Population differentiation in the European roe deer (*Capreolus capreolus*) by non-metric skull traits in Germany

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Abstract

The non-metric skull divergence between populations of the roe deer, *Capreolus capreolus* (Linnaeus, 1758) were studied using 786 complete skulls (crania and mandibles) from three wildlife research areas in Germany which were from Hakel, Fallstein and Darss. A total of 292 male and 494 female were scored for 56 cranial non-metric traits.

The dependence of incidence of characters on age, sex and correlation between variants was studied. Four traits were found to have sex dependence and eighteen traits displayed a dependence on age. Of 1540 calculated correlation coefficients between the variants only 140 (9.1%) were significantly different from zero at $p < 0.05$. But only 4 out of 140 (2.86% and 0.26% out of all) had a correlation value, equal or more than 0.3 that were excluded. After deleting all variants correlated with one another and dependence on sex and age, further analysis was based on the frequency of 34 traits.

The Mean Measures of Divergence (MMD) was used to express the interpopulation differences. The MMD values were calculated between pair populations Fallstein and Darss; Fallstein and Hakel; and Hakel and Darss as 0.04671, 0.01572 and 0.04131 respectively and all were highly significant at $P < 0.001$.

The measure of uniqueness (MU) was calculated for each sample as the sum of its epigenetic distance (MMD) and they were 0.08802, 0.06243 and 0.05703 for Darss, Fallstein and Hakel respectively.

The cluster analysis the MU values proved existence two main clusters. The first one consists of two samples (Hakel and Fallstein) with low differentiation, contrary to a distinctly separated position of the sample Darss which from Baltic coast of Germany.

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Preface

Scientists of evolutionary biology are interested in genetic and phenotypic affinities among breeding populations. One way of obtaining estimates of these relationships is through the study of skull variation among populations. The skull is a fascinating and rewarding object for phylogenetic studies, because, even in a fossil, it can provide detailed information about the biology of a mammal.

In research into investigating transformations of the genetic structure of populations the study of non-metrical morphological characters has often been used in recent years. These epigenetic methods are based on the incidence of morphological alternatives in which high heritability is assumed. In mammal minor skeletal variants are usually used, mostly studied in the skull or postcranial skeleton. The method has been used successfully for a long time in anthropological research, and in studying wild forms it has so far been applied most often in various species of rodents and carnivores.

Although the heritability of non-metric traits, mainly the presence of foramina and similar structures for blood vessels and nerves, have been evaluated as rather low, the simultaneous consideration of several traits allows estimation of epigenetic variation in time and space as a result of genetic relationship. Thus, the main use of non-metric characters has been aimed at assessing epigenetic variability and divergence among populations. Applications extend from the problem of genetic isolation of populations, the lack of reproductive contact, detection of genetic drift, systematic studies to clarify species taxonomy, to phylogenetic interpretation.

The European roe deer (*Capreolus capreolus*) has been selected as the object of this study as it is the most numerous free-living autochthonous ungulate and is one of the most important species of game animals in Germany. It shows wide ecological tolerance, and its populations occur continuously almost all over the territory and are stable at high levels. Thus the species meets the conditions under which the genetic difference between populations can be function of geographic distance. The aim of this dissertation is to examine the genetic contributions and investigate the relations between populations of roe deer from three wildlife research areas of Germany (Hakel, Fallstein and Darss) using epigenetic methods on the basis of analysis of non-metric traits.

1. Introduction

1.1. Taxonomy, morphology, geographic range and habitat use of roe deer

1.1.1. Taxonomy

The roe is a deer that phylogenetic and taxonomic positions have been the subject of considerable debate (Andersen et al. 1998). Roe deer (*Capreolus* sp.) were once classified as belonging to the Cervinae subfamily, it now seems clear that they are in fact part of the Odocoileinae (Groves and Grubb 1987, Grubb 1993).

Although occasionally treated as a mono-specific genus, it is now widely accepted that there are two allopatric species of roe: *Capreolus pygargus* (Pallas 1771), the ahu or Siberian roe (reviewed by Danilkin 1995), and C. *capreolus* (Linnaeus 1758), the European species (reviewed by Sempere et al. 1996). However, there is still confusion about their geographical and morphological boundaries (Andersen et al. 1998). The systematic classification of *Capreolus* is as follows (Danilkin and Hewison 1996):

Order **Artiodactyla**
Suborder **Ruminantia**
Family **Cervidae**
Subfamily **Odocoileinae**
Tribe **Capreolini**
Genus **Capreolus** the roe deer

- ***Capreolus pygargus*** (Pallas, 1771) - Siberian roe deer
- ***Capreolus capreolus*** (Linnaeus, 1758) - European roe deer

1.1.2. Morphology

1.1.2.1. Characterization of the Genus Capreolus

The roe deer is a small telemetacarpalian deer with a mean body length and mass which vary among populations from 100 to 145 cm and from 18 to 49 kg, respectively. Roe deer moult twice a year, once in spring and once in autumn (Danilkin and Hewison 1996). Coloration in winter is light grey to dark brown, with a large white caudal patch. The summer coat is shorter, with thinner hairs which are a bright orange-brown and the skin around the forehead and neck is thickened in males (Andersen et al. 1998). In the summer the white

caudal patch is less conspicuous or is absent (Danilkin and Hewison 1996). Fawns are spotted with white spots. Males are on average somewhat larger than females, but the degree of sexual dimorphism is relatively low. The pedicles are close together relative to orbital width, and are parallel, diverging, or occasionally even converging. Males have three-tined antlers (Andersen et al. 1998).

Antlers are shed in autumn or early winter and begin to regrow immediately afterwards. The skull is superficially similar to that of other species of deer of similar size, but there is much variation (Andersen et al. 1998). It is small with population average ranging from 180 to 245 mm but relatively elongated, with a maximum width 75-106 mm less than half its length. The permanent dental formula is typical for cervids, with regularly no upper canine: i 0/3, c 0/1, p 3/3, m 3/3, total 32. The karyotype (2n = 70-84) comprises 70 main chromosomes plus, in Siberian roe deer only, 1-14 accessory B-chromosomes (Danilkin and Hewison 1996).

1.1.2.2. The Siberian roe deer- Capreolus pygargus (Pallas, 1771)

Caprealus pygargus is larger than *C. caprealus*, with a total average population body length of between 127 and 145 cm, an average body mass of 32-49 kg, a bigger skull (condylobasallength 201-231 mm), a lower tooth row of average length 71-76 mm, and antlers generally longer than 27 cm. In summer coat, the hair of the head, like that of the rest of the body, is generally reddish. This species is distributed through Eastern Europe and Asia (Danilkin and Hewison 1996).

1.1.2.3. The European roe deer- Capreolus capreolus (Linnaeus, 1758)

Caprealus caprealus is distinguished from C. *pygargus* by its shorter body length (population average between 100 and 126 cm), smaller cranium (condylobasal length averages 180-200 mm) and shorter antlers (length 17-26 cm, span 7-14 cm). The average body weight is 18-32 kg. The length of the tooth row of the lower mandible is between 58 and 66 mm. A schematic drawing of roe deer is shown in Fig. 1.1.

When in summer coat, the hair of the head is grey or grey-brown (usually much darker than that of the body). This species consists of a single taxonomic group which is widely distributed in Europe (not farther than the Volga) and is also found in Asia Minor (Danilkin and Hewison 1996).

Fig. 1.1: A schematic drawing of roe deer (Adopted from Mitchell-Jones et al. 1999).

1.1.3. Geographic range

Fossil records suggest that both the European and Siberian roe deer forms have existed since the Pleistocene period (Danilkin and Hewison 1996). These fossils, which were found in the Ukraine, are from the Middle Miocene period (approximately 10 million years ago) (Lambert 2005). Roe deer cover an enormous geographical distribution, ranging from Great Britain and Spain to the Far East and from Kazakhstan and central Asia to northern Scandinavia and Siberia, and a large amount of data has now accumulated which reveals great variation of form over this range (Hewison and Danilkin 2001).

The range of European roe deer (*Capreolus capreolus* L.) stretches from the Atlantic coast of Europe in the west to the middle reaches of the Volga River, Caucasia and Asia Minor and Iran in the east. Its southern border runs across the Mediterranean region with scattered roe deer populations; in Sweden the northern border almost reaches the Arctic Circle (Zeijda and Koubek 1988). In general they occur in all European countries except Iceland and Ireland (Lambert 2005). The present distribution of European roe deer in Europe is shown in Fig. 1.2.

1.1.4. Habitat use

The wide variety of habitats occupied by roe deer today is the best evidence of their success. They occur in almost all of the natural habitats found in Europe, including deciduous, coniferous and Mediterranean forests, shrub lands, moorlands and marshes (Danilkin and Hewison 1996; Fruzinski et al. 1983; Telleria and Virgos 1997). Only high alpine areas over the tree line and the most open grasslands are rarely occupied. Their tolerance of human activity (Linnell and Andersen 1995) has allowed them to also succeed in occupying most man-made habitats, including plantation forests, mixed forest, farmland mosaics, the very open agricultural plains of western and Eastern Europe, and even suburban gardens (Aulak and Babinski-Werka 1990; Cibien et al. 1989; Strandgaard 1972; Tufto et al. 1996; Latham et al. 1996, 1997). On a finer scale of habitat selection, early successional habitats are generally preferred over climax habitats (Andersen et al. 1998).

Their only major habitat requirement, apart from food appears to be cover to escape from predators and man (Tufto et al. 1996). Their small body size allows them to survive in small patches of woodland or shrubs, and even tall grass, which provide the cover they need. This requirement is explained by their inability to run far and fast; roe deer are not cursorial ungulates. They are extremely tolerant of climatic extremes, from hot and dry Mediterranean through to the cold of the boreal forests; they can live in snow up to brisket height, about 1 m in extreme cases (Aragon et al. 1995). Their ability to survive drought conditions is still uncertain (Andersen et al. 1998).

1.2. Study areas

The roe deer (*Capreolus capreolus*) skulls were collected in the wildlife research areas: Darss, Hakel and Fallstein in Germany during 1957-1987. This valuable material was lies basis for a lot of scientific studies (Stubbe 1966, 1971, 1977, 1984, 1993; Stubbe et al. 1984, 1986, 1989, 1995) and also the detailed research in this academic qualification. Following would be explained some information as location, climate and flora regarding to these areas.

1.2.1. Darss

1.2.1.1. Location

Darss (also Darß) is originally a part of a peninsula in the South of Baltic Sea in the German land of Mecklenburg-Western Pomerania (Fig.1.3.) in the district Ribnitz- Damgarten (54°26 ′N, 12°44′E). Its full name is Fischland-Darß-Zingst, for these have been the names of

three regions making up the peninsula .The "Darß" originally is the name of the still giant forest there. In recent times the name "Darß" applies also to the entire peninsula. It is situated within the territory of the county of Nordvorpommern (Wikimedia Foundation, Inc. 2008). This area with 4500 ha (Konow 2000), encloses on an average 1-km width the 9-km-long western and northern part of the peninsula, from the Vordarß up to the Bernsteininsel (Leberecht et. al 1980).

The landscape is formed by fresh- and salt-water marshes, sand dunes and Bodden (Low, German: Bodden). Bodden or shallow bays are lagoons cut off from the open Baltic Sea with a mix of salt- and fresh-water (Kocka et. al 2005). Between the peninsula and the mainland there is a very shallow lagoon, which is a part of the Western Pomerania Lagoon Area National Park, just as the entire peninsula itself (Wikimedia Foundation, Inc. 2008).

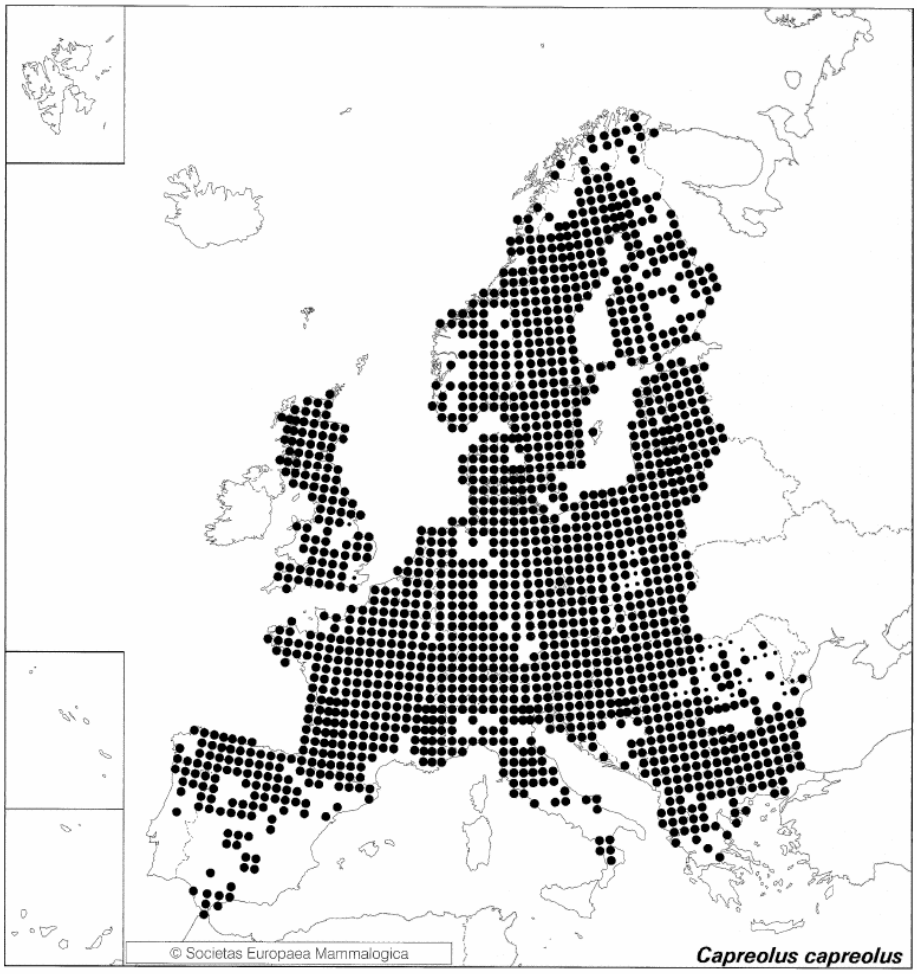


Fig. 1.2: The distribution of European roe deer in Europe (Adopted from Mitchell-Jones et al. 1999).

1.2.1.2. Protective status

In 1957 the Westdarß and Darßer Ort was officially announced as a nature reserve. In 1990 the forest of Darss as well as adjoining areas was added to the Pomerania Lagoon Area National Park. It was recognized as a European Important Bird Area in 1996. The area is famous as an important Central European resting place for cranes (Konow 2000).

1.2.1.3. Coastal Geology

The coastal area around the site is characterized by a flat coast. The current morphology is a result of strong geological movements. Sediment is removed from the cliffs in Zingst and the west coast of Darss and due to strong currents; it is transported towards the East-West or South-North and deposited in various sandbanks (Lüth and Förster 2004).

1.2.1.4. Climate

The local climate is Baltic Sea climate; weather situations with western winds prevail and bring mild Atlantic air into the area. Heavy cooling occurs when the south-western Baltic Sea is influenced by weather situations from the northern area or from Russia (Lüth and Förster 2004). The total annual precipitation is about 570 mm and the mean annual temperature of the air is 7.9-8.0°C. The mean annual variation lies at 17.3°C (monthly mean of January: below 0.1°C, monthly mean of July: 17.2°C) (Leberecht et. al 1980). The direction of the wind in Baltic Sea changes and is 25 – 28 % in the average of the year. November is the windiest and May the most windless month. Only during long cold winter the surface of the Bodden in the area of Darss freezes (Lüth and Förster 2004).

1.2.1.5. Flora

The saltwater from the North Sea and the freshwater from the rivers create a rich habitat in the Baltic Sea. A salt content of 10‰ can be found in western parts of the Baltic. The salt content as well as the abundance and diversity of organisms vary in different parts of the Baltic Sea to the streaming water, from the North Sea (Lüth and Förster 2004).

Indeed, the vegetation of the Darss forest is distinguished by a row of oceanic geoelements like European Holly (*Ilex aquifolium*), cross-leaved heath (*Erica tetralix*) and Royal Fern (*Osmunda regalis*) in plant-geographic regard, however, it gains, because of the strong supremacy of the Scots Pine or Scotch Pine (*Pinus sylvestris*) a boreal forest (Taiga) character, which is underlined as a row of other boreal geo-elements like Crowberry *(Empetrum nigrum),* chickweed wintergreen or Arctic starflower *(Trientalis europaea),* twinflower *(Linnaea borealis)* and Greater Fork-moss *(Dicranum majus)* (Leberecht et. al 1980).

1.2.2. Hakel

1.2.2.1. Location

Hakel, with a size of 1303 ha, is situated in the north-eastern foreland of Harz Mountains in central Germany, about 35 km south-west of Magdeburg (Saxony-Anhalt) (Toepfer and Stubbe 2001). The elevation is 150 to 210 m with the coordination: 51° 53′ 3″N, 11° 19′ 54″E (Fig.1.3.). Hakel, which is consists of an isolated forestland within structured agricultural land (arable land) include two subfields; the Large and Small Hakel (Hentschel et. al 1983).

1.2.2.2. Protective status

Since 1995, Hakel in its entirety is protected as a natural conservation area (Geiter and Hanelt 2003). A percentage of 66% from this area is protected as a landscape protection area and 34% as the nature reserve (European Topic Centre for Nature Protection and Biodiversity 2008b). The rich nature of these forests, especially regarding its flora, since the mid-19th century, is known in the topic of floristic and botanic studies (Eichler 1970, Weinitschke 1954, Michel and Mahn 1998). Hakel was as a former wildlife research field, also object of many zoological studies. Especially because of the rich ornitho fauna it has been recognized as an Important European Bird Area (Stubbe et al.1991, M. Stubbe 1971). This is an important site for breeding raptors and woodpeckers (Bird Life International 2007). Hakel stands completely under protection (Hentschel et. al 1983).

