the European populations were much higher (range: 28.5–68.77) than

between introduced populations (range: 1.99–22.03; Table 3). The largest percentage of correct classification was found for the Amursk region sample (96.4%), while the average amounted to 61.7% (Table 4).

The results of the canonical analysis of variables showed that 93.3% of the explained data

Table 3. F values for distances between groups for the standard discriminant analysis of raccoon dog samples based on 18 non-metric skull characters ($n = 640$). Asterisks indicate statistical significance ($p < 0.05$).

	2	3	4	5	6	7	8
1. Amur (Russia)	52.70*	55.86*	45.40*	54.65*	68.77*	42.23*	28.50*
2. Häme (Finland)		1.99*	5.36*	14.02*	19.86*	12.55*	7.51*
3. Kymi (Finland)			5.61*	14.84*	22.03*	13.94*	7.82*
4. Białowieża (Poland)				13.57*	17.18*	13.62*	9.12*
5. Mecklenburg (Germany)					12.32*	8.20*	5.32*
6. Brandenburg (Germany)						1.37	1.24
7. Pongland (Germany)							0.67
8. Hilly Country (Germany)							

Table 4. Classification matrix for standard discriminant analysis of raccoon dog samples based on 18 non-metric skull characters ($n = 640$).

	1	2	3	4	5	6	7	8	Percentage correct
1. Amur (Russia)	53	1	0	0	0	1	0	0	96.4
2. Häme (Finland)	2	37	25	16	4	11	0	0	38.9
3. Kymi (Finland)	4	18	55	9	9	11	0	0	51.9
4. Białowieża (Poland)	0	4	4	30	2	2	0	0	71.4
5. Mecklenburg (Germany)	9	0	5	0	61	18	1	2	63.5
6. Brandenburg (Germany)	3	3	1	0	16	145	10	3	80.1
7. Pongland (Germany)	0	1	0	0	2	26	12	1	28.6
8. Hilly Country (Germany)	0	0	0	1	0	17	3	2	8.7

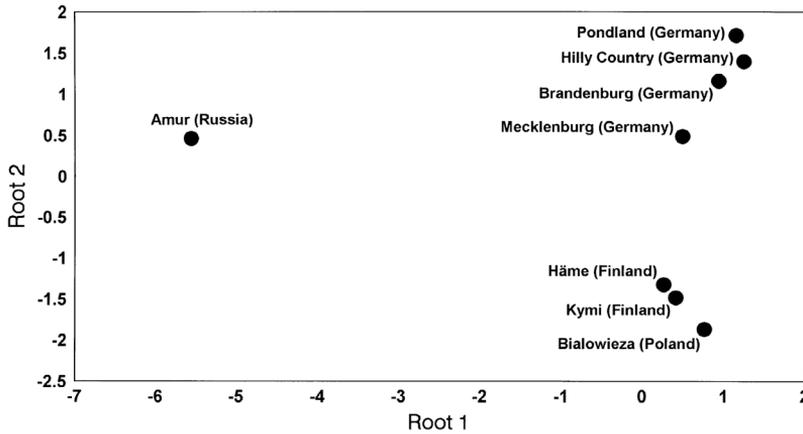


Fig. 4. Means of canonical discriminant functions for the region resulting from standard discriminant analysis of raccoon dog samples based on 18 non-metric skull characters ($n = 640$).

variability was given by the first three Canonical Discriminant Functions (CDF). Of these, 54.4% of the explained data variability was from CDF1, which separates the Amursk Region sample from all European samples of raccoon dogs (Fig. 4). CDF2 discriminated the four German samples from the Finnish and Polish populations of raccoon dogs. The main results of the standard discriminant analysis are:

1. The majority of the considered non-metric traits significantly discriminate the raccoon-dog samples.
2. The Amursk region sample shows the best correct classification and the strongest discrimination by non-metric traits from other samples.
3. German samples are significantly separated from the other European samples.

