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A CRANIOMETRIC INVESTIGATION OF THE FIELD VOLE *MICROTUS AGRESTIS* IN DENMARK - POPULATION SUBSTRUCTURE

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ABSTRACT - A craniometric investigation using univariate and principal component analysis (PCA) was performed on 91 skull traits of 472 field voles, collected on nine islands and from the Jutland peninsula in Denmark in order to reveal possible geographical differences in skull shape and size.
Because of missing values in the skulls due to various damages, only 186 individuals were measured for the dorsal side of the skull, 174 individuals for the ventral side and 154 individuals for the mandible. Of these traits, from the dorsal side of the skull 28 traits were measured, from the ventral side 33 traits and from the mandible 30 traits. With few exceptions, differences in skull shape were found between samples collected from the different islands, and also between samples from islands and samples from the Jutland peninsula. It is therefore suggested that field voles have a genetic differentiation between island and island and between island and mainland at the loci determining the shape of the skull. The field voles from some islands and the eastern part of the Jutland peninsula had the largest skulls compared with field voles from other islands and other parts of the Jutland peninsula. The origin of size differences is discussed and attributed to be due to several environmental factors as geographic variation in habitat quality but also as a consequence of the island syndrome.

Key words: morphometric differentiation, Bergman's rule, principal component analysis, island rule, environmental variability

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INTRODUCTION

The understanding of the genesis of subpopulations is an important part of our understanding of evolution and speciation. The ever present dilemma for the conservationist is on one side to preserve as much genetic diversity as possible in order to preserve the evolutionary potential, and on the other side to protect populations with specific genetic adaptations to local conditions (Higgs and Usher 1980; Wilcove et al. 1986). The combination of small population size and isolation may lower the fitness of a population because of reduced genetic diversity from drift and inbreeding (Saccheri et al. 1998;

Bijlsma et al. 2000; Keller and Waller 2002). In view of this, identification of subpopulations is an important step in the work of conserving genetic diversity (Nunney and Campbell 1993; Franklin and Frankham 1998) and local adaptations (Moritz 1994; Crandall et al. 2000). Knowledge of the genetic structure of populations may therefore inform decision-making processes concerning conservation strategies like designing of reserves or reintroductions and translocations of individuals from larger populations to threatened or extinct populations.

The field vole, *Microtus agrestis*, is a common and abundant rodent in the Danish fauna (Lund 1991) and is reported on many of the Danish islands (Christiansen and Jensen 2007). For several raptors, owls, snakes and mammal predators in the Danish fauna the field vole is an important prey (Lund 1991; Jensen 1993). Despite its name, the field voles do not prefer fields with sparse ground cover, like heath or fields with intensive grazing (Jensen and Hansen 2003). Their prime habitat consists of continuous ground cover, so the field vole can tunnel and make nests (Hansson 1977). Forest glades and areas with permanent grass cover and a humid ground soil are often the areas where field voles are most abundant (Lund 1991). However, less suitable areas can be inhabited seasonally because of increased competition for food and competition for females in the primary habitats (Myllymäki 1977).

Geographical variation in body size of a species may be a result of different predation pressure, inter and intra-specific competition for food, sexual selection, environmental factors, etc. (Alcantara 1991; Adler and Levins 1994; Renaud and Michaux 2003; Xin 2003). Skull size is regarded as a more reliable measure for body size than body weight which fluctuates daily and seasonally (Ellison et al. 1993). Skull morphology consists of different inter-dependent traits (Klingenberg and Leamy 2001), that are subjected to selection (Yom-Tov et al. 1999). The number of loci coding for shape and size of the skull is unknown, but in general the shape of a morphological character involves more loci than the size of the character (Birdsall et al. 2000, Zimmerman et al. 2000; Klingenberg et al. 2001; Chase et al. 2002; Workman et al. 2002). Workman et al. (2002) found that 18 quantitative trait loci (QTL) are associated with the shape of the mandible in mice compared with 3 QTL coding for its size. Differences in the size of a character are, therefore, more likely to be a plastic response to environmental factors, like food quality and food availability (Yom-Tov et al. 2003; Ergon et al. 2004; Pertoldi et al. 2005). In spite of being subjected to selection, a morphological characteristic may be influenced by drift during periods with low population sizes, as the strength of drift is inversely related to the effective population size (Frankham et al. 2002). To summarize, skull morphology is an effective measure to indicate whether geographical groups are genetically differentiated and vary in shape (Lynch and Hayden, 1995; Simonsen et al. 2003) and/or live in different habitats and vary in size (Renaud 1999; Yom-Tov and Yom-Tov 2004). Several studies have used the shape of a characteristic to identify species (Kooij et al.