1.2.2.3. Climate

This area according to Meusel (1952) belongs to the Central German dry area (Mitteldeutsches Trockengebiet). The annual average precipitation, measured at the station Heteborn (190 m NN) from 1955 to 1995, is 558.3 mm (301.7 - 903.9 mm). The February is in the long-term average the month with the lightest precipitation (34.5 mm), June the precipitation-richest (67.3 mm). A snow cover over 1cm lays in the mean of 36.4 days a year (Hentschel et. al 1983). The average annual temperature is 8.7°C (Meteorologische Station des Institutes für Pflanzenzüchtung und Kulturpflanzenforschung Gatersleben, Abt. Genbank adopted from Hofmann 1999).

1.2.2.4. Flora

The relatively dry but fertile soil was mostly loess-soil developed on loam materials (Kayser et al. 1998). The size of typical fields ranged from 30 to 60 ha (maximum 166 ha). Several types of fallow land accounted for 7% of the total area. Only 5% of the area consisted

of non-agricultural habitats, i. e. trees, bushes, buildings, roads, etc (Toepfer and Stubbe 2001). Hakel is an area of mixed forest (predominantly Tilia and Quercus with some Carpinus) and large part of the area is dominated by Sessile Oak (*Quercus petraea*), Small-leaved Lime (Linden) (*Tilia cordata*) mixed woodland.

At the north-western border of the Hakel a Steppe or "Steppenheide" (which are neither heath nor steppe) forest (Potentilla cinquefoil-Sessile oak-forest) is located. Also it is to be found Field Maple-Scots elm-forest and, Liverwort-Beech-forest (Michel and Mahn 1998). The beeches (*Fagus silvatica L.*) dissemination is limited to this area of subcontinent thermophil mixed deciduous woodland. Sessile oak-Lime-mixed forest, European or common hornbeam and common beech characterize the forests sight. A highly developed layer of bushes offer the wildlife (game) possibility to cover and protection (Stubbe 1965).

1.2.3. Fallstein

1.2.3.1. Location

Fallstein with a size of about 1500 ha is situated in the northern foreland of Harz Mountains in central Germany (Saxony-Anhalt) (Fig.1.3.). This area located about 40-45 km from Hakel (M.Stubbe pers. comm). The elevation is 200 to 280 m with the coordination: 52° 0′ 41″ N, 10° 44′ 9″ E (Hentschel et. al 1983).

1.2.3.2. Protective status

The entire Fallstein is since 1961 a landscape protection area. The northern zones of Osteroder wood and the central forest area above 270 m NN, protected as the nature reserve and the ways may not be left "road order" (Region Braunschweig Ostfalen 2005). A percentage of 94.34% from this area is protected as the landscape protection area and 15.97 % as the nature reserve (European Topic Centre for Nature Protection and Biodiversity 2008a).

1.2.3.3. Climate

The annual average of precipitation is 600 mm. The average annual temperature is 8.0° C. The mean annual variation lies at 17.6°C (monthly mean of January: below 0°C, monthly mean of July: 17.5°C) (Hentschel et. al 1983).

1.2.3.4. Flora

Fallstein is an outpost of the western widespread of the pure beech forests at the transition to the Central German dry area. The character of the Fallsteins woodland is dominated by pure

beech forests to mixed oak-beech forests. The layer of bushes is not as rich as in Hakel, but beneath the layer of branches and fallen wood a thick vegetation of young beech trees is to be found (Stubbe 1966). On the even clay ground of the highest elevation of Fallstein is to be found a Wood fescue-Beech forest *(Festuca altissima-Fagetum*), whose layer of trees consists out of 100-200 year old Common beeches (*Fagus silvatica*) and is mixed only with a small amount of ash *(Fraxinus excelsior),* Sycamore Maple *(Acer pseudoplatanus)* and Sessile Oak *(Quercus petraea)*. The low layer of bushes consists only out of young tree of the above-mentioned kinds. The lowest layer of grasses is dominated by Wood fescue *(Festuca altissima)* (Hentschel et. al 1983). However the habitat types in Fallstein are divided into six main categories as: broad-leaved deciduous woodland (88%), dry grassland, Steppes (1%), other arable land (1%), coniferous woodland (1%), mixed woodland (2%) and artificial forest monoculture (e.g. Plantations of poplar or Exotic trees) (7%) (European Topic Centre for Nature Protection and Biodiversity 2008a).

1.3. Morphological study

By now it is clear that phenotype is a result of the interaction between genotype and environment, in addition to variation not readily attributable to either (Peaston and Whitelaw 2006). Morphological (phenotype) variation between populations of a species can be considered as the continuous result of micro-evolutional processes which are determined by genotype or the genetical constitution of individuals and populations as well as by the ecological conditions of the environment (Grant and Price 1981, Cherry et al. 1982, Pankakoski and Nurmi 1986). Morphology of mammals often can be related to ecological adaptations. Ecogeographic rules (Mayr 1942, 1956) have been widely used to describe correlations between morphological variation and environmental characteristics (Aragon et al. 1998).

Different morphological methods and approaches often provide different pictures of the similarity or dissimilarity of geographic populations and of intraspecific differentiation. As a rule, it is difficult to distinguish between genetical and environmental determinants of an empirical pattern of morphological differentiation (Zima et al. 1989). However the phenotypic variation can provide useful indications of genetic relationships within and between populations of mammals. Indeed, variation in certain morphological characteristics of large mammals, such as: body and antler size have been shown to have a genetic basis. For example, Rees (1969,1970) estimated that each of 20 skull measurements was under the

control of approximately 10 gene loci in white-tailed deer *(Odocoileus virginianus)* (Andersen et al. 1998).

Fig. 1.3: The location of sample areas for the roe deer in Germany.

Biometric analysis of the patterns of morphological variation can help in explaining evolutionary problems, i.e. estimation of the degree of similarity between populations, effect of habitat changes into adaptive responses, tracing of historical events into observed geographic variation, among other issues (Fandos 1994). In the past, investigations on free-living mammals used biometrics as the main method to study intraspecific structure, particularly to compare dimensions of various skeletal parts and especially skull dimensions (Zima et al. 1989).

The skull is the most complex bone structure in the body and is highly variable in shape,

reflecting variation in genetic origin. Thus, cranial morphometrics is a useful tool for examining genetic variation at higher orders of organization as population, subspecies and species (Kuhn and Zeller 1987). This technique has been used to investigate the degree of the hybridization between Red Deer (*Cervus elaphus*) and Sika Deer (*Cervus nippon*) in northern Britain (Lowe and Gardiner 1975, Ratcliffe et al. 1991), the taxonomic status of British feral Muntjac (Chapman and Chapman 1982) and the genetic divergence of geographically isolated populations of Red Deer (Lowe and Gardiner 1974) in Europe (Hewison 1997).

1.3.1. Genetics aspect of morphological study on roe deer

In ungulates, the roe deer *Capreolus capreolus* (Linnaeus, 1758) constitutes a good material for the study of morphological changes in populations living under different environments (Fandos and Reig 1993). It is a wideranging Cervidae with low migration distances, but with high ecological adaptability and colonizing ability (Pielowski 1970, Stubbe and Passarage 1979, von Lehman and Sägesser 1986).

Also the roe deer has considerable ecological (von Lehmann 1958, Saez-Royuela and Telleria 1991), behavioral (Vincent and Bideau, 1992), morphological (Zima et al. 1989), and cytogenesis (Baskevich and Danilkin 1992) variability across its range. It is also one of the genetically most variable deer species yet studied (Hartl et al. 1991, Lorenzini et al. 1993) and more than 25 subspecies of roe deer have been described based on phenotypic variation (Corbet 1978, von Lehmann and Sägesser 1986). Cranial morphometries have been used extensively to investigate relationships between roe deer *Capreolus capreolus* species (Sokolov et al. 1985), subspecies (Fandas and Reig 1993), populations (Markowski and Markowska 1988; Zima et al. 1989) and 'ecotypes' (Markowski 1993).

The skull biometry of roe deer populations is well known, mainly in the prior Czech and Slovak Republik where studies of roe deer are relatively abundant (Fandos 1994). For example, the geographical variability of non-metrical characters has been studied in the roe deer by Zima (1989), Gromov and Skulkin (1986).

Also craniometric analyses suggest that the European *(Capreolus capreolus)* and the Siberian *(Capreolus pygargus)* roe deer may be considered as different species (Sokolov et al. 1985). Based on a similar approach there is a suggestion that further subdivision of both species may be justified (Danilkin et al. 1985; Markov et al. 1985), but this requires more in-depth analysis. Other studies of cranial morphometries have not supported claims for subspecific status among the roe deer of prior Czechoslovakia (Zima et al. 1989) or the Iberian

peninsula (Fandos and Reig 1993), although Hewison (1997) identified a skull morph in certain populations of northern Scotland which may correspond to an indigenous British race *(Capreolus capreolus thottii)*, the only remnants following the widespread decline of this species in the eighteenth century (Andersen et al. 1998).

Zejda and Koubek (1988) found differences in skull size between various populations of Czechoslovakian roe deer, but all individuals were assigned to a single group on the basis of shape. There are obvious dangers in assuming a relationship between phenotypic variation in size alone and in genetic variation. Environmental factors such as food availability and climate are strongly determinate of overall size. However, on the basis of similarity in both cranial size and shape, roe deer from 13 British populations were assigned to one of three well-defined groups (Hewison 1997). One group was composed of populations which were founded from German stock. A novel allelic variant for one particular enzyme locus has previously been described in these populations (Hewison 1995), suggesting that the differentiation of this group based on skull characteristics does indeed have a genetic basis (Andersen et al.1998).

Roe deer show remarkable behavioural and ecological plasticity (Danilkn and Hewison 1996). In particular, this predominantly woodland species has colonised the open agricultural plain of Central Europe, prompting certain authors to differentiate between two ecotypes (Pielowski 1977).

Further, some authors have proposed that certain aspects of morphological variation in roe deer may represent the results of changes occurring during adaptation to different habitat types, possibly mediated by human interference through hunting and management (Zejda and Koubek 1988, Zima et al. 1989). For example, Fandos and Reig (1993) proposed that the larger mandibles of roe from the Occidental Cantabric mountains, compared to elsewhere in the Iberian peninsula, was an adaptation to the more ligneous food sources in this region. However, although certain morphological characteristics do vary between 'field' and 'forest' roe (Fruzinski et al. 1982), it seems that differences in these phenotypic traits are due to environmental rather than genetic influences (Andersen et al. 1998).

1.3.2. Non- metric method as a morphological tool

Classical morphological investigations and the use of morphological characters in various lines of biological sciences constitute the basis of research from the very beginning up till now, often complemented by the prevailing 'modern' methods (Gutmann et al. 1994). Recently a true renaissance in use of morphology has arisen, due to the increasing application of non-

metric skeletal characters for population genetics and the rapid spreading of the analysis of fluctuating asymmetry in quasi-continuous traits as a measure of developmental stability. Non-metric characters have become highly attractive as a relatively simple morphological tool (Rahmel and Ruf 1994, Pertoldi et al. 2000), even to non-morphologists, because of the rapid and apparently reliable outcome in applied research (Ansorge 2001).

Geographic variation in, for example, metrical measurements and non-metrical traits can be regarded as a universal phenomenon in the animal kingdom (Wiig and Lie 1979). Estimation of genetic affinities among groups based on skeletal variation is difficult because of the influence of non-genetic factors. Continuous, or metric, variants, such as lengths and widths of body parts, are influenced to a considerable extent by environment. However, the percentage occurrence of discrete, or non-metric, variants in the mammalian skeleton may be a group characteristic that is largely genetic in nature.

In general, external factors probably influence the size of a skeletal element more than its shape; thus, shape may tend to reflect genetic background better than does size. This tendency, however; would probably be most reliable when size is fairly constant. There are complicating factors when size differences are prominent. For example, relative thickening of leg bones in large vertebrates is necessitated by size (volume) increase in order to support the increase in weight (Gould 1966). Shape difference, in this case, is a result of mechanical or structural response associated with size difference and would not, therefore, reflect genetic background any better than size does (Ress1969).

In the other hand, non-metric characters are potentially more useful than metric characters for discriminating populations of mammals because (1) a large number of relatively independent characters can more easily be defined and (2) the characters are supposedly more "neutral" in relation to adaptation and therefore good indicators of degree of gene flow between populations (Sjøvold 1973, Hartman 1980). An additional attraction of non-metric characters is that they are less affected by preparation methods and specimen handling; for example, dolphin skulls in museums very frequently have broken rostra or dried tissue remaining on the rostrum tips, broken pterygoids, etc., and this often leads to missing values for several measurements (Perrin et al 1994).

The following advantages of using this technique for assessing variation at the population level also were derived by Markowski (1995):

(1) They can be rapidly and easily scored, without large-scale equipment on mammalian

skeletons, being frequent objects of study, have been kept in all museum collections in large numbers.

(2) Non-metric variation is not affected by sex, age and ontogenetic developmental stage of the specimens.

(3) Non-metric variants are usually not inter-correlated among traits.

The acceptance of the second and especially of the third argument led to the statement that "computation of multivariate statistics is much simpler than it is the case for metrical characters and there are grounds for believing that estimates of divergence between samples based on differences in non-metrical variant incidence more accurately reflect genetical differences than statistics calculated from metrical data" (Berry 1968).

1.3.3. History of non-metric method

It would mean going too far back to seek the roots of noting non-metric characters in the qualitative morphological approach of the earlier naturalists. It is, nevertheless, worth mentioning the attention of Aristotle to the bilateral asymmetry of claws in crustaceans more than 2300 years ago (Palmer 1996). The first steps in using non-metric characters according to our modern understanding were taken by anthropologists at the end of the 19th century (Ansorge 2001). Minor variations in the ossicles, foramina and ridges of the cranium have aroused the curiosity of anatomists for many decades (e.g. Le Double 1903).

It was Wood Jones (1930-31), however, who first proposed that the differing incidences of these minor variants which occurred in different races might be useful in anthropological studies. Laughlin and Jørgensen (1956) put this idea into practice and in 1967 Berry and Berry suggested that a wide range of these variants could be used to calculate a distance statistic between population samples (Berry 1968).

After cautious preliminaries around the middle of the last century, profound research became focussed on genesis and heritability of non-metric traits (Ansorge 2001). Numerous genetical studies on minor variants of the skeleton have been performed by Grüneberg and his co-workers in inbred strains of mice (Grüneberg 1950, 1952, 1955), who showed that these traits are determined by polygenes. The latter produce a continuous variation as to the expression of a non-metric trait, but the ultimate realization of this variation is dependent on whether or not the respective genetic alterations do exceed a particular physiological threshold (Falconer 1960).

Numerous further authors (Berry 1963 1968 1979, Hilborn 1974, Self and Leamy 1978,

Jablokov 1980, Cheverud and Buikstra 1981, 1982, Yablokov 1982, Richtsmeier and McGrath 1986) paved the way for the wide use of qualitative morphological variants as an epigenetic polymorphism for evaluating diversity and differences among and within populations and species in manifold lines. Instigated by Berry (1963), researches in relation to non-metric characters progressed rapidly and spread out to numerous mammal species, resulting in the subsequent publication of a bibliography on 'non-metrical variation in wild mammals' (Bachau 1988) comprising more than one hundred articles.

Ever since, this course of research has gained wider dimensions through practical applications. Beyond simple differentiation of populations, the degree of reproductive isolation and phylogenetic bottlenecks were discovered (Kozakiewicz 1993), as well as lines of historic colonisation (Spitzenberger et al. 1999), leading to analyses of complex geographic or phylogenetic intraspecific relations (Ventura and Sans-Fuentes 1997, Hartl et al. 1993, Suchentrunk 2000). Last not least, the frequencies of qualitative traits have been used for systematic clarification near the species level (Lyalyukhina et al. 1991).

In an analogous process to the above mentioned research a field, another practice, i.e., the use of fluctuating asymmetry has developed more recently. At the same time when the genetic background of non-metric traits was established, Ludwig (1932) directed attention to the presence of asymmetry in morphological characters by the "Rechts-Links-Problem im Tierreich", but in a more descriptive way. It did not become certain until the fifties that deviations from symmetry might supply insight into developmental stability (see Palmer and Strobeck 1986).

When tools for analyzing fluctuating asymmetry became more popular, an explosion of applications by biologists of very different disciplines set off within the last twenty years. Most of the extensive research, especially which, in the nineties, dealt with fluctuating asymmetry as an epigenetic measure of stress in general (Parsons 1990), often related to anthropogenic influences and switched over ultimately to the status of virtually being a field method and biomonitoring tool (Rahmel and Ruf 1994).

However, very thorough studies are also currently aimed at conservation biology, detecting lower genetic diversity in endangered populations (Gilligan et al. 2000), the influence of hybridisation (Auffray et al. 1996), or uncovering connections to population dynamics (Zakharov et al. 1991). The hard-to-interpret but nevertheless upcoming fluctuating asymmetry has provoked serious critique of the method and its sometimes exaggerated applications (Merilä and Björklund 1995, Palmer 1996), but this youngest issue of research in

non-metric traits is still in progress (Møller and Swaddle 1997).