These relations correspond very well to the results of the UPGMA cluster of the mean measure of divergence.

Discussion

The analysis of epigenetic variability within and between populations has become more and more attractive. Knowledge is needed for conservation and to solve the questions of genetic isolation, inbreeding or bottleneck effects, especially with regard to endangered species (Ansorge & Stubbe 1995, Baranov *et al.* 1997, Pertoldi *et al.* 1997, Pertoldi *et al.* 1998, Grobler *et al.* 1999, Pertoldi

et al. 2000). However, comparably little attention has been paid to the exceptional genetic condition of newly established or introduced species. Especially problems of founder effects or the course of historic immigration and colonisation have to be taken into consideration as well as questions of phylogenetic, micro-evolutionary or ecogenetic causation (Wiig & Lie 1979, Pankakoski 1985, Suchentrunk *et al.* 1998). The present study of the raccoon dog, a neobiotic flagship species, deals with all these problems. This is due to the long and complicated history of introduction and immigration of the species within Europe. It is most probable that all animals introduced within the former Soviet Union are descendants of the Amursk region population (Judin 1977). Although first introductions started in 1928 at least in the Ukraine (Nowak 1993) and the raccoon dog had arrived in Finland by the second half of the 1930s (Helle & Kauhala 1987), the majority of the approximately 9000–10 000 captive-bred animals had been released at several places in the beginning of the 1950s. In addition, all farmed animals had been set free during the Second World War. However, after the 1950s, the raccoon-dog populations dispersed spontaneously without active human support. Thus, these introductions should have resulted in several repeated founder effects as well as broad gene transfer. Furthermore, after expansion to Eastern Germany, for example, the raccoon dogs were scarce for about 30 years (Stubbe 1989, Ansorge 1998) and might have been affected by rabies, parasites and heavy hunting pressure. This may have been the cause

for bottleneck effects or ecogenetic and micro-evolutionary processes. The recent population increase probably resulted from reproduction of the few resident animals with decreased mortality rate as well as from immigrating raccoon dogs. All these facts and possibilities were taken into account during evaluation of the epigenetic analysis results of the raccoon dog as an unintentional genetic field experiment.

Against this background, the pattern of epigenetic variability within the populations seems easier to interpret. The epigenetic variability of most European populations is well around that of the original Amursk population (region 1), probably due to the high number of released founder animals and the long time period of population establishment. Newly colonised areas in Germany show the same range of epigenetic variability as the two Finnish populations established about two decades earlier. There is no indication of lower epigenetic variability in more recently established populations of the raccoon dog. Only raccoon dogs from the Białowieża Primeval Forest have a slightly lower variability, even lower than the Amursk population, but giving no indication of any potential founder effect. This estimation is mainly based on the overall slightly lower epigenetic variability of the raccoon dog (0.30) than that of other European carnivore species. According to data from Upper Lusatia in eastern Germany, the native carnivores such as the polecat *Mustela putorius* (0.32), the pine marten *Martes martes* (0.35), the Eurasian otter *Lutra lutra* (0.41) and the badger *Meles meles* (0.45) show higher variability than the raccoon dogs from the same region (0.31–0.32) (Ansorge 1992, Ansorge 1994, Ansorge & Stubbe 1995, Eichstädt *et al.* 1997). Low allozyme variability of German raccoon dogs was also found by Suchentrunk *et al.* (2002). The results of the present study, i.e. even lower epigenetic variability in the Amursk population, confirms their hypothesis that this low level “might be typical for this species, rather than a consequence of a founder effect following its introduction to Europe” (Suchentrunk *et al.* 2002). This corresponds well with the generally low adaptability of the non-metric characters (Bachau 1988).