1997; Caumul and Polly 2005), subpopulations (Lynch et al. 1996; Renaud and Michaux 2003; Simonsen et al. 2003) or even confirm demographic bottlenecks (Pertoldi et al. 2005), and size differences of a character have yielded information on possible increased fragmentation (Schmidt and Jensen 2003, 2005) and/or changing quality of the habitat (Yom-Tov et al. 2003).

Field voles rarely disperse over distances of more than 350 meters, and only occasionally are barriers such as water ways crossed (Manniche 1935). It may therefore be questionable how genetically connected the field voles from different Danish islands and from the different regions of the Jutland peninsula are. In this study we try to reveal whether the field voles in Denmark are geographically subdivided, by investigating the shape and the size of the skull. Differences in the shape of the skull and the mandible will be used to indicate genetically different subpopulations, and the size to investigate environmental differences between geographic regions.

MATERIALS AND METHODS

1. Sampling, preparation and measurement of the skull

472 specimens of the field vole stored in the Natural History Museum (Aarhus, DK) and Zoological Museum (Copenhagen, DK) were utilized for the morphometric investigation. In the preparation process the sex was noted and the reproductive status was determined from the thickness of the uterus and vagina in females and the size of the testicles in males. The head was separated from the body at the neck and the brain was removed by flushing water through the cervical vertebra. Afterwards the head was boiled for approx. 6 minutes. Tissue and muscles were removed with a pair of tweezers and the skulls were placed in sodium hypochloride (15% solution) and H_2O_2 for approx. 5 minutes to remove the remaining tissue.

The field voles were assigned to geographical regions consisting of nine different islands in Denmark and five regions on the Jutland peninsula (*Fig. 1*). The *a priori* assignment of the field voles from the Jutland peninsula into geographical regions was made because of no earlier knowledge of size and shape differences in skulls within Jutland. The regions within Jutland roughly represent different habitats within Jutland (Enghoff and Nielsen 1977). Individuals from Jutland, assigned to an UTM-square next to the demarcation between two regions, were omitted. Because of the smaller area, the individuals from Upper Jutland West were all included. The islands investigated were connected to mainland Europe until approx. 8,000 years ago, at a time where field vole presumably already inhabited the area.

A standardized digital photo was taken of the three parts of the skull: the mandible, the dorsal side and the ventral side of the skull. The software program ImageJ (http://rsb.info.nih.gov/ij/) was used to generate the coordinates in two dimensions. All the distances between the coordinates (inter-landmark linear distances) were calculated (truss method), (see Zelditch et al., 2004).

From the dorsal side of the skull 28 traits were measured, 33 from the ventral side and 30 for the mandible traits (*Fig. 2*). The measuring process was repeated, so each trait was measured three times. The median of each trait was chosen for further analyses. The average measuring error for the traits on the dorsal side was 0.97%, for the ventral side 0.88% and for the mandible 1.5%.

For this investigation only adult individuals were used, i.e. reproductive or post-reproductive individuals.