1.3.4. Forms for the occurrence of non-metric character

The term 'non-metric character' in this context stands for various kinds of discontinuous variants in different parts of the skeleton, the incidence of which should be obviously independent of growth and not be induced by direct external influences. Because their characters are very informative, the skulls have been most under consideration. Characters of the vertebrae as well as of the whole appendicular skeleton have been widely neglected, certainly owing to entire skeletons being more rarely collected than skull series (Ansorge 2001). A general classification of the group of non-metric traits is hard to establish without the possibility of any exceptions.

However, following Ossenberg (1969) with some generalizations, most of non-metric traits can be classified as belonging to one of the following four categories of skeletal variation:

*Hypostotic variation***.** Hypostotic traits are characterized by either incomplete lack of ossification, or by arrested development, the retention of an immature or embryonic stage. (i.e., weak osseous development, arrested morphogenesis, retention of infantile features).

Hyperostotic variation. Traits being Hyperostotic are characterized by excess of ossification, in some instances by excess of ossification over the non-anomalous condition; (i.e., excess of ossification, not reaching the pathological condition).

Supernumerary sutures and centers of ossification. These sutures are confined to the skull, most often enclosing a sutural ossicle, that is, associated with supernumerary ossification center. Sutural ossicles may occur in every suture of the skull, although the vault sutures show by far the highest incidences. Supernumerary ossification centers may be found in connection with many bones of the skeleton, rarely forming separate bones. With respect to the supernumerary sutures, some of the sutures or sutural remains, apparently Supernumerary, represent arrested growth between parts of the skull and belong to the group of hypostatic traits.

Foramina, canals and grooves for blood vessels and nerves. Most commonly-used traits among minor skeletal variants constitute the foramina, natural holes in the skull or bones, through which nerves and blood vessels pass.

Non-metric traits cannot be measured. As such, these traits tend to be scored or ranked. The most common made of scoring is presence / absence of trait (Truesdell 2005). Often non-metrical variants appear in more than two states, *e.g.* a foramen may be absent, single, double,

triple *etc.,* which indicate the existence of more than one threshold on the liability axis. However, the mathematical properties of multistate characters have not been solved (Sjøvold 1977), and thus several states have been pooled to obtain only two alternatives (i.e. absent versus present) when a mean measure of divergence has been calculated in population studies (Wiig and Andersen 1988).

Often a certain single foramen is recorded as merely being present or absent or being single or multiple. Sometimes the occurrence of an additional minor foramen is looked for, or the total number of unspecified foramina is counted within a small defined area of the bone. More rarely, variation in relative position of a foramen on the bone is considered. However, foramina are so numerous and of such importance, that several investigations exclusively regarding this type of non-metric characters have been done (Wiig and Lie 1979, Andersen and Wiig 1982).

Nevertheless, in most analyses, further categories of qualitative traits complete the methodical foundation. Because of their good recognisability, all kinds of hyperostotic variation are applied even though they occur infrequently. This excess of ossification can be found, e.g., as a small bone bridge within a foramen like that in the divided-looking foramen ovale (Ansorge and Stubbe 1995). The overgrowth of bones can produce canals from grooves and, in few cases, small additional processes, both of which can be regarded as non-metric characters.

In contrast, hypostotic variation is characterised by the lack of ossification. Expressions reach from true fenestration, e.g., of the vomer (Gao and Gaskin 1996), openings in the mandible's coronoid process, or in the alveoli at the molar roots (Pankakoski and Hanski 1989) through to the total absence of Os interparietale (Hartl et al. 1993) or of the Processus pterygoideus (Berry 1963). Among the fusion traits, supernumerary sutures enclosing a sutural ossicle are more useful than missing sutures. The latter usually evade analysis of epigenetic variation because of their frequent fusion during normal growth (Ansorge 2001).

1.3.5. Variable heritability of non-metric characters

The non-metric traits of skull also known as discontinuous, discrete, quasi-continuous variables or epigenetic polymorphism (Gualdi-Russo et al. 1998). Theoretically, minor cranial variants being "epigenetic" (i.e., controlled by environmental as well as hereditary factors) they also could be affected by such changes (Berry 1979).

Epigenetic phenomena associated with phenotypic variation at the biochemical, cellular,

tissue, and organism level are now well recognized and are likely to contribute to the ‘‘intangible variation’’ alluded to. However, much mammalian phenotypic variance cannot be attributed to single-gene effects, and other sources include multigene effects, environmental influences, noise, and epigenetic effects (Peaston and Whitelaw 2006).

Epigenetic effects are those effects caused by chemical modifications to DNA that do not alter the DNA sequence but do alter the probability of gene transcription. Such modifications include direct covalent modification of the DNA by methylation of cytosines in symmetric or asymmetric contexts and modification of the proteins that bind to DNA. Such modifications may alter DNA accessibility to transcription complexes at a local level and affect higher order chromatin structure at regional and genomewide levels, thus linking genome structure and transcriptional regulation. The extent to which epigenotype contributes to variable phenotype is somewhat controversial and is difficult to disentangle from genetic and environmental contributions in outbred natural populations. Much evidence is indirect; being the genetic equivalent of an epigenetic effect is a strong candidate source of phenotype variability after genetic and environmental effects have been ruled out (Peaston and Whitelaw 2006).

Today, non-metric characters or epigenetic variants have become widely accepted as genetic markers (Bachau 1988). It is claimed that they are highly heritable in nature, and may be employed in phylogenetic studies (Uhlikova 2004). Frequency analysis of non-metric variants has in the meanwhile become a well-established technique having substantial advantages (Markowski 1995, Lazarova 1999). Extensive studies by Grüneberg (1963) on inbred strains of mice and by Berry (1968) on humans and laboratory mice proved the complex multigenic background of minor skeletal variants, which is corroborated by the strong heritability of these traits as found, e.g., by Hilborn (1974), Berry (1978) and Cheverud and Buikstra (1981).

It seems convincing that the minor variants of non-metric skeletal characters are of lower importance for an organism than selectively more relevant traits concerning feeding or the reproductive system. It has been assumed that such traits are therefore exposed to a minimum of selection pressure (Pankakoski and Hanski 1989). The variants are believed to be caused by the accumulating effects of a high number of alleles acting at several loci, as well as of various non-genetic factors. It seems therefore reasonable to assume that the total effect, called liability (Falconer 1981) is normally distributed (Sjøvold 1977). A variant is manifested when its liability exceeds the threshold [see Falconer (1981) for discussion of threshold characters]. Each variant may be presumed to be under the control of at least ten gene loci (Berry and

Jacobson 1975, Berry 1986) and they are usually uncorrelated with each other (Truslove 1961, Sjøvold 1977), indicating that different variants are controlled by different loci (Ansorge 2001).

Nevertheless, the results mentioned above were never been confirmed on real wild-living animals (Berry and Jacobson 1975). Moreover, the genetic background of only a few single non-metric characters such as dentition in mice has hitherto been clarified. Even though a true genetic background has not been proved for non-metric characters in general, the assumption that they are genetically controlled has been applied to many different traits (Ansorge 2001).

However, the fact of a rather low heritability of non-metric traits, e.g., as emphasised by Self and Leamy (1978), or Richtsmeier and McGrath (1986), has led to realisation of the importance of the influence of certain, intangible non-genetic factors. These are paraphrased with 'prematernal' or 'intra-uterine environment' (Berry 1978), and seem to be connected with diet, age of females or birth order. This results in obvious differences in trait incidence between sexes and ages, or in correlation among characters. Some of the respective characters affected predominantly by environmental influences must be excluded. As it is impossible to prove that genetic factors are solely involved in character formation, the existence of potential environmental influence has to be accepted (Ansorge 2001).

Furthermore, in simultaneous analysis of a number of traits, the genetic determinants should assert themselves more distinctly, and intangible environmental influences should be less considered (Sjøvold 1977). Howe and Parsons (1967) found while investigating mice that individual traits were significantly affected by the environment. When several traits were combined in the study, the environmental effects were not significant (Wiig and Lie 1979).

Accordingly, consideration of numerous characters is a prerequisite for the epigenetic value of non-metric traits, which are the basis for a useful method for evaluating population variation with a mainly genetic expression (Ansorge 2001).

1.3.6. Non-metric variability and population divergence

The use of non-metrical variants as genetical markers in mammalian population studies is a well established technique (Berry 1969a 1969b, Berry and Warwick 1974, Sjøvold 1977, Berry et al. 1978, Andersen and Wiig 1982, Wiig and Lie 1984, Pankakoski and Nurmi 1986, Berry 1986). An important use of non-metric variants is based on their occurrence in separate samples of individuals or populations. They have been widely used for analyzing diversity within and among populations and species (Markowski 1995). The analysis of a large number

of characters makes it possible to determine the epigenetic population variation and thus, the epigenetic divergence between populations (Sjøvold 1977).

High variability in the frequency of trait expression between populations is considered to imply a large degree of epigenetic divergence. To express the degree of separation, Sjøvold (1977) further developed the theoretical foundation of the C.A.B. Smith's 'mean measure of divergence' (MMD) derived from the Mahalanobis-distances. This parameter is widely applied and preferred to any other measures of divergence. Modifications and adjustments have led to an unbiased estimate of divergence independent of sample size. Epigenetic population distances have been verified so far for more than 50 mammalian species (Ansorge 2001).

1.3.7. Population divergence in Roe deer

In the *Cervidae* family Rees (1969) studied the epigenetic variability of cranial characters in white-tailed deer *(Odocoileus virginianus*) based on 16 non-metric variants .The geographical variability of non-metrical characters has been studied in the roe deer (*Capreolus capreolus*) by Zima (1989) in the prior Czech and Slovak Republic. Also the variability of non- metrical traits in roe deer was studied by Gromov and Skulkin (1986).

Their paper concerns intraspecific classification. It is however, based on a different method of distinguishing variants, which limits making comparisons with them. Meanwhile, the other non-metric study on roe deer is non-metrical variation in three populations of roe deer in Poland by Markowski and Markowska (1988). They were unable to detect any significant differences in 76 cranial non-metric traits between field and forest populations, suggesting that this distinction does not have a genetic basis. Obviously, adaptations to changing environmental conditions were not connected with micro-evolutionary processes. This underlines the sensitiveness of non-metric characters even to ecological matters in genetic context (Ansorge 2001).

1.4. Study objectives

The general aim of this dissertation is, with the aid of epigenetic methods, to examine the genetic contributions and investigate the relations between populations of roe deer from three wildlife research areas of Germany (Hakel, Fallstein and Darss). The minor aim is to study geographical, as well as sex- and age-dependent, variation in this species based on epigenetic features of the skull.

2. Material and Method

The material consisted of 786 (494♀, 292♂) complete roe deer skulls (crania and mandibles). The skulls, from the three sampling sites were investigated. The skull collections of three wildlife research areas in Germany were from Hakel, Fallstein and Darss including 316 (40.20%), 401 (51.02%) and 69 (8.78%) skull samples respectively. The more details information of these areas was explained in the first chapter. These collections were established mainly in during the period 1957-1987 (Table 2.1) and were various age-class (Table 2.2) and sex (Table 2.3).

Age of samples was determined at the collecting time; consequently their age were known and aged from 1 to 13.5 year old. The specimens were considered as a sample if they are more than one year of age.

To determine the morphological differentiation with regard to the epigenetic distance, after having studied the test sample, 56 non-metrical traits (48 foramina and 8 sutures or morphological variations of particular skull bones) were identified. Among them, 51 traits were bilateral and the remaining 5 unilateral. These traits that could be scored objectively or were coded as discrete variables, have been chosen according to the some investigation on roe deer and own preliminary studies. In this study 42 of the total 56 traits used were taken from earlier studies (Rees 1969, Markowski and Markowska 1988 and Zima 1989), but 14 were new to this work (Table 2.4). Each trait was scored on the left and the right side of the skull (regarding to the median line of the skull), as present or absent (dichotomously). That means, bilateral traits were taken from both sides of the skull and incidence of traits were recorded, independently on both sides. Therefore, the denominator ranges up to twice the number of skulls observed. There is a disagreement in the literature as to the appropriateness of this artificial doubling of sample size. The numbers of observations as well as the absolute frequencies obtained for each bilateral trait should be considered in order to facilitate later comparisons. This was done in the present study, because all traits in a sample were not based on exactly the same number of observations, which is given. The theoretical considerations involved are discussed in Green et al. (1979) and Sjøvold (1973, 1977).

Frequencies of bilateral traits were separately as well as together calculated (i.e. the trait was considered as present if the trait was expressed at least on the one side) according to the total number of sides examined. Since several skulls were damaged, the frequency of some traits did not correspond to the total sample size. To reduce inter-observe difference or subjectivity in scoring, all the data were collected by myself.

All of traits are described below and they are regarded as bilateral if not, otherwise stated. The location of non-metric traits of the roe deer skulls were shown in figures 2.a, 2.b, 2.c and 2.d and the list of non-metric traits scored on roe deer skulls with authors from earlier studies and acronym were shown in Table 2.4.

2.1. The list of non-metric traits

1. Internal hypoglossi foramen (double) (Fig. 2.1.a): The hypoglossi foramen enters the cranium on the ventral side between the occipital condyle and the jugular processes. It opens into the caudal cranial fossa orally behind the edge of the foramen magnum. The foramen may be simple or double.

2. Accessory internal hypoglossi foramen (present) (Fig. 2.1.a): A small distinct foramen situated endocranially anterior to the hypoglossi foramen. It may be missing or present.

3. Internal condylar foramen (present) (Fig. 2.1.a): The opening of the condyloid canal lies aborally behind the foramen hypoglossi. It may be missing or present.

4. External condylar foramen (present) (Fig. 2.1.a): A small distinct foramen situated on the ventral side as external side of the hypoglossi, between the occipital condyle and the jugular processes. It may be present or absent.

5. External supraoccipital foramen (present) (Fig. 2.1.a): Two foramina situated symmetry at the both external sides of crista nuche (external occipital crest) on supraoccipital bone which becomes the squamous part of the occipital. It may be absent or present.

6. Medial supraoccipital foramen (present) (Fig. 2.1.a): A small distinct foramen located on the median line of the ventral surface of supraoccipital, direct under ridge of the crista nuche. It may be missing or present. It is a unilateral trait.

7. Infra medial supraoccipital foramen (present) (Fig. 2.1.a): A distinct foramen situated under medial supraoccipital foramen and in the supraoccipital region above foramen magnum. It may be missing or present. It is a unilateral trait.

8. Mastoid foramen (present) (Fig. 2.1.a): This foramen is located on the posterior aspect of the skull near the junction of the occipital and the temporal. The position and size of this foramen are very variable; it is not always present.

9. Mastoid foramen (double) (Fig. 2.1.a): A small accessory foramen opens into the mastoid foramen.

10. Meatus temporal foramen (present) (Fig. 2.1.b): A foramen on the temporal bone close to the meatus temporal. It may be missing or present.

11. Meatus temporal foramen (double) (Fig. 2.1.b): A small accessory foramen opens into meatus temporal foramen.

12. Postglenoid (supraglenoid) foramen (present) (Fig. 2.1.b): The Postglenoid foramen may lie anywhere along the junction of the zygomatic process of the temporal with the main body of the squamosal (squamous temporal). It varies greatly in size and may be absent or present.

13. Two and more foramina in sutura parietemporalis (present) (Fig. 2.1.a): A series of foramina lie on sutura between the parietal and the temporal bones. Those may be missing or present.

14. Two and more foramina in parietal bone (present) (Fig. 2.1.a): These foramina located on the external surface of the parietal bone, between the sutura parietemporalis and the coronal suture. Those may be missing or present.

15. Anterior accessory foramen near foramen oval (present) (Fig. 2.1.a): A small foramen situated anterior to the foramen oval at the base of the pterygoid bone. It may be missing or present.

16. Posterior accessory foramen near foramen oval (present) (Fig. 2.1.a): A small vascular foramen situated between the posterior margin of foramen oval and the suture between alisphenoid and the tympanic bulla. It may be absent or present.

17. Supra accessory foramen near foramen optic (present) (Fig. 2.1.a): A foramen located in front of foramen optic in the sphenoid bone. It may be missing or present.

18. Infra accessory foramen near foramen optic (present) (Fig. 2.1.a): This foramen located inferior to the supra accessory foramen near foramen optic. It may be present or missing.

19. Ethmoid foramen (present) (Fig. 2.1.b): The ethmoid foramen is a large foramen, typically situated on the part of the medial orbital wall. It is most often present.

20. Supraorbital inferior I foramen (double) (Fig. 2.1.a): The supraorbital foramen is a bony elongated path located above the orbit (eye socket). The supraorbital inferior I foramen lies directly inside of the supraorbital foramen. It may be sometimes missing.

21. Supraorbital inferior II foramen (present) (Fig. 2.1.a): The supraorbital inferior II foramen lies posterior to the supraorbital foramen, on dorsal surface of frontal bone. It may be absent or present.

22. Supraorbital bridge (present) (Fig. 2.1.b): A bar or plate of bone that bridges the supraorbital groove at or near the supraorbital foramen. In the region of the supraorbital foramen on the dorsal side of the skull there are usually several foramina, often joined

together. There is a bony bridge which sometimes forms at the junctions.

23. Zygomatic anterior foramen (present) (Fig. 2.1.b): This foramen on the ventral surface of the zygomatic process of the jugal (zygomatic bone), may be absent or present. Its size varies greatly.

24. Accessory zygomatic anterior foramen (present) (Fig. 2.1.b): A small foramen is located in front of the zygomatic anterior foramen. It may be missing or present.

25. Zygomatic posterior foramen (present) (Fig. 2.1.b): A distinct vascular foramen situated on the dorsal surface of the base of the zygomatic. It may be absent or present.

26. Accessory zygomatic posterior foramen (present) (Fig. 2.1.b): A small foramen is located in front of the zygomatic posterior foramen. It may be present or missing.