Analysis of the epigenetic distances between raccoon-dog populations obtained from both

methods — mean measure of divergence and discriminant analysis — resulted in a clear and absolute correspondence. The epigenetic distances of the studied populations were generally high, being at levels hardly found in any other mammalian species (Ansorge 2001). Even considering the relatively low epigenetic variability of the raccoon dog, this should obviously be the effect of the colonisation history of the species. In particular, the original raccoon dogs of the Amursk region are completely distinct from the European populations. This is most likely a consequence of the complete reproductive isolation of about 60 years as well as of repeated founder effects in the European pioneer populations, which can no longer be detected from recent specimens. Any kind of short-term evolution induced by environmental influences like the general changes in size of the acclimatised raccoon dogs (Sorokin 1953) must be excluded as the reason of epigenetic divergence because of the high heritability of non-metric characters and their minimal exposition to any selection pressure (Bachau 1988, Pankakoski & Hanski 1989). Although there are some hints of differentiating non-metric characters related to environmental changes in reacclimatised populations of the Siberian sable *Martes zibellina* (Monakhov 2001) and in acclimatised muskrats *Ondatra zibethicus* (Vasilyev *et al.* 1999) these single traits cannot influence the evolutionary process regarding the majority of conservative constant characters.

Within the European populations, the cluster tree and the canonical discriminant function means show identical and very interesting patterns. The German samples form a separate cluster with a rather high epigenetic distance to the Finnish-Polish group. Considering the geographic situation, a distinct Finnish cluster was to be expected. The Finnish regions and the Białowieża region were colonised by the raccoon dog much earlier than Germany (Nowak & Pielowski 1964), possibly during the same immigration phase after the Second World War. However, it is only certain that the German raccoon dogs do not descend from the established population from the Białowieża Primeval Forest. Also the relatively large distances between the raccoon dogs from Mecklenburg and those from

the three southern German regions indicate different immigration lines of the species in time and space. Interestingly, small epigenetic differences between the American mink *Mustela vison* populations in Norway had been found by Wiig and Lie (1979), although this invasive carnivore species escaped several times from many distant farms over a period of about 30 years. After the American mink had attained a continuous distribution in Norway, gene-flow could occur and minimise the founder effects in the opinion of the authors. In contrast, in the present study, the raccoon dog shows a well-differentiated epigenetic population structure depending mainly on the colonisation history responsible for the huge epigenetic distances and on the geographic distance, which impeded the genetic exchange up to now.

Acknowledgments

We express our gratitude to the curators of the Zoological Museum of the Moscow State University for the access to the Amursk collection material. We honestly thank Leena Lindström and the anonymous reviewers for their helpful comments. Sincere thanks go to David Russell (Görlitz) and Herbert Boyle (Görlitz) for English revision. Parts of the study were supported by the research program Collegium Pontes 2004.