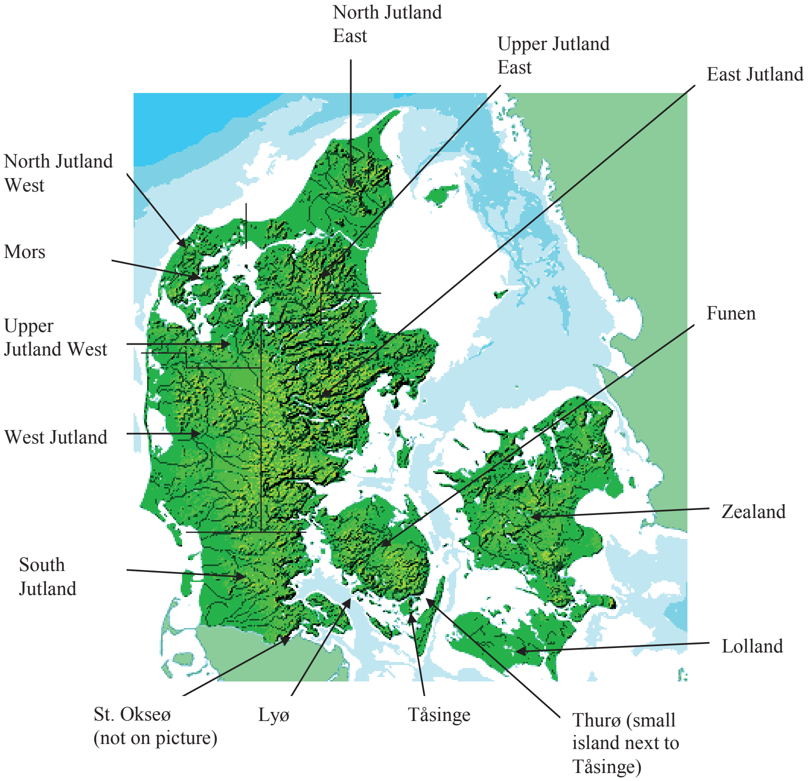


Figure 1 - The geographical regions in Denmark used for this study.

Some skulls were partly damaged or had location marks difficult to spot. Traits affected by such problems were not measured and a gap was left in the dataset. In those instances when the trait was based on the average of two distances from each side of the symmetrical line, but only one side could be measured, the gap was filled in with the distance from this side.

2. Univariate analysis

The geographical groups with the largest sample sizes (Zealand, East Jutland, Upper Jutland West, North Jutland East, North Jutland West, Funen) were tested for normal distribution of the traits by a Shapiro-Wilk test followed by a Bonferroni correction for multiple comparisons (Rice 1989). The tests for normal distribution were made in order to check the homogeneity of the samples. Significant deviations from kurtosis and skewness could in fact indicate heterogeneity in our sample due to environmental differences and/or genetic substructures within the *a priori* defined samples. To determine whether there was sexual dimorphism, two-tailed t-tests were carried out to check for single trait differences. The geographical groups with a minimum of five adult specimens in each sex were chosen for the test. The t-tests were performed separately for the dorsal and the ventral side of the skull, the mandible and the ratio/angle-traits, to test for single trait differences between the populations.