27. Intersutura fontanele between lacrimal and zygomatic (present) (Fig. 2.1.b): A deep grooved shape that sometimes situates on sutura between the lacrimal and the zygomatic bones is located within basal surface of orbit cavity. It may be missing or present.

28. Inferior zygomatic foramen (present) (Fig. 2.1.b): Sometimes there is a foramen situated in the lateral wall of the zygomatic bone in the orbit cavity, as judged by the position of the sutura with the lacrimal and the zygomatic bones next to the edge of last bone. It may be present or missing.

29. Infra lacrimal foramen (present) (Fig. 2.1.b): The large lacrimal foramen is placed in the lower part of the orbital edge, on the facial parts of lacrimal bone. It is the opening of the tear duct, and visible in lateral view. It may be absent or present.

30. Supra lacrimal foramen (present) (Fig. 2.1.b): The lacrimal foramen is a small circular foramen lies outside the orbit, on the facial parts of lacrimal bone, and clearly present at the upper part of orbital margin. It may be missing or present.

31. Lacrimal foramen fused (Fig. 2.1.b): Two tear ducts have their mouths on the edge of the ocular cavity above the lacrimal fossa, and may sometimes have a common foramen.

32. Foramen penetrating nasal bone (present) (Fig. 2.1.b): Vascular foramen is opening on the outer surface of each nasal bone. It may be missing or present.

33. Premaxilla bone connected with nasal bone (Fig. 2.1.b): The nasal and premaxilla bones on either side of the rostrum may touch, or have a gap between them.

34. Nasal bone protruding from the distal line of maxilla bone (Fig. 2.1.b): The nasal bone may pass from the junction of the maxilla bone and the premaxilla bone.

35. Infraorbital foramen (double) (Fig. 2.1.b): Infraorbital foramen is oval shape foramen that is situated bilaterally on the maxilla bone in front of the first premolar. The foramen may be divided into two parts by a bony septum. That means a tiny distinct foramen

situated just inside the opening.

36. Accessory infraorbital foramen (present) (Fig. 2.1.b): An accessory foramen could sometimes be anterior to the infraorbital foramen.

37. Supra accessory infraorbital foramen (present) (Fig. 2.1.b): A small distinct foramen is sitting immediately above the infraorbital foramen. It may be missing or present. Its location varies greatly.

38. Foramen maxilla above PM1 (present) (Fig. 2.1.b): A tiny, vascular foramen situated above the first upper premolar on the lateral surface of maxilla bone. It may be absent or present.

39. Two foramina maxilla above PM2 (present) (Fig. 2.1.b): Two foramina situated vertically to each other, on the lateral surface of maxilla bone approximately above the second premolar and the third premolar**.** Those may be missing or present.

40. First premolar extra (present) (Fig. 2.1.d): There are usually 32 teeth in the permanent dentition of the roe deer. In the upper jaw there are only three premolars on each side and three molars. On each side of the lower jaw there are three incisors and a canine in front, behind which there are again three premolars and three molars, divided from the incisors and the canine by a diastema. Deviations from this normal formula are relatively frequent in this species. An example of typical polyodontia is the occurrence of the first premolar in the diastema of the lower jaw (PM1) (see Zima 1988). In this study rarely an extra PM1 present in the mandible of specimens.

41. Sutura intermaxilla serrated (Fig. 2.1.a): The line of junction between the two maxilla of the upper jaw bone, sometimes is serrated and jagged or sometimes is smooth and straight .It is a unilateral trait and visible in ventral view.

42. Foramina inside Sutura intermaxilla (present) (Fig. 2.1.a): It is possible to being to see some foramina on the suture between the intermaxilla .It is an unilateral trait.

43. Foramen maxilla (present) (Fig. 2.1.c): The foramen maxilla lies in the ventral part of the maxilla and is usually on the margin interalveolar between the rostrum and the first premolar. It may be missing or present.

44. Foramen by PM1 on maxilla (present) (Fig. 2.1.c): A tiny foramen situated on the palatine process of maxilla, in front of the first premolar and near the lateral margin. It is visible in ventral view and may be missing or present.

45. Foramen infraorbitopalatine (present) (Fig. 2.1.c): This term will indicate a foramen located on the horizontal process of the maxilla and may occur bilaterally along the row of teeth, in the vicinity of the second and third premolars, most often on the level of the second

premolar. It is the ventral opening of a small passage that usually leads to the infraorbital canal. The foramen may be absent or present.

46. Caudal major palatine foramen (present) (Fig. 2.1.a): A relatively big size foramen is immediately ventral to the sphenopalatine foramen at the junction of the horizontal and perpendicular plates.

47. Accessory caudal major palatine foramen (present) (Fig. 2.1.a): Maybe a small additional foramen there is near the caudal major palatine foramen. It may be missing or present.

48. Posterior palatal foramen (present) (Fig. 2.1.c): The paired anterior palatine foramina are located in the palatomaxillary suture relatively near the midline. The palatine foramina are opposite the third premolars. There is often a larger foramen that is usually in the suture lateral and posterior to the anterior palatine foramen. This larger foramen will be referred to as the posterior palatine foramen. It is the anterior opening of a canal from the pterygopalatine fossa that conducts a neurovascular bundle which, when the posterior foramen and canal are not present, must continue ventrad to reach the ventral surface of the horizontal part of the palatine bone before passing rostrad on the palate. The posterior palatine foramen may be present or absent.

49. Accessory foramen near posterior palatal foramen (present) (Fig. 2.1.c): A small distinct foramen situated in the opening of the caudal palatine foramen. It may be missing or present.

50. Angle of the median palatine suture (Fig. 2.1.c and 2.1.a): There is a junction (angle) that two lateral wings of the palatal bone reach along the median palatine suture. The connection between them can be either smooth curved surfaces or sharp–edge surfaces. It is a unilateral trait

51. Mental foramen (double) (Fig. 2.1.d): The foramen mental anterior lies on the buccal side of the mandible, in the region of the diastema, between the incisors and the premolars. The foramen is usually single, but it may be double.

52. Superior accessory mental foramen (present) (Fig. 2.1.d): A small foramen sitting above the mental foramen. It may be missing or present.

53. Inferior accessory mental foramen (present) (Fig. 2.1.d): A somewhat smaller foramen sitting at the base of the incisors, anterior to the mental foramen.

54. Posterior mental foramen (present) (Fig. 2.1.d): This distinct foramen on the body (horizontal ramus) of the mandible is positioned back to the mental foramen, in the vicinity of the first premolar may be absent or present.

55. Accessory posterior mental foramen (present) (Fig. 2.1.d): A small foramen situated in front of the posterior mental foramen. It may be missing or present.

56. Foramen mandible (double) (Fig. 2.1.d): The foramen mandible lies on the lingual side of the ramus mandible. Below the main foramen there is may be an accessory foramen.

2.2 Preliminary tests of non-metric traits

Population distance is an expression of morphological similarity between two or more populations, based on a statistical treatment of the selected variables. In choosing these variables, one assumes that they are (1) largely under genetic control and (2) minimally affected by environmental or nutritional conditions (Corruccini 1974). In the other hand, among the assumptions behind the use of non-metrical variants in population studies are that they are uncorrelated, independent of sex and age and that the correlation between sides in bilateral variants is negligible. These assumptions have, however, been proven to fail for particular traits (Sjøvold 1977). Among them, age and sex are important considerations in the analysis of non-metric variation and researchers have demonstrated that there are significant sex and age variation in non-metric traits. It is to be necessary, the traits should be tested for age and sex depended (Buikstra 1972, Corruccini 1974, Garn et al. 1966, Konigsberg 1987, Scott 1977).

Consequently, prior to the calculation of the Mean Measure of Divergence (MMD), non-metric variants were examined for sex dependence (to exam the homogeneity of the distribution of traits between sexes) and for age dependence (to assess the relation between age of variability and the trait frequencies) by the chi-square test. Thus, traits that exhibited significant dependency with sex and age were eliminated. However, when the expected frequency of the variant in group was lower than 5, the result of the test is not quite reliable as an indication of the statistical significance of the differences (Hruby 1961).

The dependence of the occurrence of variants on sex was measured using sample from Fallstein, which was the largest sample evaluated containing both sexes.

The dependence of the incidence of variants on age was evaluated in a sample of specimens from Hakel. Age of specimens from Hakel, was divided into 6 aged classes: (1), (2), (3), (4), (5-6) and (≥ 7). As explained before, they were considered as a sample if they are more than one year of age (12 months). Thus, age class 0 (age less than 12 months) was not considered. The six age classes are: age class 1, which are individuals between 13 and less than 24 months of age. Age-class 2 which are individuals between 2 and less than 3 years of age. Age-class 3 which are between 3 and less than 4 years of age. Age-class 4 which are between 4 and less

than 5 years of age. Age-class 5-6 which are between 5 and less than 7 years of age and aged class ≥ 7 when aged 7 years or older. Its details are shown in the Table 2.2.

The other test of the homogeneity prior to the calculation of MMD is that traits not providing significant information about population divergence should be excluded. Such a selection may be based on tests of homogeneity between the samples, omitting the traits the incidences of which do not differ significantly from homogeneity (Sjøvold 1977, Hanski and Kuitunen 1986). Therefore, the third homogeneity test was utilized based on chi-square tests between the samples, omitting traits which do not differ significantly between at least two of the populations.

The application of cumulating formulas for determining the divergence between samples required employing non correlated variants. Interdependence between traits or the degree of correlation between the variants was calculated by Pearson's correlation test based on a chi-square approach ($P < 0.05$) for the whole sample (786 individuals) (Sjøvold 1977). It is to be expected that at least some of the non-metric variants that were scored would be highly correlated with others. In such cases, it may be possible to drop one or more characters without losing significant information. Also, when correlations involved only a pair of variants, the character more difficult to score was discarded.

It is notable that all computations and statistical tests were performed by Statistical Analysis System (SAS, 2003) software and chi-square test were statistically significant if their error probability to signify were less than 0.05 ($p<0.05$).

For computing the epigenetic distances, the formula of MMD proposed and derived by Freeman and Tukey (1950) and Sjøvold (1977) could be used which employ somewhat different approaches. Variance and standard deviation (SMMD) of the MMD are necessary to prove statistic significance by MMD > 2 SMMD. That means differences were statistically significant if the MMD value was twice higher than the standard deviation of the MMD (Sikorski 1982).

In a skeletal sample of size n, different non-metrical traits selected for study are scored and both the absolute frequencies x, as well as the relative incidence $p = x/n$, are recorded. The sample size n refers to the total number of observations made, and x to the number of observations possessing the trait. If the trait is unilateral, n denotes the number of individuals for whom the trait could be scored; if it is bilateral, n refers to the number of observable sides. The observed relative incidence $p = x/n$ is consequently modified according to the following formula giving the new p-values.

$$P_1 = \frac{x_1}{n_1}$$

Where, x_1 = Traits, n_1 = Number of Trait observations, r = Number of Traits.

The following are the used formulas to calculate MMD (2.1) and SMMD (2.2) between two populations 1 and 2 with p-values p_1 and p_2 respectively:

$$MMD = \frac{1}{r\sum(\theta_1 - \theta_2)^2 - V_{12}} \tag{2.1}$$

and

$$SMMD = \sqrt{\frac{(2\sum V_{12}^2)}{r^2}} \tag{2.2}$$

By Sjøvold (1977):

$$V_{12} = \frac{1}{n_1} + \frac{1}{n_2}$$

$$\theta_1 = Arc\sin(1 - 2P_1)$$

By Freeman and Tukey (1950):

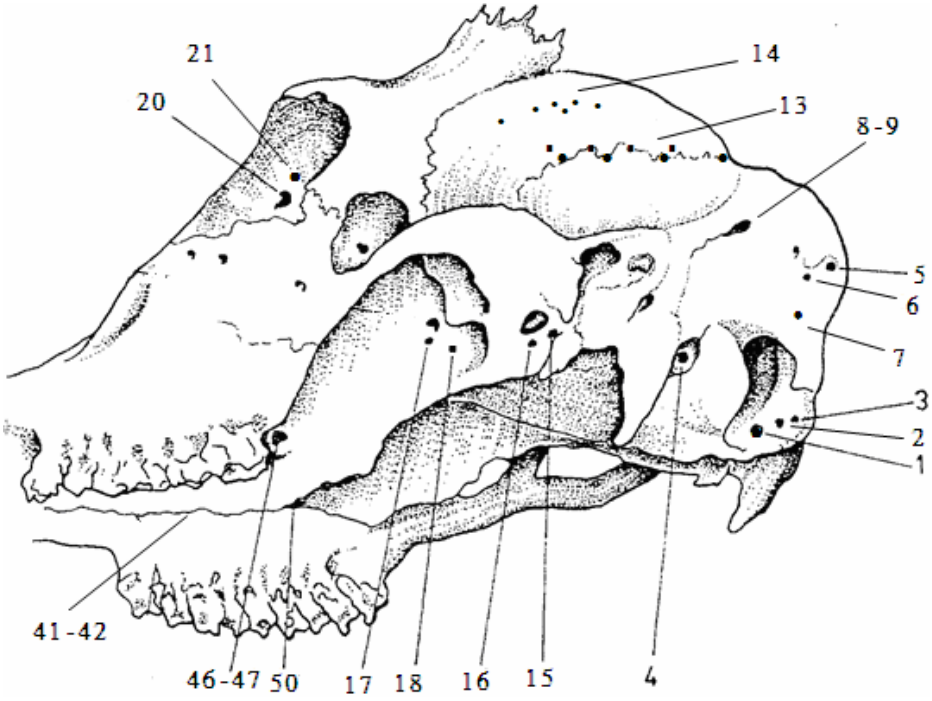
$$V_{12} = \frac{1}{(n_1 + 0.5)} + \frac{1}{(n_2 + 0.5)}$$

$$\theta_1 = \frac{1}{2}\left(Arc\sin\left(1 - \frac{2x_1}{n_1 + 1}\right) + Arc\sin\left(1 - \frac{2(x_1 + 1)}{n_1 + 1}\right)\right)$$

The measure of uniqueness (MU) was calculated as the sum of its epigenetic distance (MMD) computed by following formula (formula 2.3):

$$MU_k = \sum_{j=1}^{u} MMD_{kj} \tag{2.3}$$

Where j is the number of sample which compared with kth sample. Based on the mean measure of divergence a dendrogram of epigenetic similarity was constructed by the clustering of the MMD matrix. The cluster analysis was done by use of a simple but popular clustering algorithm for distance data named Unweighted Pair Group Method using Arithmetic averages (UPGMA) introduced by Sneath and Sokal (1973) via SAS (2003). The MMD and SMMD formulas applied in the present work were carried out by help of the computer program of the state Museum of Natural history Görlitz, Germany.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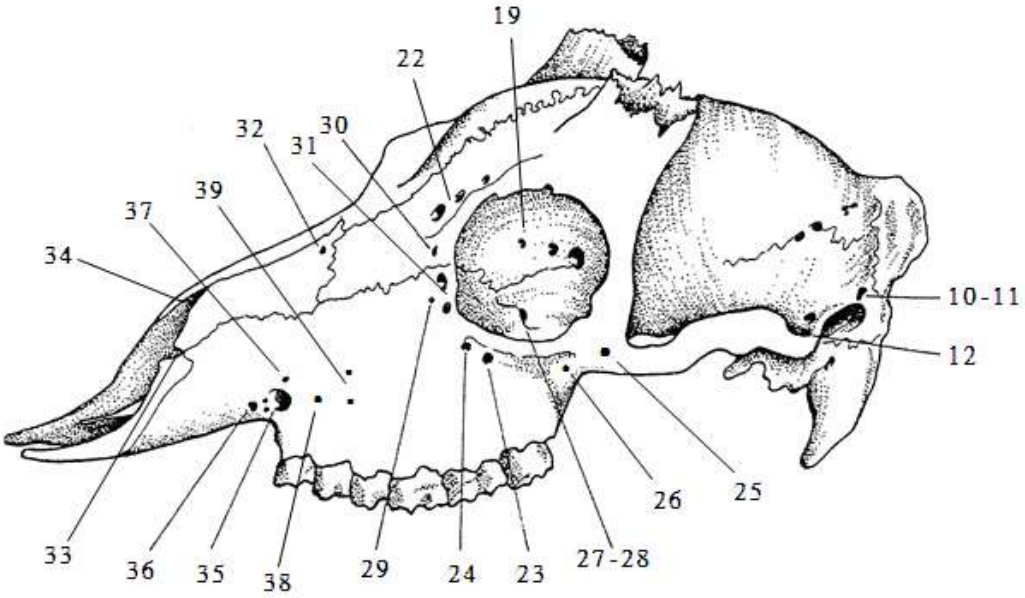
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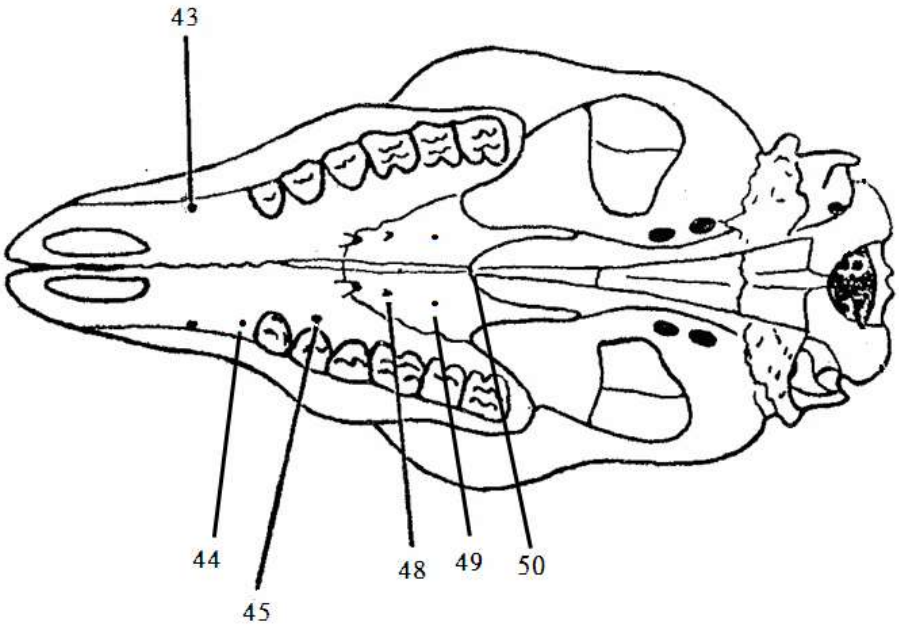