References

- Ansorge, H. 1992: Craniometric variation and nonmetric skull divergence between populations of the pine marten, *Martes martes*. — *Abhandlungen und Berichte des Naturkundemuseums Görlitz* 66: 9–24.
- Ansorge, H. 1994: Verbreitung und Biologie des Iltis, *Mustela putorius*, in der Oberlausitz. — *Abhandlungen und Berichte des Naturkundemuseums Görlitz* 68: 1–16.
- Ansorge, H. 1995: Notizen zur Altersbestimmung nach Wachstumslinien am Säugetierschädel. — *Wissenschaftliche Beiträge der Universität Halle* 1995: 95–102.
- Ansorge, H. 1998: Biologische Daten des Marderhundes aus der Oberlausitz. — *Abhandlungen und Berichte des Naturkundemuseums Görlitz* 70: 47–61.
- Ansorge, H. 2001: Assessing non-metric skeleton characters as a morphological tool. — *Zoology* 104: 268–277.
- Ansorge, H. & Stiebling, U. 2001: Die Populationsbiologie des Marderhundes (*Nyctereutes procyonoides*) im östlichen Deutschland — Einwanderungsstrategie eines Neubürgers? — *Beiträge zur Jagd- und Wildforschung* 26: 247–254.
- Ansorge, H. & Stubbe, M. 1995: Nonmetric skull divergence in the otter — assessing genetic insulation of populations. — *IUCN Otter Specialist Group Bulletin* 11: 17–30.
- Bachau, V. 1988: Non-metric variation in wild mammals: a bibliography. — *Mammal Review* 18: 195–200.
- Baranov, A. S., Pucek, Z., Kiseleva, E. G. & Zakharov, V. M. 1997: Developmental stability of skull morphology in European bison *Bison bonasus*. — *Acta Theriologica* 42, suppl: 79–85.
- Berry, A. C. 1978: Anthropological and family studies on minor variants of the dental crown. — In: Butler, P. M. & Joysey, K. A. (eds.), *Development, function and evolution of teeth*: 81–98. Academic Press, London.
- Berry, A. C. & Berry, R. J. 1967: Epigenetic variation in the human cranium. — *Journal of Anatomy* 101: 361–379.
- Boman, S., Grapputo, A., Lindström, L., Lyytinen, A. & Mappes, J. 2008: Quantitative genetic approach for assessing invasiveness: geographic and genetic variation in life-history traits. — *Biological Invasions*, doi: 10.1007/s10530-007-9191-0.
- Cirovic, D. 2006: First record of the raccoon dog (*Nyctereutes procyonoides* Gray, 1834) in the former Yugoslav Republic of Macedonia. — *European Journal of Wildlife Research* 52: 136–137.
- Dehnel, A. 1956: Nowy ssak dla fauny polskiej *Nyctereutes procyonoides* (Gray). — *Chrońmy Przyrodę Ojczystą* 12: 17–21.
- Driscoll, K. M., Jones, G. S. & Nichy, F. 1985: An efficient method by which to determine age of carnivores, using dentine rings. — *Journal of Zoology, London* 205: 309–313.
- Drygala, F., Mix, H. M., Stier, N. & Roth, M. 2000: Preliminary findings from ecological studies of the raccoon dog (*Nyctereutes procyonoides*) in eastern Germany. — *Zeitschrift für Ökologie und Naturschutz* 9: 147–152.
- Eichstädt, H., Ansorge, H., Hofmann, T. & Stubbe, M. 1997: Populationsdifferenzierung beim Dachs — epigenetische Unterscheidung. — *Berichte der Naturforschenden Gesellschaft der Oberlausitz* 6, suppl: 11.
- Grobler, P. J., Taylor, P. J., Pretorius, M. D. & Anderson, C. P. 1999: Fluctuating asymmetry and allozyme variability in an isolated springbok *Antidorcas marsupialis* population from the Chelmsford Nature Reserve. — *Acta Theriologica* 44: 183–193.
- Helle, E. & Kauhala, K. 1987: Supikoiran leviämishistoria ja kantojen nykytila. — *Suomen Riista* 34: 7–21.
- Helle, E. & Kauhala, K. 1995: Reproduction in the raccoon dog in Finland. — *Journal of Mammalogy* 76: 1036–1046.
- Heptner, V. G. & Naumov, N. P. 1974: *Die Säugetiere der Sowjetunion*, vol. 2. — Fischer Verlag, Jena.
- Hollmann, H. & Schröpfer, R. 1999: Ein Vergleich von Bisam- (*Ondatra zibethicus*) DNA aus unterschiedlichen Regionen in den Niederlanden und Nordwest-Deutschland. — *Zeitschrift für Säugetierkunde* 64, suppl.: 16–17.
- Jahodová, Š., Trybusch, S., Pyšek, P., Wade, M. & Karp, A. 2007: Invasive species of *Heracleum* in Europe: an insight into genetic relationships and invasion history. — *Diversity and Distribution* 13: 99–114.