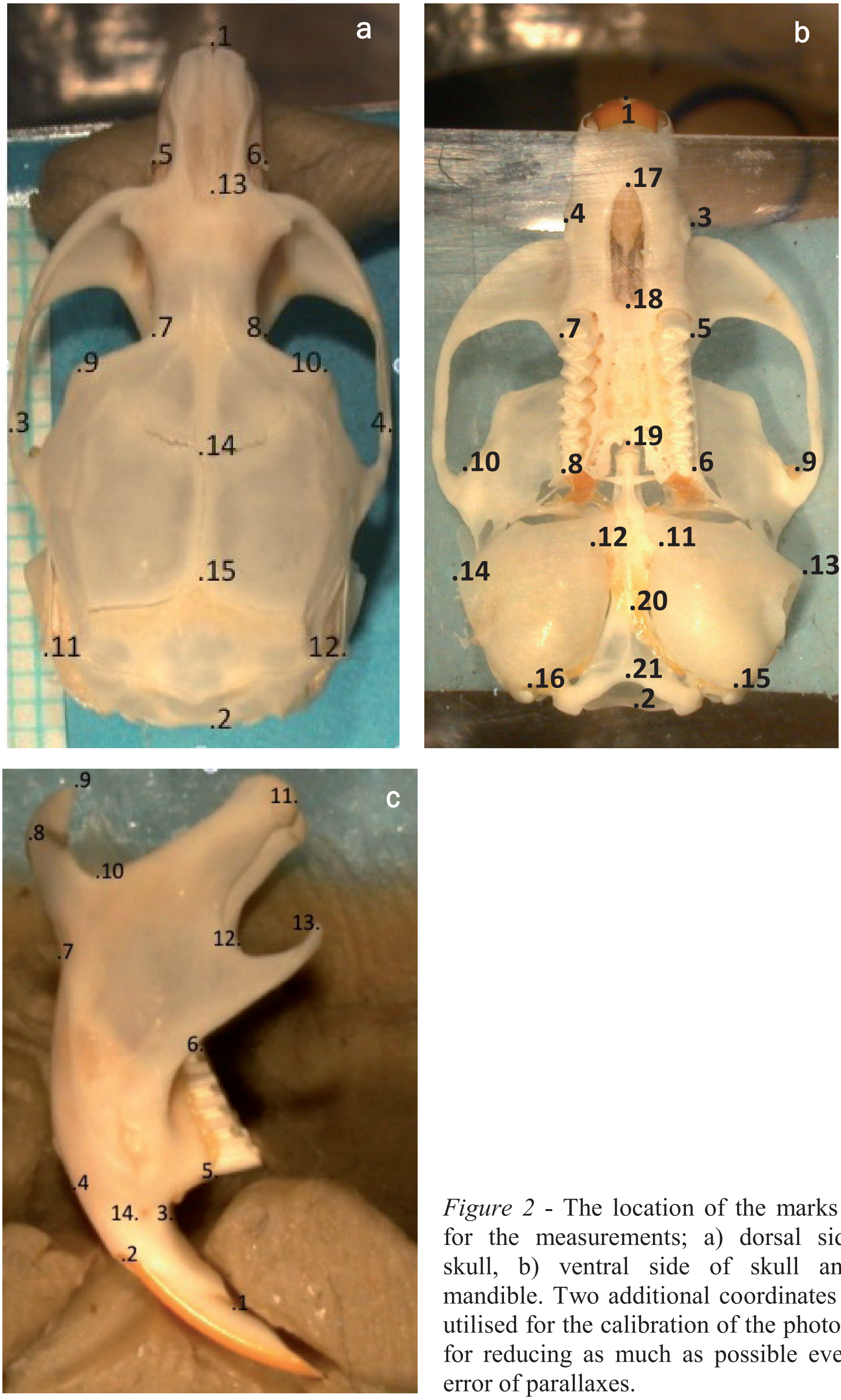


Figure 2 - The location of the marks used for the measurements; a) dorsal side of skull, b) ventral side of skull and c) mandible. Two additional coordinates were utilised for the calibration of the photos and for reducing as much as possible eventual error of parallaxes.

For the pairwise comparisons, 185 adult individuals were used. To find possible correlations between month of catch and skull length (trait 3-4, dorsal side), Spearman's r was calculated for those samples caught during different parts of the year (Upper Jutland West, East Jutland, North Jutland East, Funen and West Jutland).