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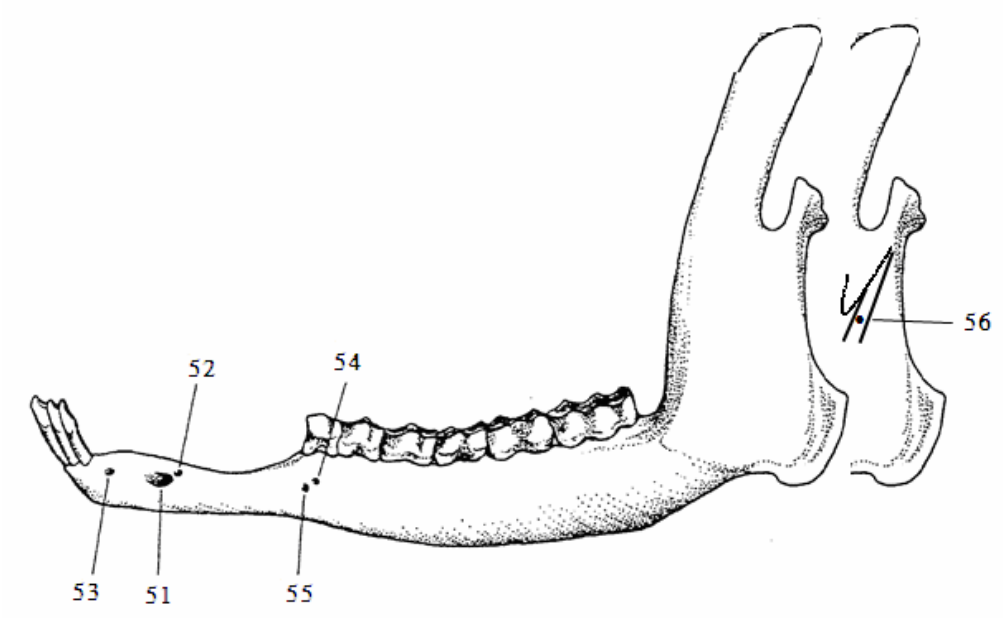

2.1.d

Fig. 2.1: The location of non-metric traits of the roe deer skull: a) vetrolateral view, b) dorsolateral view, c) ventral view and d) mandible (Fig. a., b. and d. adopted from Markowski and Markowska 1988 and Fig. c. adopted from Zima 1989). Numbers refer to non-metric traits listed in the Table 2.4.

Table 2.1: Number (N) and Percentage (P) of samples for year distributions in each area.

Year	Darss		Fallstein		Hakel	
	N	**P**	**N**	**P**	**N**	**P**
1957			14	3.49	3	0.95
1958	23	33.33	35	8.73	16	5.06
1959	13	18.84	94	23.44	19	6.01
1960	6	8.70	24	5.99	27	8.54
1961	6	8.70	42	10.47	38	12.03
1962	7	10.14	85	21.20	39	12.34
1963	5	7.25	63	15.71	42	13.29
1964	8	11.59	44	10.97	13	4.11
1965	1	1.45			25	7.91
1966					17	5.38
1967					5	1.58
1968					2	0.63
1969					19	6.01
1970					17	5.38
1971					3	0.95
1972					9	2.85
1975					1	0.32
1976					1	0.32
1977					3	0.95
1979					2	0.63
1980					3	0.95
1981					8	2.53
1986					3	0.95
1987					1	0.32

Table 2.2: Number (N) and Percentage (P) of samples for age-class in each area.

Age class	N	P	Darss		Fallstein		Hakel	
			N	P	N	P	N	P
1	375	47.71	32	4.071	163	20.738	180	22.90
2	116	14.76	10	1.272	65	8.270	41	5.216
3	118	15.01	14	1.781	70	8.906	34	4.326
4	67	8.524	5	0.636	37	4.707	25	3.181
5-7	66	8.397	4	0.509	37	4.707	25	3.181
≥ 7	44	5.598	4	0.509	29	3.690	11	1.399
Total	**786**	**100**	**69**	**8.779**	**401**	**51.02**	**316**	**40.20**

Table 2.3: Number (N) and Percentage (P) of samples for each sex in the three areas.

Area	Female		Male	
	N	**P**	**N**	**P**
Darss	42	5.344	27	3.435
Fallstein	316	40.20	85	10.81
Hakel	136	17.30	180	22.90
Total	**494**	**62.85**	**292**	**37.15**

Table 2.4: The list of non-metric traits scored on roe deer skulls by present study.

N	The non-metric traits (character names)	Authors	Acronym
1	Internal hypoglassi foramen (double)	(Markowski and Markowska 1988)	Fiterhypd
2	Accessory internal hypoglassi foramen (present)	(Markowski and Markowska 1988)	aFiterhyp
3	Internal condylar foramen (present)	(Zima 1989)	FinterCon
4	External condylar foramen (present)	(Markowski and Markowska 1988)	FexCon
5	External supraoccipital foramen (present)	(Markowski and Markowska 1988)	FexSupocci
6	Medial supraoccipital foramen (present)	(Markowski and Markowska 1988)	FmedSupocci
7	Infra medial supraoccipital foramen (present)		InfraFmedSupocci
8	Mastoid foramen (present)	(Zima 1989) , (Ress 1969)	Fmast
9	Mastoid foramen (double)		Fmastd
10	Meatus temporale foramen (present)	(Markowski and Markowska 1988)	Fmeatte
11	Meatus temporale foramen (double)	(Ress 1969)	Fmeatted
12	Postglenoid (supraglenoid) foramen (present)	(Zima 1989) , (Ress 1969)	Fpostgl
13	Two and more foramina in sutura parietemporalis (present)	(Markowski and Markowska 1988)	2FsutuPtem
14	Two and more foramina in parietal bone (present)	(Zima 1989) , (Ress 1969)	2Fparietal
15	Anterior accessory foramen near foramen oval (present)	(Ress 1969)	aaFfoval
16	Posterior accessory foramen near foramen oval (present)	(Markowski and Markowska 1988)	paFfova
17	Supra accessory foramen near foramen optic (present)	(Markowski and Markowska 1988)	suaFfopt
18	Infra accessory foramen near foramen optic (present)	(Markowski and Markowska 1988)	infraaFfopt
19	Ethmoid foramen (present)	(Markowski and Markowska 1988)	Fethmo
20	Supraorbital inferior I foramen (double)	(Markowski and Markowska 1988)	inferFsuorbId
21	Supraorbital inferior II foramen (present)	(Markowski and Markowska 1988)	inferFsuorbII
22	Supraorbital bridge (present)	(Zima 1989)	suorbBirdg
23	Zygomatic anterior foramen (present)	(Markowski and Markowska 1988)	anFzygo
24	Accessory zygomatic anterior foramen (present)	(Markowski and Markowska 1988)	aanFzygo
25	Zygomatic posterior foramen (present)	(Ress 1969)	postFzygo
26	Accessory zygomatic posterior foramen (present)		apostFzygo
27	Intersutura fontanele between lacrimal and zygomatic(present)		intSutuFontal
28	Inferior zygomatic foramen (present)		inferFzygom
29	Infra lacrimal foramen (present)	(Markowski and Markowska 1988)	infraFlacrim
30	Supra lacrimal foramen (present)	(Markowski and Markowska 1988)	supFlacrima
31	Lacrimal foramen fused	(Zima 1989)	Flacrimfus
32	Foramen penetrating nasal bone (present)	(Markowski and Markowska 1988)	Fnasal
33	Prermaxilla bone connected with nasal bone	(Ress 1969)	Premax_nas
34	Nasal bone protruding from the distal line of maxilla bone		Nasprocess
35	Infraorbital foramen (double)	(Zima 1989) ,(Ress 1969)	Finfraorbitd
36	Accessory infraorbital foramen (present)	(Markowski and Markowska 1988)	aFinfraorbi
37	Supra accessory infraorbital foramen (present)		suaFinfraorbi
38	Foramen maxilla above PM1 (present)	(Markowski and Markowska 1988)	Fmaxabopm1
39	Two foramina maxilla above PM2 (present)		2Fmaxabopm2
40	First premolar extra (present)		PM1ex
41	Sutura intermaxilla serrated	(Markowski and Markowska 1988)	sutintmax
42	Foramina inside Sutura intermaxilla (present)	(Markowski and Markowska 1988)	Fsutintmax
43	Foramen maxilla (present)	(Zima 1989)	Fmax
44	Foramen by PM1 on maxilla (present)		Fmaxundpm1
45	Foramen infraorbitopalatine (present)	(Zima 1989), (Ress 1969)	Finforbipala
46	Caudal major palatine foramen (present)		Fcaumpal
47	Accessory caudal major palatine foramen (present)		aFcaumpal
48	Posterior palatal foramen (present)	(Zima 1989)	postFpalat
49	Accessory foramen near posterior palatal foramen (present)		apostFpalat
50	Angle of the median palatine suture		Palat +
51	Mental foramen (double)	(Zima 1989)	Fmentald
52	Superior accessory mental foramen (present)	(Markowski and Markowska 1988)	supaFmental
53	Inferior accessory mental foramen (present)	(Markowski and Markowska 1988)	inferaFmental
54	Posterior mental foramen (present)	(Zima 1989) , (Ress 1969)	postFmental
55	Accessory posterior mental foramen (present)	(Markowski and Markowska 1988)	apostFmental
56	Foramen mandible (double)	(Zima 1989)	Fmandd

3. Results

3.1. Homogeneity to variants'occurrence depending on sex and age

As stated before, the first phase of the study was aimed at broadening the knowledge of the variability of non-metrical traits according to test which factor affected them. To do this, the X^2 test at $p< 0.05$ was applied to check the dependence of trait occurrence in sex and age-class.

Sex dependence variations were examined in Fallstein sample by the computation of Chi-square statistics. The results of this analysis and the incidence of the traits studied in the two sexes are presented in Table 3.1. Four traits were found to have a sex dependence including traits Nos. 6, 7, 34 and 53. So it would be slightly more than can be attributed to chance. The Fig. 3.1 shows the histogram of relative frequencies for these four traits in both male and female sexes separately.

As it is observable at the Fig 3.1 for sex dependence traits expect of trait No. 7, incidence of non-metric characters for female was more frequent than male. It implies on existence of a different pattern of ossification in some part of skulls for male and female sexes of roe deer in this study or it may result from a significant difference in sex dimorphism.

Age dependence variations were evaluated for the possible effect of age on the expression of the traits in Hakel sample by the computation of Chi-square statistics. The results for this analysis and the incidence of the 56 traits studied in individual age-class are reported in Table 3.2. Eighteen traits displayed an essential dependence on age. Traits number including 4, 6, 7, 9, 16, 27, 28, 30, 31, 32, 36, 42, 43, 44, 45, 49, 50 and 52. In general, the considered traits seem to be influenced by age.

In order to survey the variation of the age dependent traits during life career diagram 3.2 has been drawn by three age class. It was done due to omit the probable random variation and to bring into view the age effect on relative frequency of the influenced traits as it is possible. In this figure, the age class 1 is similar to before and the age class 2 is included age class 2 and 3 and the age class 3 consists on three age classes (4, 5 and 6).

Looking at the Fig. 3.2, one can find three general forms for the traits that their frequencies vary according to the animal's age. The first form that includes traits no. 4, 6, 32, 43 and 44; have lower occurrence at the beginning the life and then increase in mid-age and after that remain constant or decline. The second class of traits continuously increases during life period of animals (no. 16, 32, 36, 42, 45, 49, and 50). The third and last class of these traits, there is frequently when animals are younger and thereafter gradually decrease by aging animals (no. 7, 9, 27, 28, 30, and 52).

3.2. Correlation between variants

Correlation between variants was calculated for the entire material (786 individuals) on the basis of two-way tables. Taking advantage of the fact that the product of the square correlation coefficient and abundance coefficient values had a X^2 distribution at one degree of freedom, the Pearson coefficient values at $p < 0.05$ were calculated.

Of 1540 calculated correlation coefficients between the variants only 140 (9.1%) were significantly different from zero at $p < 0.05$. But only 4 out of 140 (2.86% and 0.26% out of all) had a correlation value, equal or more than 0.3. It was for correlation value 0.2 or more as 8 out of 140 (5.72% and 0.52% out of all). And finally there was 54 correlation ≥ 0.1 (38.57% and 3.51% out of all). Regarding to significant correlations, 41 of the correlations were negative and 99 were positive, what seems to be at random. Most of the correlations ranged from -0.07 to 0.18 with mean 0.0583, but two of the correlations with high value were highly significant ($p<0.001$). These were between traits Nos. 33 and 34 ($r= 0.547$) in the nasal bone and between Nos. 48 and 49 ($r = 0.506$) in the palatal bone. The mean absolute values of negative correlation coefficients and their highest absolute value were 0.097 and 0.193 respectively. Those for positive correlations were 0.1223 and 0.547.

3.3. Selection of traits evaluated

Traits for final evaluation were selected on the basis of the dependence of their incidence on age and sex and correlation between traits. Consequently, the evaluation of epigenetic characteristics and mean measures of divergence (MMD) and their standard deviations (SMMD) was performed on the basis of the incidence of the 34 traits displaying no dependence with age, sex and correlation between them.

Traits Nos. 4, 6, 7, 9, 16, 27, 28, 30, 31, 32, 34, 36, 42, 43, 44, 45, 49, 50, 52 and 53 were eliminated because they were dependence with sex and age at statistical significant level of $p < 0.05$. Considering a sufficient Pearson correlation value minimum 0.5 and statistical significant level $p<0.001$ for correlation two traits (34 and 49) should be discarded but these traits were automatically omitted before because of the dependence of trait occurrence in sex and age respectively.

Among the remaining 36 traits, two of the traits were additionally excluded as well, the one regarding the presence of an extra premolar (trait no. 40) and the other concerning the presence of the Ethmoid foramen (trait no.19). In the first case, the trait was only observed once, and in the second case, it occurs in all skull samples as pointed out by Sjøvold (1977:p70). In concerning to the existence an extra premolar (trait no. 40) which is a kind of polyodontia, it is notable that polyodontia as oligodontia (congenital tooth loss) are

hereditarily based, but the development of the character of the resultant morphological shape is influenced by environmental factors such as maternal influences in the process of embryogenesis. Quantitative changes in dentition can therefore be classified as epigenetic traits (Grahnén 1956, Grüneberg 1963, Berry 1968), and their incidence used to characterize the characteristics of the gene pool of populations and to assess their relations to each other (Zima 1988). Some examples of typical polyodontia in roe deer are the occurrence of upper canines (C) (Chaplin and Atkinson 1968, Meyer 1975, Borg 1985), or of the first premolar in the diastema of the lower jaw (P_1) (Virchow 1940, Wetzel and Rieck 1972, Bubenik and Wurtzinger 1967, Meyer 1985).

However, a number of investigators observed the low prevalence of different types of dental anomalies in roe deer. Wallroth (1941), who studied 850 mandibles and 230 hemimandibles of roe deer from Germany, found no case of extra incisiform teeth. The same applies to Kratochvil (1984), whose material comprised 1,140 mandibles of roe deer from Czechoslovakia, and to Markowski and Markowska (1990); who studied 432 skulls of Polish roe deer. In a sample of 2,603 roe deer skulls from Germany, Stubbe (1969) found one specimen (= 0.038%) with extra incisiform teeth. Meyer (1975), who studied approximately 6,000 roe deer mandibles from Germany, observed only one specimen (~ 0.017%) with an extra left third incisor or canine and Zima (1988) found 12 cases with extra incisiform teeth (= 0.074%) in a sample of 16,177 roe deer mandibles from Czechoslovakia (Kierdorf and Kierdorf 2002).

In order to reveal the effect of consideration of homogeneity between the samples (significant difference in frequency of traits between at least two samples or population) on results, MMD were computed two times, first with condition (sex and age dependency and correlation between traits only) and second (sufficient difference between pair populations in addition to earlier conditions).

However, of the 56 traits, those were excluded that did not show significant between-population variation. To do this, it was selected a conservative criterion: if a variant had a significant (at $p< 0.05$) difference in frequency between one or more pairs of populations, the trait was included in the analysis. This aspect agrees with what has been implemented previously (Sjøvold 1977, Hanski and Kuitunen 1986).Traits Nos. 7, 24, 36, 37, 43, 47, 48, 50 and 53 were showed significant between-population variation by the computation of Chi-square statistics at $p< 0.05$. The results for this analysis and the P-value for chi-square test between pairs of populations by the traits are reported in Table 3.3. Finally to calculate of MMD with condition (sex-age dependency, and correlation between traits and being areas

sufficient difference) only traits Nos. 24, 37, 47 and 48 remained in the analysis because they varied in some samples and displaying no dependence with age, sex and correlation between traits. The MMD and SMMD resulted by these both excluding strategies have been displayed in Table 3.6.