- Jędrzejewska, B. & Jędrzejewski, W. 1998: *Predation in vertebrate communities: the Białowieża Primeval Forest as a case study*. — Ecological Studies 135, Springer Verlag, Berlin Heidelberg New York.
- Jeschke, J. M. & Strayer, D. L. 2006: Determinants of vertebrate invasion success in Europe and North America. — *Global Change Biology* 12: 1608–1619.
- Judin, W. G. [Юдин, В. Г.] 1977: [The raccoon dog of the Primorje and the Amur region]. — Nauka, Moskva. [In Russian].
- Kauhala, K. 1996: Reproductive strategies of the raccoon dog and the red fox in Finland. — *Acta Theriologica* 41: 51–58.
- Kauhala, K. & Helle, E. 1990: Age determination of the raccoon dog in Finland. — *Acta Theriologica* 35: 321–329.
- Kauhala, K. & Saeki, M. 2004: Raccoon dogs. — In: Sillero-Zubiri, C., Hoffmann, M. & Macdonald, D. W. (eds.), *Canids: foxes, wolves, jackals and dogs. Status survey and conservation action plan*: 136–142. IUCN/SSC Canid Specialist Group, Gland Cambridge.
- Kowalczyk, R., Zalewski, A., Jędrzejewska, B. & Jędrzejewski, W. 2003: Spatial organization and demography of badgers *Meles meles* in Białowieża Forest (Poland) and the influence of earthworms on badger densities in Europe. — *Canadian Journal of Zoology* 81: 74–87.
- Kruska, D. & Schreiber, A. 1999: Comparative morphometrical and biochemical-genetic investigations in wild and ranch mink (*Mustela vison* : Carnivora : Mammalia) — *Acta Theriologica* 44: 377–392.
- Lavrov, N. P. [Лавров, Н. П.] 1971: [The results of the introductions of the raccoon dog (*Nyctereutes procyonoides*) in different provinces in the USSR]. — *Trudy Katedry Biologii, Moskovskii Gosudarstvennyi Zaochnyi Pedagogicheskij Institut* 29: 101–160. [In Russian].
- Lindeman, H. R., Merenda, P. F. & Gold, R. 1980: *Introduction to bivariate and multivariate analysis*. — Scott, Foresman & Co., New York.
- Monakhov, V. G. 2001: Phenetic analysis of aboriginal and introduced populations of sable (*Martes zibellina*) in Russia. — *Russian Journal of Genetics* 37: 1074–1081.
- Multrus, F. & Lucyga, D. 1996: *Einführung in Statistica*. — Lucius & Lucius, Stuttgart.
- Nasimovich, A. A. [Насимович, А. А.] 1984: [Ecological aspects of acclimatization of the raccoon dog in the European part of the USSR]. — *Bulletin of Moscow Society of Naturalists, Biological series* 89: 8–19. [In Russian].
- Nowak, E. 1984: Verbreitungs- und Bestandsentwicklung des Marderhundes, *Nyctereutes procyonoides* (Gray, 1834) in Europa. — *Zeitschrift für Jagdwissenschaft* 30: 137–154.
- Nowak, E. 1993: *Nyctereutes procyonoides* Gray, 1834 — Marderhund. — In: Niethammer, J. & Krapp, F. (eds.), *Handbuch der Säugetiere Europas, Bd. 5/2*: 213–248. Aula-Verlag, Wiesbaden.
- Nowak, E. & Pielowski, Z. 1964: Die Verbreitung des Marderhundes in Polen im Zusammenhang mit seiner Einbürgerung und Ausbreitung in Europa. — *Acta Theriologica* 9: 81–110.
- Pankakoski, E. 1985: Epigenetic asymmetry as an ecological indicator in muskrats. — *Journal of Mammalogy* 66: 52–57.
- Pankakoski, E. & Hanski, I. 1989: Metrical and non-metrical skull traits of the common shrew *Sorex araneus* and their use in population studies. — *Annales Zoologici Fennici* 26: 433–444.