3. Multivariate analysis

Multivariate analyses do not allow gaps in the data matrix. Therefore individuals with more than 20% of the values missing were excluded from further multivariate analyses. For the other individuals gaps were filled by the mean of the geographical sample calculated for each trait. The problem of missing data or absent landmarks is a persistent problem in morphometric studies. Geometric methods require the same set of homologous landmarks on all specimens. Unfortunately, specimens can be missing landmarks if they are broken, poorly preserved, or structures are articulated differently. Options are limited in these cases. Landmarks which are missing in some specimens are either eliminated from the analysis (effectively reducing shape information), or damaged specimens' missing landmarks are eliminated from the data set when rare (which is not our case as most of our specimens have broke or missing parts), or missing landmarks are estimated using sample means if the missing landmarks are rare (which is not our case) (Adams et al. 2004). Reducing the number of landmarks in our geometric analyses would have considerably reduced the power to distinguish groups using the geometric data, compared to the traditional methods where power is influenced by the number of traits considered. Using sample means for the missing landmarks would have been a wrong approach as it would have created strong deviations of the landmarks position from the real ones, due to the fact that sometimes the number of missing landmarks in a specimen were high, and as the more missing landmarks there are, the higher is the level of uncertainty of the estimation of the correct position of the landmarks (Adams et al. 2004). The problems mentioned above are an additional reason for choosing a traditional morphometric approach using traits rather than landmarks for our analyses.

North Jutland West was excluded from the analysis of the mandible, since two of the traits were only represented by one individual. Regions with a sample size of two adult individuals were included. In total 186 individuals for the dorsal side of the skull, 174 individuals for the ventral side and 154 individuals for the mandible were available for the multivariate analysis. The statistical program PAST (Hammer et al.,2001) was used for the multivariate analyses.

To identify if population substructure is due to variation in size and/or shape, a principal component analysis (PCA) was carried out. This analysis classifies phenotypic variation into independent components that can be used to dissect genetic networks regulating complex biological systems (Chase et al.,2002). If size variation is present in the data and the loadings of principal component 1 (PC1) are either all positive or all negative, PC1 can be said to summarise the within-sample size variation (Bookstein 1989). Shape can be defined as the subspace of dimensions one less than the number of measured variables and quantifies the variation that cannot be explained by size variation and allometric relationships.

The variation from the first principal component (PC1) is explained by allometric size variation (Manly 1986; Bookstein 1989). Since the rest of the variation is not correlated with size, the variation from the forthcoming principal components explain shape variation. We considered only the first two PCs (PC1 and PC2) in our analysis because they were the only two PCs with eigenvalues above the Jollife cut-off value (Jolliffe 1986). The Jolliffe cut-off value gives an informal indication of how many principal components should be considered significant (Jolliffe 1986). Components with eigenvalues smaller than the Jolliffe cut-off may be considered insignificant. Hence, by making pairwise comparisons of the geographical groups on PC1 and PC2, the geographical groups can be tested for possible differentiation by size and/or shape.

The PC1 and PC2 from the variance/covariance matrix were tested for normal distribution and equal variance. Depending on result from the test for normality and the sample size of the population a parametric t-test, which compares the means, or a non-parametric Mann-Whitney U-test, which compares the medians, were run to test for significant differences. In case equal variance was rejected, a resample t-test (1,000 permutations) was performed. However, geographic samples with less than five specimens were always compared with other samples using a Mann-Whitney U-test. To prevent type-1-errors an overall Bonferroni correction was made (Rice 1989). The fact that the analyses performed contain many pairwise comparisons, makes the Bonferroni correction quite conservative. Furthermore, many of the traits measured in this investigation are highly correlated with each other, making the pairwise tests not independent from each other. Therefore, the results before and after the Bonferroni correction are presented, but only the results before the Bonferroni correction are discussed.

To find possible correlations between islands area and skull sizes, the PC1 and the log area of the islands were correlated by the non-parametric Spearman's r. The PC1 was generated by running all the island samples together.

RESULTS

1. Univariate analysis

1a. Test for normal distribution

The samples tested for normality had between 1% and 13% of the 91 traits significantly deviating from normal distribution ($0.0005 < p < 0.05$). After a Bonferroni-correction ($K = 30$) was carried out, the samples from Upper Jutland West and North Jutland East still had one and two traits deviating from normal distribution respectively. Overall, the result suggests that no subpopulation structure is found within each geographical group. Pairwise t-tests were therefore carried out to test for single trait differences between the geographical samples.