It is notable that further results were extracted only by the earlier condition (sex and age dependency and correlation between traits) with no regard to being difference between samples because of the aim of losing as little information as possible.

3.4. The results of the population divergence

The final remaining 34 non-metric characters contributed to the computation of MMD and SMMD for the different areas listed in Table 3.4. The frequencies of these variants in the three investigated populations are presented in Table 3.5. On the basis of these frequencies the MMD and their standard deviations were calculated and presented in Table 3.6. Differences between pairs of compared areas are statistically significant (MMD> 2SMMD). The measure of uniqueness (MU) was calculated for each sample as the sum of its epigenetic distance (MMD) from the other samples. According to the MU a dendrogram of epigenetic distance was constructed by the clustering of the MMD matrix. The cluster analysis was done by use of the UPGMA method.

However, all MMD amounts resulted by Roe deer are highly significant at $P < 0.001$ in three sample areas and the sample from Darss have the greatest and significant distances to all the other samples. Two main clusters of samples are obviously confirmed by the dendrogram of epigenetic distance in Fig. 3.3 The first one consists of two samples (Hakel and Fallstein) with low differentiation, contrary to a distinctly separated position of the sample Darss which from Baltic coast of Germany.

The highest value of MU was found in the Darss population, a lower one in that of Fallstein and the lowest one in that of Hakel (Table 3.7).

Table 3.1: Distribution frequency of the non-metric traits of both sexes in the sample Fallstein (D.F=1)

N	Traits	Female	Male	Total	X^2	P
1	Fiterhypd	281/632	78/170	44.76	0.022	0.881
2	aFiterhyp	409/632	122/170	66.21	0.364	0.546
3	FinterCon	480/632	126/170	75.56	0.022	0.881
4	FexCon	557/632	147/170	87.78	0.016	0.900
5	FexSupocci	570/632	145/170	89.15	0.137	0.712
6	FmedSupocci	106/316	12/85	29.43	7.918	0.005**
7	InfraFmedSupocci	5/219	30/85	11.51	29.00	0.000***
8	Fmast	628/632	170/170	99.50	0.002	0.964
9	Fmastd	2/438	2/148	0.68	0.443	0.506
10	Fmeatte	519/632	128/170	80.67	0.296	0.586
11	Fmeatted	97/632	20/170	14.59	0.474	0.491
12	Fpostgl	572/632	143/170	89.15	0.234	0.629
13	2FsutuPtem	632/632	168/170	99.75	0.007	0.934
14	2Fparietal	294/632	82/170	46.88	0.031	0.860
15	aaFfoval	457/632	114/170	71.20	0.198	0.656
16	paFfova	37/632	4/170	5.11	1.494	0.222
17	suaFfopt	614/632	165/170	97.13	0.000	0.995
18	infraaFfopt	370/632	80/170	56.11	1.249	0.264
19	Fethmo	632/632	170/170	100.00	0.000	1.000
20	InferFsuorbId	607/632	165/170	96.26	0.005	0.942
21	InferFsuorbII	465/632	118/170	72.69	0.121	0.728
22	suorbBirdg	156/632	38/170	24.19	0.115	0.734
23	anFzygo	488/631	140/170	78.40	0.158	0.691
24	aanFzygo	138/632	48/170	23.19	0.818	0.366
25	postFzygo	578/631	156/170	91.64	0.000	0.990
26	apostFzygo	500/631	122/170	77.65	0.370	0.543
27	intSutuFontal	176/628	61/170	29.70	0.966	0.326
28	inferFzygom	154/594	47/170	26.31	0.055	0.814
29	infraFlacrim	488/631	129/169	77.13	0.007	0.935
30	supFlacrima	347/632	105/170	56.36	0.403	0.525
31	Flacrimfus	9/632	0/170	1.12	1.424	0.233
32	Fnasal	23/627	4/165	3.41	0.254	0.614
33	Premax_nas	198/610	55/161	32.81	0.044	0.835
34	Nasprocess	384/598	71/163	59.79	3.959	0.047*
35	Finfraorbitd	576/632	147/170	90.15	0.123	0.726
36	aFinfraorbi	167/630	30/170	24.63	1.778	0.182
37	suaFinfraorbi	162/629	26/170	23.53	2.666	0.103
38	Fmaxabopm1	480/632	148/170	78.30	0.757	0.384
39	2Fmaxabopm2	243/596	79/170	42.04	0.372	0.542
40	PM1ex	0/632	0/170	0.00	-	-
41	Sutintmax	98/316	20/85	29.43	1.027	0.311
42	Fsutintmax	107/316	21/85	31.92	1.431	0.232
43	Fmax	1/632	0/170	0.12	0.158	0.691
44	Fmaxundpm1	208/632	41/170	31.05	1.356	0.244
45	Finforbipala	23/632	3/170	3.24	0.650	0.420
46	Fcaumpal	631/632	170/170	99.88	0.000	0.991
47	aFcaumpal	140/632	33/170	21.57	0.181	0.671
48	postFpalat	99/632	15/170	14.21	1.911	0.167
49	apostFpalat	31/632	2/170	4.11	2.286	0.131
50	Palat+	128/303	35/85	42.01	0.014	0.907
51	Fmentald	401/632	112/170	63.97	0.046	0.831
52	supaFmental	16/632	3/170	2.37	0.137	0.711
53	inferaFmental	253/632	41/170	36.66	3.948	0.047*
54	postFmental	424/632	87/170	63.72	2.141	0.143
55	apostFmental	8/632	1/170	1.12	0.248	0.619
56	Fmandd	170/629	58/170	28.54	0.822	0.365

In this table, *, ** and *** mean that the frequency is significantly greater than 5 % at $P < 0.05$, $P < 0.01$ and $P < 0.001$ respectively. Numerator: number of findings of a trait in each sex and denominator: total number of observable sides in each sex. D.F = 1 means one degree of freedom.

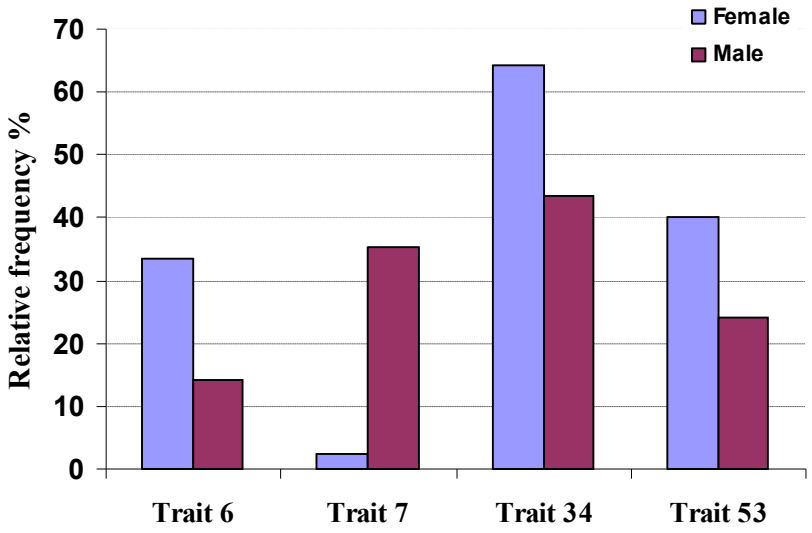

Fig. 3.1: The histogram of relative frequencies for sex-dependent traits in male and female sexes.

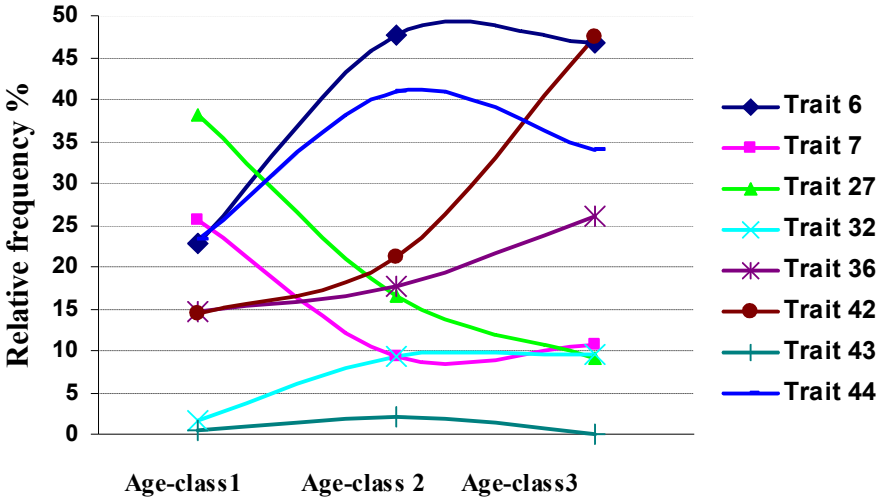

Fig. 3.2: The diagram of relative frequencies for age-dependent traits.

Table 3.2: Distribution frequency of the non-metric traits in individual age-class in the sample Hakel (D.F=5)

N	Traits	Age- class						Total	X^2	P
		1	2	3	4	5	6			
1	Fiterhypd	187/360	30/82	30/68	19/50	24/50	10/22	47.47	3.913	0.562
2	aFiterhyp	192/360	48/82	38/68	32/50	22/50	11/22	54.27	4.422	0.490
3	FinterCon	293/360	67/82	48/68	37/50	35/50	18/22	78.80	2.125	0.832
4	FexCon	303/360	73/82	59/68	45/50	31/50	13/22	82.91	12.641	0.027*
5	FexSupocci	328/360	74/82	67/68	47/50	50/50	22/22	93.04	1.032	0.960
6	FmedSupocci	41/180	21/41	15/34	14/25	12/25	4/11	33.86	16.615	0.005**
7	InfraFmedSupocci	46/180	4/41	3/34	5/25	3/25	0/11	19.30	31.840	0.000***
8	Fmast	358/360	82/82	68/68	50/50	50/50	22/22	99.68	0.003	1.000
9	Fmastd	11/360	0/82	2/68	0/50	3/50	0/22	2.53	15.004	0.010*
10	Fmeatte	282/360	69/82	52/68	37/50	34/50	14/22	77.22	3.660	0.599
11	Fmeatted	31/360	8/82	5/68	2/50	7/50	1/22	8.54	8.428	0.134
12	Fpostgl	281/360	66/82	55/68	39/50	36/50	16/22	78.01	0.943	0.967
13	2FsutuPtem	355/360	81/82	66/68	48/50	50/50	22/22	98.42	0.131	1.000
14	2Fparietal	148/360	40/82	35/68	27/50	29/50	14/22	46.36	5.687	0.338
15	aaFfoval	289/360	58/82	50/68	36/50	35/50	16/22	76.58	0.932	0.968
16	paFfova	5/360	1/82	2/68	1/50	2/50	2/22	2.06	12.705	0.026*
17	suaFfopt	357/360	81/82	68/68	50/50	50/50	22/22	99.37	0.015	1.000
18	infraaFfopt	210/360	39/82	34/68	23/50	27/50	14/22	54.91	4.314	0.505
19	Fethmo	360/360	82/82	68/68	50/50	50/50	22/22	100.00	0.000	1.000
20	InferFsuorbId	345/360	79/82	67/68	50/50	48/50	22/22	96.68	0.199	0.999
21	InferFsuorbII	257/360	65/82	48/68	31/50	40/50	15/22	72.15	3.250	0.661
22	suorbBirdg	74/360	10/82	12/68	9/50	9/50	7/22	19.15	10.856	0.054
23	anFzygo	254/360	53/82	46/68	35/50	36/50	17/22	69.78	1.290	0.936
24	aanFzygo	69/360	13/82	14/68	9/50	4/50	6/22	18.20	10.940	0.053
25	postFzygo	330/360	72/82	64/68	46/50	43/50	22/22	91.30	1.329	0.932
26	apostFzygo	280/360	63/82	48/68	41/50	41/50	18/22	77.69	1.292	0.936
27	intSutuFontal	137/360	14/82	11/68	4/50	5/50	2/22	27.37	38.685	0.000***
28	inferFzygom	69/360	21/82	6/68	6/50	7/50	2/22	17.56	14.390	0.013*
29	infraFlacrim	219/359	64/82	46/68	33/50	36/50	18/22	65.93	4.275	0.511
30	supFlacrima	210/360	55/82	33/68	18/50	31/50	15/22	57.28	13.503	0.019*
31	Flacrimfus	0/360	0/82	0/68	0/50	0/50	0/22	0.00	-	-
32	Fnasal	6/349	4/82	9/66	8/50	4/49	1/22	5.18	19.220	0.002**
33	Premax_nas	115/330	26/80	18/66	18/50	25/49	10/22	35.51	10.151	0.071
34	Nasprocess	211/328	51/80	40/66	28/50	29/47	12/22	62.56	1.358	0.929
35	Finfraorbitd	319/359	73/82	54/68	46/50	43/50	20/22	87.96	1.180	0.947
36	aFinfraorbi	53/360	11/82	15/68	5/50	25/50	4/22	17.88	49.872	0.000***
37	suaFinfraorbi	71/359	16/82	8/68	7/50	13/50	6/22	19.18	9.763	0.082
38	Fmaxabopm1	281/359	62/82	46/68	35/50	31/50	21/22	75.44	9.052	0.107
39	2Fmaxabopm2	158/359	28/82	23/68	22/50	17/50	10/22	40.89	4.250	0.514
40	PM1ex	1/360	0/82	0/68	0/50	0/50	0/22	0.16	1.389	0.926
41	sutintmax	60/180	13/41	11/34	8/25	6/25	4/11	32.28	2.662	0.752
42	Fsutintmax	26/180	9/41	7/34	10/25	12/25	6/11	22.15	40.844	0.000***
43	Fmax	2/360	0/82	3/68	0/50	0/50	0/22	0.79	18.916	0.002**
44	Fmaxundpm1	84/360	43/82	20/68	21/50	14/50	7/22	29.91	16.756	0.005**
45	Finforbipala	10/360	2/82	2/68	4/50	1/50	0/22	3.01	11.685	0.039*
46	Fcaumpal	359/360	82/82	68/68	50/50	50/50	22/22	99.84	0.001	1.000
47	aFcaumpal	88/360	18/82	12/68	12/50	9/50	3/22	22.47	4.491	0.481
48	postFpalat	83/359	15/82	16/68	14/50	7/49	4/22	22.06	5.750	0.331
49	apostFpalat	21/359	4/82	3/68	4/50	0/50	3/22	5.55	16.644	0.005**
50	Palat+	26/178	6/41	12/34	7/25	10/25	4/11	20.70	22.204	0.000***
51	Fmentald	206/359	59/82	37/66	31/50	28/50	14/22	59.62	3.109	0.683
52	supaFmental	7/359	3/82	0/66	0/50	2/50	0/22	1.91	11.115	0.049*
53	inferaFmental	150/359	29/82	22/66	18/50	16/50	7/22	38.47	1.969	0.853
54	postFmental	281/360	70/82	54/66	41/50	36/50	17/22	79.21	1.376	0.927
55	apostFmental	5/360	2/82	1/66	0/50	1/50	0/22	1.43	4.238	0.516
56	Fmandd	103/359	17/82	15/66	10/50	8/50	6/22	25.28	4.996	0.416

In this table, *, ** and *** mean that the frequency is significantly greater than 5 % at $P < 0.05$, $P < 0.01$ and $P < 0.001$ respectively. Numerator: number of findings of a trait in each age-class and denominator: total number of observable sides in each age-class. D.F = 5 means five degree of freedom.

Table 3.3: The P-value for chi-square test between pairs of populations by the traits (D.F=1).

N	Traits	Pairs P-values		
		D - F	D - H	F - H
1	Fiterhypd	0.842	0.934	0.778
2	aFiterhyp	0.091	0.545	0.277
3	FinterCon	0.513	0.693	0.794
4	FexCon	0.806	0.899	0.709
5	FexSupocci	0.598	0.811	0.773
6	FmedSupocci	0.216	0.496	0.577
7	InfraFmedSupocci	0.325	0.019*	0.161
8	Fmast	0.972	0.982	0.990
9	Fmastd	0.409	0.112	0.302
10	Fmeatte	0.449	0.302	0.783
11	Fmeatted	0.292	0.836	0.209
12	Fpostgl	0.611	0.724	0.389
13	2FsutuPtem	0.986	0.911	0.925
14	2Fparietal	0.279	0.256	0.957
15	aaFfoval	0.525	0.847	0.658
16	paFfova	0.804	0.365	0.254
17	suaFfopt	0.960	0.913	0.873
18	infraaFfopt	0.097	0.122	0.909
19	Fethmo	1.000	1.000	1.000
20	InferFsuorbId	0.829	0.853	0.976
21	InferFsuorbII	0.222	0.239	0.964
22	suorbBirdg	0.292	0.772	0.444
23	anFzygo	0.468	0.986	0.479
24	aanFzygo	0.002**	0.019*	0.437
25	postFzygo	0.855	0.836	0.980
26	apostFzygo	0.971	0.974	0.998
27	intSutuFontal	0.850	0.905	0.758
28	inferFzygom	0.792	0.114	0.187
29	infraFlacrim	0.624	0.154	0.349
30	supFlacrima	0.403	0.357	0.931
31	Flacrimfus	0.289	-	0.289
32	Fnasal	0.617	0.277	0.546
33	Premax_nas	0.666	0.449	0.744
34	Nasprocess	0.124	0.074	0.802
35	Finfraorbitd	0.765	0.643	0.869
36	aFinfraorbi	0.002**	0.034*	0.301
37	suaFinfraorbi	0.002**	0.013*	0.505
38	Fmaxabopm1	0.951	0.865	0.817
39	2Fmaxabopm2	0.757	0.663	0.900
40	PM1ex	-	0.691	0.691
41	Sutintmax	0.896	0.816	0.717
42	Fsutintmax	0.240	0.878	0.184
43	Fmax	0.046*	0.117	0.486
44	Fmaxundpm1	0.228	0.177	0.884
45	Finforbipala	0.680	0.613	0.925
46	Fcaumpal	0.993	0.991	0.998
47	aFcaumpal	0.060	0.045*	0.892
48	postFpalat	0.023*	0.001**	0.193
49	apostFpalat	0.263	0.124	0.645
50	Palat+	0.001**	0.456	0.007**
51	Fmentald	0.279	0.141	0.696
52	supaFmental	0.927	0.895	0.823
53	inferaFmental	0.036*	0.059	0.834
54	postFmental	0.182	0.968	0.195
55	apostFmental	0.112	0.153	0.848
56	Fmandd	0.350	0.623	0.657

In this table, *, ** and *** mean that the frequency is significantly greater than 5 % at $P < 0.05$, $P < 0.01$ and $P < 0.001$ respectively. D= population of Darss area, F= population of Fallstein area and H= population of Hakel area. D.F = 1 means one degree of freedom.