- Pertoldi, C., Loeschcke, V., Braun, A., Madsen, A. B. & Randi, E. 2000: Craniometrical variability and developmental stability. Two useful tools for assessing the population viability of Eurasian otter (*Lutra lutra*) populations in Europe. — *Biological Journal of the Linnean Society* 70: 309–323.
- Pertoldi, C., Loeschcke, V., Madsen, A. B. & Randi, E. 1997: Developmental stability in the Eurasian Otter (*Lutra lutra*) in Denmark. — *Annales Zoologici Fennici* 34: 187–196.
- Pertoldi, C., Madsen, A. B., Randi, E., Braun, A. & Loeschcke, V. 1998: Variation of skull morphometry of Eurasian otters (*Lutra lutra*) in Denmark and Germany. — *Annales Zoologici Fennici* 35: 87–94.
- Rohlf, F. J. 1994: *NTSYS-pc. Numerical Taxonomy and Multivariate Analysis System*. — Exeter Software, New York.
- Sjøvold, T. 1977: Non-metrical divergence between skeletal populations. — *Ossa* 4, suppl.: 1–133.
- Smith, M. F. 1981: Relationships between genetic variability and niche dimensions among coexisting species of *Peromyscus*. — *Journal of Mammalogy* 62: 273–285.
- Sorokin, M. G. [Сорокин, М. Г.] 1953: [Acclimatization of the raccoon dog in the Kaliningrad district]. — *Priroda* 6: 106–107. [In Russian].
- Strayer, D. L., Eviner, V. T., Jeschke, J. M. & Pace, M. L. 2006: Understanding the long-term effects of species invasions. — *Trends in Ecology and Evolution* 21: 645–651.
- Stubbe, M. 1989: Neue Erkenntnisse zur Verbreitung und Ökologie des Marderhundes *Nyctereutes procyonoides* (Gray, 1834) in der DDR. — *Beiträge zur Jagd- und Wildforschung* 16: 261–267.
- Suchentrunk, F., Ansorge, H., Stier, N. & Nitze, M. 2002: First account on allozymic variability of raccoon dogs, *Nyctereutes procyonoides*, immigrating into central Europe. — *Mammalian Biology* 67, suppl.: 37–38.
- Suchentrunk, F., Hartl, G. B., Flux, J. E. C., Parkes, J., Haiden, A. & Tapper, S. 1998: Allozyme heterozygosity and fluctuating asymmetry in brown hares *Lepus europaeus* introduced to New Zealand: developmental homeostasis in populations with a bottleneck history. — *Acta Theriologica* 43: 35–52.
- Vasilyev, A. G., Bolshakov, V. N., Malafeev, Y. M. & Valyaeva, E. A. [Васильев, А. Г., Большаков, В. Н., Малафеев, Ю. М. & Валяева Е. А.] 1999: [Evolutional and ecological processes in populations of the muskrat during acclimatization under northern conditions]. — *Ekologija* 6: 433–441. [In Russian].
- Weber, E. 1980: *Grundriß der Biologischen Statistik*. — Fischer Verlag, Jena.
- Weber, J. M., Fresard, D., Capt, S. & Noel, C. 2004: First records of raccoon dog, *Nyctereutes procyonoides* (Gray, 1834), in Switzerland. — *Revue suisse de Zoologie* 111: 935–940.

Wiig, O. & Lie, R. W. 1979: Metrical and non-metrical skull variations in Norwegian wild mink (*Mustela vison* Schreber). — *Zoologica Scripta* 8: 297–300.

Zachos, F. E., Cirovic, D., Rottgardt, I., Seiffert, B., Oeking,

S., Eckert, I. & Hartl, G. B. 2007: Geographically large-scale genetic monomorphism in a highly successful introduced species: the case of the muskrat (*Ondatra zibethicus*) in Europe. — *Mammalian Biology* 72: 123-126.