1b. Sexual dimorphism

For each skull part (dorsal side, ventral side and mandible) most geographical samples had 0-6% of the traits with a significant sexual dimorphism ($0.001 < p < 0.05$), but no sex was consistently larger than the other in a sample. The populations on Lyø (male > female) and Thurø (male < female) did show signs of sexual skull dimorphism with 19 - 20% of the traits being significantly different. However, none of the differences were significant after the Bonferroni correction. Therefore, the two sexes were pooled in all geographical samples.

1c. Dorsal side of skull

34%t of all single trait comparisons between the geographical samples on the dorsal side were significant ($p < 0.05$). The sample from West Jutland had the shortest skulls (mean = 25.494 ± 0.68 mm) and the sample from Funen the smallest zygomatic breadth (mean = 14.49 ± 0.47 mm). The sample from Mors had both the largest skull length (mean ± S.E. = 26.988 ± 0.95 mm) and the largest zygomatic breadth (mean = 15.323 ± 0.41 mm) and had a significantly larger skull length than the samples from West Jutland and Upper Jutland West ($p < 0.001$), which had the shortest skulls. On the zygomatic breadth, the sample from Mors was significantly larger than the sample from Funen, West Jutland and Upper Jutland West ($p < 0.001$).

The Lyø and Mors samples had a surplus of traits which were significantly larger in each pairwise comparison (see *Table 1a*). However, the Lyø sample was the only one being significantly larger on two traits (traits 9-11 and 10-12, $p < 0.001$) and significantly smaller on two other traits (traits 9-15 and 10-15, $p < 0.05$) compared with all the other geographical samples, thereby indicating that the sample from Lyø differed in shape from the other samples. In *Table 1a*, the Funen and West Jutland samples had a surplus of traits, which were significantly smaller in each pairwise comparison.

Some traits clearly contained more inter-sample differences than other traits. For instance, in 36 out of the 55 sample comparisons there was a significant difference ($p < 0.05$) in trait 11-12. 22 out of these 36 differences were highly significant ($p < 0.001$). The trait with least inter-sample differences was trait 13-14, where only two significant differences ($p < 0.05$) were found. Another trait, which varied only slightly between samples, was the length of parietal (trait 15 - 16) with five significant differences ($p < 0.05$). The correlations between month of catch and skull length (trait 3-4, dorsal side) from the East Jutland, the North Jutland East, the Funen and West Jutland samples were not significant (Spearman's r: $-0.117 < r < 0.522$, $0.06 < p < 0.971$).

1d. Ventral side of skull

29% of all single trait comparisons between the geographical samples on the ventral side were significant ($p < 0.05$). The sample from the geographical group of North Jutland East had the longest teeth rows (TR1: mean = 6.045 ± 0.21 mm; TR2: mean = 6.075 ± 0.20 mm) and the sample from West Jutland had the shortest (TR1: mean = 5.706 ± 0.35 mm; TR2: mean = 5.75 ± 0.28 mm). The difference between the sample from North Jutland East and the two samples with the shortest teeth rows, West Jutland and Upper Jutland West, was significant ($p < 0.001$). The Thurø sample had the largest palate breadth (PW1: mean = 4.643 ± 0.13 mm; PW2: mean = 3.636 ± 0.17 mm) and the Upper Jutland West sample the smallest (PW1: mean = 4.346 ± 0.12 mm; PW2: mean = 3.335 ± 0.15 mm). The Thurø sample had a significantly larger palate breadth than the two samples with the smallest palate breadth, West Jutland and Upper Jutland West ($p < 0.001$).