Table 3.4: The list of selected traits for calculation of MMD.

N	Traits number	The non-metric traits (character names)	Acronym
1	1	Internal hypoglassi foramen (double)	Fiterhypd
2	2	Accessory internal hypoglassi foramen (present)	aFiterhyp
3	3	Internal condylar foramen (present)	FinterCon
4	5	External supraoccipital foramen (present)	FexSupocci
5	8	Mastoid foramen (present)	Fmast
6	10	Meatus temporale foramen (present)	Fmeatte
7	11	Meatus temporale foramen (double)	Fmeatted
8	12	Postglenoid (supraglenoid) foramen (present)	Fpostgl
9	13	Two and more foramina in sutura parietemporalis (present)	2FsutuPtem
10	14	Two and more foramina in parietal bone (present)	2Fparietal
11	15	Anterior accessory foramen near foramen oval (present)	aaFfoval
12	17	Supra accessory foramen near foramen optic (present)	suaFfopt
13	18	Infra accessory foramen near foramen optic (present)	infraaFfopt
14	20	Supraorbital inferior I foramen (double)	InferFsuorbId
15	21	Supraorbital inferior II foramen (present)	InferFsuorbII
16	22	Supraorbital bridge (present)	suorbBirdg
17	23	Zygomatic anterior foramen (present)	anFzygo
18	24	Accessory zygomatic anterior foramen (present)	aanFzygo
19	25	Zygomatic posterior foramen (present)	postFzygo
20	26	Accessory zygomatic posterior foramen (present)	apostFzygo
21	29	Infra lacrimal foramen (present)	infraFlacrim
22	33	Prermaxilla bone connected with nasal bone	Premax_nas
23	35	Infraorbital foramen (double)	Finfraorbitd
24	37	Supra accessory infraorbital foramen (present)	suaFinfraorbi
25	38	Foramen maxilla above PM1 (present)	Fmaxabopm1
26	39	Two foramina maxilla above PM2 (present)	2Fmaxabopm2
27	41	Sutura intermaxilla serrated	sutintmax
28	46	Caudal major palatine foramen (present)	Fcaumpal
29	47	Accessory caudal major palatine foramen (present)	aFcaumpal
30	48	Posterior palatal foramen (present)	postFpalat
31	51	Mental foramen (double)	Fmentald
32	54	Posterior mental foramen (present)	postFmental
33	55	Accessory posterior mental foramen (present)	apostFmental
34	56	Foramen mandible (double)	Fmandd

Table 3.5: Percentage frequencies of non-metric traits (%) in three samples.

N	Traits	Frequency		
		Darss	**Fallstein**	**Hakel**
1	Fiterhypd	46.63	44.76	47.47
2	aFiterhyp	48.18	66.21	54.27
3	FinterCon	83.82	75.56	78.80
5	FexSupocci	96.32	89.15	93.04
8	Fmast	100.00	99.50	99.68
10	Fmeatte	90.58	80.67	77.22
11	Fmeatted	9.42	14.59	8.54
12	Fpostgl	82.48	89.15	78.01
13	2FsutuPtem	100.00	99.75	98.42
14	2Fparietal	57.97	46.88	46.36
15	aaFfoval	78.99	71.20	76.58
17	suaFfopt	97.83	97.13	99.37
18	infraaFfopt	39.86	56.11	54.91
20	InferFsuorbId	99.28	96.26	96.68
21	InferFsuorbII	58.70	72.69	72.15
22	suorbBirdg	17.39	24.19	19.15
23	anFzygo	69.57	78.40	69.78
24	aanFzygo	6.52	23.19	18.20
25	postFzygo	94.09	91.63	91.30
26	apostFzygo	78.12	77.65	77.69
29	infraFlacrim	83.33	77.12	65.93
33	Premax_nas	29.41	32.82	35.51
35	Finfraorbitd	94.20	90.15	87.95
37	suaFinfraorbi	6.52	23.53	19.18
38	Fmaxabopm1	77.54	78.30	75.44
39	2Fmaxabopm2	44.93	42.04	40.89
41	Sutintmax	30.43	29.43	32.28
46	Fcaumpal	100.00	99.88	99.84
47	aFcaumpal	10.87	21.57	22.47
48	postFpalat	4.41	14.21	22.07
51	Fmentald	76.81	63.97	59.62
54	postFmental	79.71	63.72	79.21
55	apostFmental	5.07	1.12	1.43
56	Fmandd	21.84	28.52	25.27

Table 3.6: Mean measures of divergence (MMD) and its standard division (SMMD) of the Roe deer samples from three regions of Germany.

Areas	Number of traits	Comment	MMD	SMMD
F_H	4	Sex, age and Area	0.01441	0.00200
D_H	4	Sex, age and Area	0.16432	0.00626
D_F	4	Sex, age and Area	0.16496	0.00603
F_H	34	Sex and age	0.01572	0.00072
D_H	34	Sex and age	0.04131	0.00224
D_F	34	Sex and age	0.04671	0.00216

In this table: D = population of Darss area, F = population of Fallstein area and H = population of Hakel area. Sex, age and Area = MMD with condition (sex-age dependency, and correlation between traits plus being areas sufficient difference), Sex and age = MMD with condition (sex and age dependency and correlation between traits) without being areas sufficient difference. All MMDs are highly significant at $P < 0.001$.

Table 3.7: MMD matrix and its standard deviation (SMMD) between samples and MU value as sum and mean[1].

Area	Fallstein	Hakel	MU Sum	MU Mean
Darss	0.04671 *0.00216*	0.04131 *0.00224*	0.08802	0.0440
Fallstein	-	0.01572 *0.00072*	0.06243	0.0312
Hakel	-	-	0.05703	0.0285

1- In this table SMMD are given below in italics digits.

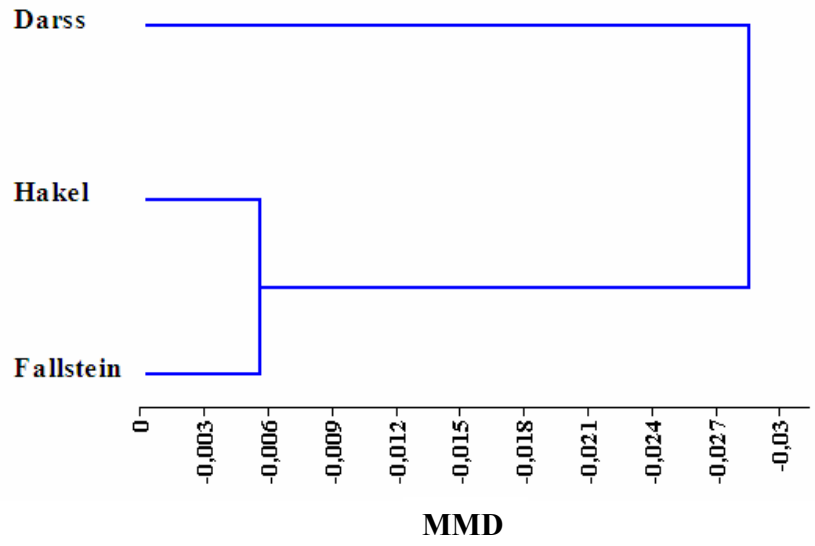

Fig. 3.3: Dendrogram of epigenetic distance (MMD) of Roe deer populations from Germany.

4. Discussion and conclusion

Morphological variation in animal populations partly reflects the historical background of the population, in which, e.g., different spreading routes or the degree of isolation are influential (Berry 1964, Berry et al. 1978, Davis 1983). Morphology is therefore (more or less) closely connected with ecology. The study of morphological variation can thus give insight into the factors that are essential in the ecology of the species studied, and at its best also into general evolutionary mechanisms (Pankakoski and Nurmi 1986).

A major problem in the study of morphological variation is the difficulty of separating the effects of the environment and heredity as the causes of variation (Gould and Johnston 1972, Atchley et al. 1981). In studies based on samples from natural populations the genetic structure of the populations can evidently be better characterized by epigenetic features such as nerve foramina, bone joints or molar patterns, than by continuous variables (Berry 1963, Rees 1969, Berry et al. 1978, Hartman 1980, Andersen and Wiig 1982).

As the morphological variables depend on age and sex, the effect of these characters should be eliminated in studies of geographical variation (Pankakoski and Nurmi 1986).

The European roe deer (*Capreolus capreolus*) has been selected as the object of this study as it is the most numerous free-living ungulate and is one of the most important species of game animals in Germany. It shows wide ecological tolerance, and its populations occur continuously almost all over the territory and are stable at high levels. Thus the species meets the conditions under which the genetic difference between populations can be function of geographic distance.

The general aim of this dissertation is, with the aid of epigenetic methods, to examine the genetic contributions and investigate the relations between populations of roe deer from three wildlife research areas of Germany (Hakel, Fallstein and Darss). The minor aim of the present research is to study geographical, as well as sex- and age-dependent, variation in this species based on epigenetic features of the skull.

The information available in the literature on relationships between non-metric traits, sex and age is debatable. Sex difference in traits incidences seems to occur in connection with various traits, the number and pattern seeming to vary from one study to other (Sjøvold 1977).

The implemented research on many skeletons of *Mus musculus,* both from wild populations and laboratory strains revealed a correlation with sex in some non-metric traits (Berry and Jakobson 1975). The same results of dependences between non-metrical traits and sex was

found by Sjøvold (1977) in red fox, Sikorski (1982) in striped field mouse, Wiig and Lie (1984) in hooded seal, Wiig and Andersen (1988) in lynx. It is assumed that these correlations result from the occurrence of dimorphic differences, which is also supported by the existence of connections between some of these traits and body size (Sjøvold 1977, Wiig and Andersen 1988). By contrast, other studies did not show a disparity between the sexes. For example, Wiig and Lie (1979) did not find any correlation of non-metric traits with sex in the wild mink *Mustela vison* and also Uhlikva (2004) in the common vole *Microtus arvalis*.

In the non-metric study of roe deer, Gromov and Skulkin (1986) found no differences in any of the 15 characters studied as regards the incidence between males and females and also Zima (1989) in the 32 non-metric traits.

This result was also confirmed by the ones of study of the roe deer by Markowski and Markowska (1988). They pointed out that "lack of correlations between non-metrical variants and sex in the roe deer of the this study may result from small differences in sex dimorphism which were recorded in this species with respect to body size (Fruzinski et al. 1982) as well as to skull size".

In present study, four traits were found to have a sex dependence including traits Nos. 6, 7, 34 and 53 and the percentage of traits related to sex in the roe deer sample investigated was 7.14% of all traits. The trait No. 6 (medial supraoccipital foramen) reported by Markowski and Markowska (1988), trait No. 7 (infra medial supraoccipital foramen), trait No. 34 (Nasal bone protruding from the distal line of maxilla bone) and trait No. 53 (Inferior accessory mental foramen), have shown statistically significant difference ($p<0.05$) between two sexes.

The results, in general, were in contrast with ones from others (Markowski and Markowska 1988, Zima 1989) which found no statistically difference for varying the non-metric characters between both sexes in roe deer. Although, it can be for using larger sample size (401 skulls from Fallstein area) to do chi-square test but however, there are also other causes to justify. However it is not possible at present to assume a definitive position on the influence of sex on the expression of non-metric traits.

In fact, there are significant differences both in these results and in those reported by other authors, but the traits involved vary according to the population studied. It should be remembered that the results obtained from various samples of a population can be different, as also emphasised by Corruccini (1974) and Sjøvold (1984).

However, since there is not a clear indication of the role played by sex, so it is preferable, where possible, to consider in future studies on non-metric traits and biological distance.

The dependence of the incidence of non-metrical cranial characters on age is apparently generally low, and ten traits displayed an essential dependence on age. Traits number including 4 (external condylar foramen), 6 (medial supraoccipital foramen), 7 (infra medial supraoccipital foramen), 9 (mastoid foramen), 16 (posterior accessory foramen near foramen oval), 27 (intersutura fontanele between lacrimal and zygomatic), 28 (inferior zygomatic foramen), 30 (supra lacrimal foramen), 31 (lacrimal foramen fused), 32 (foramen penetrating nasal bone), 36 (accessory infraorbital foramen), 42 (foramina inside sutura intermaxilla), 43 (foramen maxilla), 44 (foramen by PM1 on maxilla), 45 (foramen infraorbitopalatine), 49 (accessory foramen near posterior palatal foramen), 50 (angle of the median palatine suture) and 52 (superior accessory mental foramen). The percentages around 30% of the all traits were age dependent in the roe deer sample from Hakel area.

A prominent study by Ossenberg (1969) using non-metric traits found that the presence of non-metric traits differed considerably by age. However, the differences encountered varied mostly in sub-adult individuals and were due to developmental changes.

Age dependence of traits can be explained by changes in the skeletal structure during postnatal development, which is strongly influenced by both genetic effects and environmental factors (Berry 1968, Pucciarelli 1974). Many foramina appear together with a progressive development of the faciocranium, especially in nasal, maxillary or dental bones (Markowski 1995). This is supported by the results of Wiig and Andersen (1988), who found altogether 13% of the traits analyzed in *Lynx lynx* correlated with age, but this value decreased to 5.6% when only individuals older than 18 months were considered. Similar results were obtained in the study on populations of *Capreolus capreolus* by Markowski and Markowska (1988) where 3.6% of the traits were age dependent in the total material, but none of these correlations were found in animals older than two years. In the other study of roe deer reported by Zima (1989) 21.8% studied traits have shown the age dependence. Since, in this study the animals sampled were adults, with a minimum age of 12 months and was not carried out no comparison between them and adulated animals, so it is not possible have such as finding for the present study.

Although, non-metric traits should behave in same manner, but it seems that ossifications process in some of these traits is different from others. In other words, the lack of age dependence in adult is perhaps to be expected if the traits are considered to be in-built characteristics of the skeletal system of the individual. However, some trait may be very late in appearing (Berry 1975) although, the opposite status is also possible to be. This aspect agrees with what has been observed previously (Korey 1970) and confirms the opinion that there is correlation between age and some traits if prepubertal material is used.

Therefore, age should be taken into acount when dealing with non-metric traits as some authors confirmed (Brothwell 1981, Buikstra 1972, Saunders 1989).

In this study, correlations between traits have been calculated to remove them from biological distance analyses which use statistics, such as Smith's Mean Measure of Divergence (MMD) because this statistics can only accommodate uncorrelated traits (Cheverud and Buikstra 1981). That means; the use of non-metrical variants in population studies necessitates the lack of correlations between them (Sjøvold 1977).

Quantitative genetic theory states that the correlation between any two traits is due to both genetic and environmental correlations (Falconer 1960). Since the correlations due to both genetic and environment are the sum of positive and negative effects, a correlation between traits may equal zero, even though there are common genes and environmental factors which effect the development of both simultaneously. For this reason it seems best to use more than one population when studying inter-trait correlations (Cheverud et al. 1979), as was accomplished here with all three samples (786 skulls).

Relevant to measuring correlations among traits, sample size has some important role in the detection and treatment of correlated traits (Conner 1990, Konigsberg 1990, Sciulli 1990). In this case, previous studies (Truslove 1961, Berry and Berry 1967, Kellock and Parsons 1970, Berry 1976, Sjøvold 1977, Sikorski 1982, Prowse and Lovell 1995) have shown that the correlation between traits in the various studies has rarely reached statistical significance in small samples contrary to large samples which show higher incidence significant correlations (Molto 1985). To answer the question that emerges as to a satisfactory minimum sample size for this type of analysis. Molto (1985) recommend 300 skulls as a baseline sample size.

As a result, in roe deer the number of correlations between the variants of non-metrical traits was low and did not exceed 9.1% (at $p<0.05$). Consistent with previous studies, the results reported here indicate that correlation among non-metric cranial traits is low, but significant as authors reported (Berry and Berry 1967, Corruccini 1974, Suchey 1975, Sjøvold 1977, Sikorski 1982, Sikorski and Bernshtein 1984, Molto 1985, Pankakoski and Nurmi, 1986, Wiig and Andersen 1988, Markowski and Markowska 1988). However, the higher incidence of significant positive correlations in this study probably reflects the larger sample size used.

These correlation were between traits Nos. 33 and 34 ($r= 0.547$) in the nasal bone and between Nos. 48 and 49 ($r = 0.506$) in the palatal bone. This implies the existence of a stronger correlation among adjacent bones than non-adjacent bones.

In this regard, a number of authors have stressed the occurrence of significant correlations among traits belonging to the same topographic and topological group on the skull (Cheverud and Buikstra 1981, Rössing 1982, Molto 1985, Česnys 1988).