The Lyø and Mors samples had many traits which were significantly larger in each pairwise comparison (see *Table 1b*). The Lyø sample was also smaller in one trait on the ventral side of the skull (trait 20-21, $p < 0.001$ (exceptions: Funen and West Jutland, p = n.s.) and larger in another (trait 21-22, $p < 0.05$) compared with all the other geographical samples. The samples from Upper Jutland West and West Jutland both had many traits, which were significantly smaller in each pairwise comparison (see *Table 1b*).

On the ventral side, the two traits containing most inter-sample differences were trait 5-6 with 31 significant differences out of 55 sample comparisons and trait 20-21 (palate length) with 30 significant differences. In trait 5-6, 16 sample comparisons were highly significant ($p < 0.001$) and in trait 20-21, 13 comparisons were highly significant ($p < 0.001$). Traits 8-17, 10-18, 8-13 and 10-14 contained least inter-sample differences, with five significant differences ($0.001 < p < 0.05$).

1e. Mandible

30% of all single trait comparisons between the geographical samples of the mandible were significant ($p < 0.05$). The Zealand and Mors samples had many traits, which were significantly larger in each pairwise comparison (see *Table 1c*). Whereas, the samples from Upper Jutland West and West Jutland both had a surplus of traits, which were significantly smaller in each pairwise comparison (see *Table 1c*).

2. Multivariate analysis

2a. Principal Component Analysis – Test for size

PC 1 explained between 53% and 59% of the total variation from the three parts of the skull, when all geographical samples were pooled.

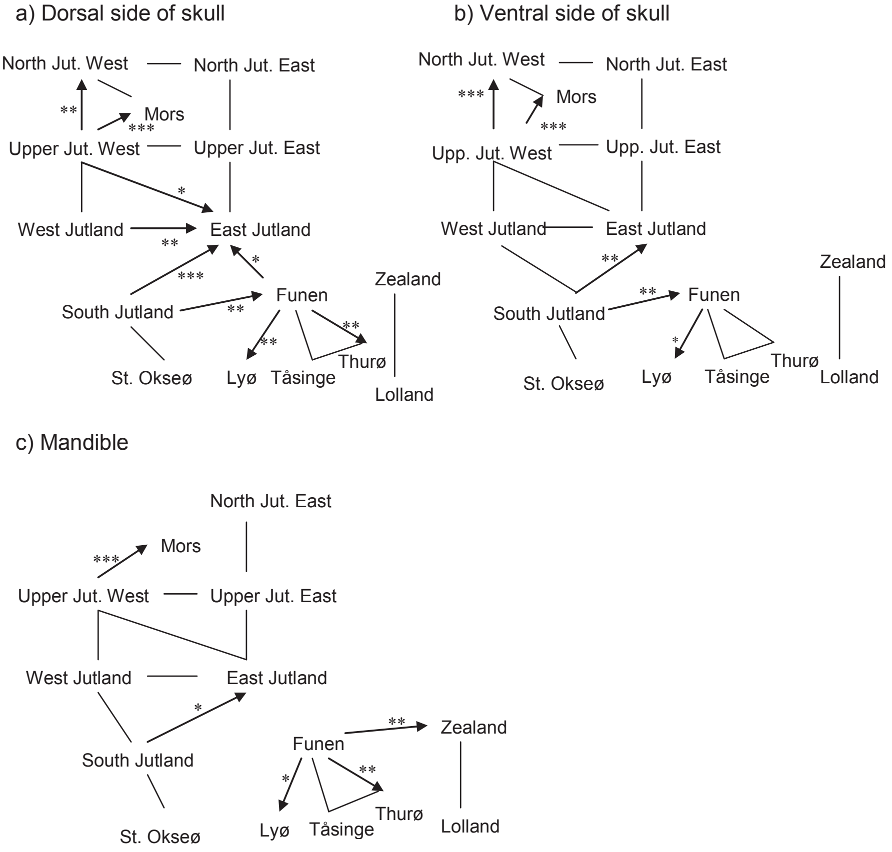
From the following pairwise comparisons, the significant differences in PC1 between neighbouring geographical samples are shown in *Figure 3*. The samples from Mors, North Jutland West, East Jutland and Thurø had the largest PC1 (see *Table 2*). The geographical samples with the smallest PC1 were West Jutland, South Jutland and St. Okseø. The most extreme difference found for PC1 was between the Mors sample and the sample from South Jutland (dorsal side: $t = 5.972$, $p < 0.0001$; ventral side: $t = 5.996$, $p < 0.0001$; mandible: $t = 4.455$, $p < 0.001$). The neighbouring geographical samples which were not significantly different in any part of the skull (the mandible, the ventral and dorsal side of the skull) were then pooled (*Figure 4*). In Jutland the PC1 became smaller from north to south and from east to west (see *Figure 3*).