It seems in a large sample as here, inter-correlations between non-metric traits, while low seem significant enough to consider in biological distance calculations.

Recently, interest in evaluating biological distance of mammal populations using the qualitative morphological skull characters has increased because of several advantages. They are of different biological relevance than dimensions of the skull. These non-metrical traits, seems to be slightly influenced by environmental factors. Therefore, some aspects of qualitative variants depend on genetic relationship. The degree of similarity of several recent populations of a species helps to explore the course of historic immigration and related issues (Ansorge 2001). It is assumed that the range of variability of non-metrical characters reflects the general genetic variability in the samples studied (Berry 1979, Smith 1981).

Non-metric variation is referred to as epigenetic variation, so the biological distance based on difference in incidences of non-metric traits will be referred to as epigenetic distance (Ress 1969). Hence the morphological differentiation by non-metric characters gives a certain measure of the epigenetic populations distance.

The results obtained in this investigation as the divergence estimate indicated that the major epigenetic split of German roe deer populations is between two groups representing the central and the northern part of the area studied. The highest epigenetic uniqueness and, at the same time, the highest value of epigenetic variability was exhibited by Darss the population northern part of the area studied from Baltic Sea.

Despite being two main clusters among samples (Fig 3.3), the populations from the territory studied in Germany can be considered epigenetically relatively homogeneous and uniform, especially between Hakel and Fallstein samples because of small achieved MMD values and consequently it shows existence of low genetic differentiation among them.

Concerning the divergence grouping (Fig 3.3) and the geographic situation as can be seen in the map (Fig 1.3), there is a cluster of two adjacent territories such as Hakel and Fallstein. These regions show just low epigenetic distances. This can be explained as being due to the fact that it is not probable the occurrence any influence of genetic variability, for instance, as a result of inbreeding or genetic drift. This would support, thus the fact that gene pool stabilization has taken place there. Therefore the roe deer of these areas do not belong to separate populations and live in reproductive connection as a logical result of small distance between them about 40-45 km.

However, from what has been reported in the literatures as Markowski and Markowska (1988) and Zima (1989), there is no correlation between genetic differentiations and geographical distance of close populations. In other words, the degree of difference between studied populations does not depend on their distance. In study carried out by Markowski and Markowska (1988) the two closely situated populations (about 50 km apart) differed more from each other than the populations with distance about 215 km.

However, it is difficult to find an unambiguous explanation for this correlation. Thus, considering results from above research and ones from literatures, it seems probable that the other factors have played a major role in shaping the divergence of roe deer populations.

The expected results were established by comparing morphological skull differentiation between Darss and the inland populations (Hakel and Fallstein). There is a high degree of divergence segregating the roe deer of the coast (Darss population) from all the others. The large epigenetic distances point to a lower reproductive contact. A possible cause for this differentation can be the finding stated by Zachos et al. (2007). They reported overhunting the complete North-German roe deer population that leaded to a bottleneck (an evolutionary event that drastically reduces a population) in the middle of the nineteenth century, but there have been numerous introductions since.

Similar morphological differences were found by Ansorge and Stubbe (1995) between the populations of otters *Lutra lutra*. The epigenetic divergence between East German regions was low, except the otters from the Baltic coast, which differ significantly from other German otter populations. They pointed out it could be assumed that these coastal otters belong to a separate population, caused perhaps by a different migration line along the coast. However, assessment of non-metric characters shows clearly that there is neither a general genetic isolation nor any indication of population splitting.

Something more positive can be said about the present study gives a good view of the differentiation of roe deer populations in Germany by their non-metric skull characters. It should be also mentioned that there is not a general genetic insulation among the centers populations. They seem to have reproductive contact during life period of those populations, perhaps inconsistent with the roe deer from the Baltic coast.

The achieved interpopulation differences that are resulted by dynamic ecological processes modifying the gene pool in each of three populations show the adaptability of this species to changing environmental circumstances.

It is clear that further analysis would be desirable to confirm the pattern revealed. In

particular, molecular markers would be valuable to elucidate genetic variation and history of these populations. Further work on both the ecology and morphology of the populations is needed and can help to understand the dynamics of semi-isolated populations like roe deer from the Baltic coast in Darss.

5. Summary

5.1. Introduction

The skull is the most complex bone structure in the body and is highly variable in shape, reflecting variation in genetic origin. Thus, cranial morphometrics is a useful tool for examining genetic variation at higher orders of organization as population, subspecies and species (Kuhn and Zeller 1987). Recently a true renaissance in use of morphology has arisen, due to the increasing application of non-metric skeletal characters for population genetics. Non-metric characters have become highly attractive as a relatively simple morphological tool (Rahmel and Ruf 1994, Pertoldi et al. 2000), even to non-morphologists, because of the rapid and apparently reliable outcome in applied research (Ansorge 2001).

The use of non-metrical variants as genetical markers in mammalian population studies is a well established technique (Berry 1969a 1969b, Berry and Warwick 1974, Sjøvold 1977, Berry et al. 1978, Andersen and Wiig 1982, Wiig and Lie 1984, Pankakoski and Nurmi 1986, Berry 1986). An important use of non-metric variants is based on their occurrence in separate samples of individuals or populations. They have been widely used for analyzing diversity within and among populations and species (Markowski 1995). The analysis of a large number of characters makes it possible to determine the epigenetic population variation and thus, the epigenetic divergence between populations (Sjøvold 1977).

High variability in the frequency of trait expression between populations is considered to imply a large degree of epigenetic divergence. To express the degree of separation, Sjøvold (1977) further developed the theoretical foundation of the C.A.B. Smith's mean measure of divergence (MMD) derived from the Mahalanobis-distances. This parameter is widely applied and preferred to any other measures of divergence (Ansorge 2001).

Among the assumptions behind the use of non-metrical variants in population studies are that they are uncorrelated, independent of sex and age and that the correlation between sides in bilateral variants is negligible. These assumptions have, however, been proven to fail for particular traits (Sjøvold 1977). Therefore, it is to be necessary, the traits should be tested for age and sex depended (Buikstra 1972, Corruccini 1974, Garn et al. 1966, Konigsberg 1987, Scott 1977).

The European roe deer (*Capreolus capreolus*) skulls were collected in the wildlife research areas: Darss, Hakel and Fallstein in Germany. This material was lies basis for a lot of scientific studies and also the detailed research in this academic qualification.

Darss (also Darß) is originally a part of a peninsula in the South of Baltic Sea in the German land of Mecklenburg-Western Pomerania in the district Ribnitz- Damgarten (54°26 ′N, 12°44′E). Hakel, with a size of 1303 ha, is situated in the north-eastern foreland of Harz Mountains in central Germany, about 35 km south-west of Magdeburg (Saxony-Anhalt) (Toepfer and Stubbe 2001). The coordination is 51° 53′ 3″N, 11° 19′ 54″E. Fallstein with a size of about 1500 ha is situated in the northern foreland of Harz Mountains in central Germany (Saxony-Anhalt). This area located about 40-45 km from Hakel (M.Stubbe pers. comm). Its coordination is 52° 0′ 41″ N, 10° 44′ 9″ E (Hentschel et. al 1983).

To do this, European roe deer has been selected as the object of this study due to be free-living endemic ungulate and one of the most important species of game animals in Germany. The species satisfies the conditions under which the genetic difference between populations can be function of geographic distance. The general aim of this dissertation is, with the aid of epigenetic methods, to examine the genetic contributions and investigate the relations between populations of roe deer from three wildlife research areas of Germany (Hakel, Fallstein and Darss) and to determine the extent of epigenetic variability in various samples on the basis of analysis of non-metric traits.

5.2. Material and methods

The material consisted of 786 (494♀, 292♂) complete roe deer skulls (crania and mandibles) which were collected in during the period 1957-1987 with various age-class and sex. The skull collections of three wildlife research areas in Germany were from Hakel, Fallstein and Darss including 316 (40.20%), 401 (51.02%) and 69 (8.78%) skull samples respectively. Age of samples was determined at the collecting time; consequently their age were known and aged from 1 to 13.5 year old. The specimens were considered as a sample if they are more than one year of age.

To determine the morphological differentiation with regard to the epigenetic distance, 56 non-metrical traits were identified. Among them, 51 traits were bilateral and the remaining 5 unilateral. These traits that could be scored objectively, have been chosen according to the some investigation on roe deer and own preliminary studies. In this study 42 of the total 56 traits used were taken from earlier studies (Rees 1969, Markowski and Markowska 1988 and Zima 1989), but 14 were new to this work. Each trait was scored on the left and the right side of the skull, as present or absent. Frequencies of bilateral traits were separately as well as together calculated (i.e. the trait was considered as present if the trait was expressed at least on the one side) according to the total number of sides examined.

Prior to the calculation of the Mean Measure of Divergence (MMD), non-metric variants were examined for sex dependence (to exam the homogeneity of the distribution of traits between sexes) and for age dependence (to assess the relation between age of variability and the trait frequencies) by the chi-square test. Thus, traits that exhibited significant dependency with sex and age were eliminated.

The dependence of the occurrence of variants on sex was measured using sample from Fallstein, which was the largest sample evaluated containing both sexes.

The dependence of the incidence of variants on age was evaluated in a sample of specimens from Hakel. Age of specimens from Hakel, was divided into 6 aged classes as: age class 1 (13 to < 24 months), age-class 2 (2 to < 3 year old), age-class 3 (3 to <4), age-class 4 (4 to < 5), age-class 5-6 (5 to < 7), age-class ≥ 7 (≥ 7 year old).

The application of cumulating formulas for determining the divergence between samples required employing non correlated variants. Interdependence between traits or the degree of correlation between the variants was calculated by Pearson's correlation test based on a chi-square approach ($P < 0.05$) for the whole sample = 786 individuals.

It is notable that all computations and statistical tests were performed by Statistical Analysis System (SAS 2003) software and chi-square test were statistically significant if their error probability to signify were less than 0.05 ($p<0.05$).

For computing the epigenetic distances the formula of MMD proposed and derived by Sjøvold (1977). Variance and standard deviation (SMMD) of the MMD are necessary to prove statistic significance by MMD > 2 SMMD (Sikorski 1982). The following are the used formulas to calculate MMD and SMMD between two populations:

$$MMD = \frac{1}{r\sum(\theta_1 - \theta_2)^2 - V_{12}} \qquad SMMD = \sqrt{\frac{(2\sum V_{12}^2)}{r^2}}$$

The measure of uniqueness (MU) was calculated as the sum of its epigenetic distance (MMD) computed by Sjøvold (1977) formula:

$$MU_k = \sum_{j=1}^{u} MMD_{kj}$$

Based on the mean measure of divergence a dendrogram of epigenetic similarity was constructed by the clustering of the MMD matrix. The cluster analysis was done by use UPGMA method. The MMD and SMMD formulas applied in the present work were carried out by help of the computer program of The Staatliches Museum für Natutkunde Görlitz, Germany.

5.3. Results

Sex dependence variations were examined in Fallstein sample. Four traits were found to have a sex dependence including traits Nos. 6, 7, 34 and 53. So it would be slightly more than can be attributed to chance. Age dependence variations were evaluated for the possible effect of age on the expression of the traits in Hakel sample. Ten traits displayed an essential dependence on age. Traits number including 6, 7, 27, 32, 36, 42, 43, 44, 49 and 50. In general, the considered traits seem to be influenced by age.

Of 1540 calculated correlation coefficients between the variants only 140 (9.1%) were significantly different from zero at $p < 0.05$. But only 4 out of 140 (2.86% and 0.26% out of all) had a correlation value, equal or more than 0.3 and the two of the correlations with high value were highly significant ($p<0.001$). These were between traits Nos. 33 and 34 ($r= 0.547$) in the nasal bone and between Nos. 48 and 49 ($r = 0.506$) in the palatal bone.

The evaluation of epigenetic characteristics and mean measures of divergence (MMD) and their standard deviations (SMMD) was performed on the basis of the incidence of the 34 traits displaying no dependence with age, sex and correlation between them.

Traits Nos. 6, 7, 27, 32, 34, 36, 42, 43, 44, 49, 50 and 53 were eliminated because they were dependence with sex and age at statistical significant level of $p < 0.05$. Considering a sufficient Pearson correlation value minimum 0.5 and statistical significant level $p<0.001$ for correlation two traits (34 and 49) should be discarded but these traits were automatically omitted before because of the dependence of trait occurrence in sex and age respectively.

Among the remaining 36 traits, two of the traits were additionally excluded as well, the one regarding the presence of an extra premolar (trait no. 40) and the other concerning the presence of the Ethmoid foramen (trait no.19). In the first case, the trait was only observed once, and in the second case, it occurs in all skull samples as pointed out by Sjøvold (1977). The final remaining 34 non-metric characters contributed to the computation of MMD and SMMD for the different areas.

However, all MMD amounts resulted by Roe deer are highly significant at $P < 0.001$ in three sample areas and the sample from Darss have the greatest and significant distances to all the other samples (Table 3.7). Two main clusters of samples were achieved by the dendrogram of epigenetic distance (Fig. 3.3). The first one consists of two samples (Hakel and Fallstein) with low differentiation, contrary to a distinctly separated position of the sample Darss which from Baltic coast of Germany. The highest value of MU was found in the Darss population, a lower one in that of Fallstein and the lowest one in that of Hakel.

5.4. Discussion and conclusion

In present study, four traits were found to have a sex dependence including traits Nos. 6, 7, 34 and 53 and the percentage of traits related to sex in the roe deer sample investigated was 7.14% of all traits. The trait No. 6 (medial supraoccipital foramen) reported by Markowski and Markowska (1988) and the trait No. 7 (infra medial supraoccipital foramen) defined by own study, shown highly significant difference ($p<0.05$) between two sexes.

The results, in general, were in contrast with ones from others (Markowski and Markowska 1988, Zima 1989) which found no statistically difference for varying the non-metric characters between both sexes in roe deer. Although, it can be for using larger sample size (401 skulls from Fallstein area) to do chi-square test but however, there are also other causes to justify. However, since there is not a clear indication of the role played by sex, so it is preferable, where possible, to consider in future studies on non-metric traits and biological distance.

The dependence of the incidence of non-metrical cranial characters on age is apparently generally low, and ten traits displayed an essential dependence on age. Traits number including 6 (medial supraoccipital foramen), 7 (infra medial supraoccipital foramen), 27 (intersutura fontanele between lacrimal and zygomatic), 32 (foramen penetrating nasal bone), 36 (accessory infraorbital foramen), 42 (foramina inside sutura intermaxilla), 43 (foramen maxilla), 44 (foramen by PM1 on maxilla), 49 (accessory foramen near posterior palatal foramen) and 50 (angle of the median palatine suture). The percentages 17.86% of the all traits were age dependent in the roe deer sample from Hakel area.

Age dependence of traits can be explained by changes in the skeletal structure during postnatal development, which is strongly influenced by both genetic effects and environmental factors (Berry 1968, Pucciarelli 1974). Many foramina appear together with a progressive development of the faciocranium, especially in nasal, maxillary or dental bones (Markowski 1995). Although, non-metric traits should behave in same manner, but it seems that ossifications process in some of these traits is different from others. However, some trait may be very late in appearing (Berry 1975) although, the opposite status is also possible to be. Therefore, age should be taken into acount when dealing with non-metric traits as some authors confirmed (Brothwell 1981, Buikstra 1972, Saunders 1989).

These correlation were between traits Nos. 33 and 34 ($r= 0.547$) in the nasal bone and between Nos. 48 and 49 ($r = 0.506$) in the palatal bone. This implies the existence of a stronger correlation among adjacent bones than non-adjacent bones. In this regard, a number of authors have stressed the occurrence of significant correlations among traits belonging to

the same topographic and topological group on the skull (Cheverud and Buikstra 1981, Rössing 1982, Molto 1985, Česnys 1988).

Based on results of the cluster analysis using MMD matrix, two main clusters inclusing north sample (Darss) and central sample (Fallstein and Hakel) were achieved. Despite being two main clusters among samples, the populations from the territory studied in Germany can be considered epigenetically relatively homogeneous and uniform, especially between Hakel and Fallstein samples because of small achieved MMD values and consequently it shows existence of low genetic differentiation among them.

Concerning the divergence grouping and the geographic situation as can be seen in the map, there is a cluster of two adjacent territories such as Hakel and Fallstein. These regions show just low epigenetic distances. This can be explained as being due to the fact that it is not probable the occurrence any influence of genetic variability, for instance, as a result of inbreeding or genetic drift. However, it is difficult to find an unambiguous explanation for this correlation. Thus, considering results from above research and ones from literatures, it seems probable that the other factors have played a major role in shaping the divergence of roe deer populations.

The expected results were established by comparing morphological skull differentiation between Darss and the inland populations (Hakel and Fallstein). There is a high degree of divergence segregating the roe deer of the coast (Darss population) from all the others. The large epigenetic distances point to a lower reproductive contact.

The present study gives a good view of the differentiation of roe deer populations in Germany by their non-metric skull characters. It should be also mentioned that there is not a general genetic insulation among the centers populations. They seem to have reproductive contact during life period of those populations, perhaps inconsistent with the roe deer from the Baltic coast.

It is clear that further analysis would be desirable to confirm the pattern revealed. In particular, molecular markers would be valuable to elucidate genetic variation and history of these populations. Further work on both the ecology and morphology of the populations is needed and can help to understand the dynamics of semi-isolated populations like roe deer from the Baltic coast in Darss.

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Eidesstattliche Versicherung

Hiermit erkläre ich, Atefeh Rahbar, an Eides statt, dass die vorliegende Dissertation selbstständig und ohne fremde Hilfe verfasst wurde. Alle verwendeten Hilfsmittel und Quellen wurden als solche gekennzeichnet.

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