The Lyø, Mors and Thurø samples all had a larger PC1 than their proximate mainland samples or the sample from the larger neighbouring island Funen. The samples with small sample size - Okseø ($n = 3$), Tåsinge ($n = 2$) and Lolland ($n = 5$) - showed no size differences from the neighbouring samples.

2b. Principal Component Analysis – Test for shape

In *Table 2* the significant results from the PCA analysis are shown. PC2 explained 7-11% of the variation from the three parts of the skull, when all geographical groups were pooled.

66% of the pairwise comparisons between the island samples were signifi-



a) Dorsal side of skull

b) Ventral side of skull

c) Mandible

Figure 3 - Test for size between neighbouring geographic samples based on PC1 (see *Table 3*). D – dorsal side of skull, V – ventral side of skull, M – mandible. The geographic sample with the arrowhead is the larger region; *p<0.05, **p<0.01, ***p<0.001.

cantly different in PC2 on one or more skull parts ($p < 0.05$). Between the island samples and the neighbouring mainland, including Funen and Zealand, 62% of the comparisons were significantly different ($p < 0.05$) (*Figure 5*). However, the samples from Okseø ($n = 3$) and Tåsinge ($n = 2$), which had very small sample sizes, were not different from the neighbouring sample. 56% of all the comparisons between island samples and all the samples from the Jutland peninsula were significantly different ($p<0.05$). Within the Jutland peninsula, 30% of the sample comparisons were significantly different ($0.001 < p < 0.05$). The samples from the islands Thurø, Lyø and Zealand were significantly different from all the other samples. However, a few exceptions with no differences between samples with small sample sizes (St. Okseø, Tåsinge and South Jutland) and the Thurø and Lyø samples were found

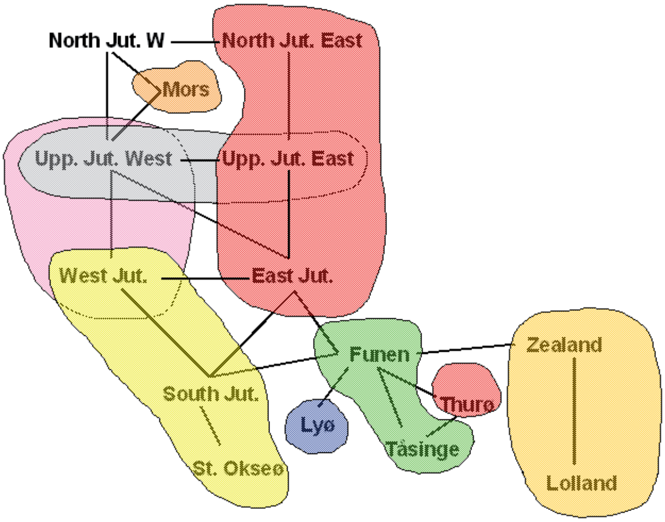


Figure 4 - Regions with no size difference are pooled. North Jutland West was excluded from the analysis of the mandible. On the dorsal and the ventral side of the skull no significant differences in PC1 were found between North Jutland West and respectively Mors and North Jutland East.

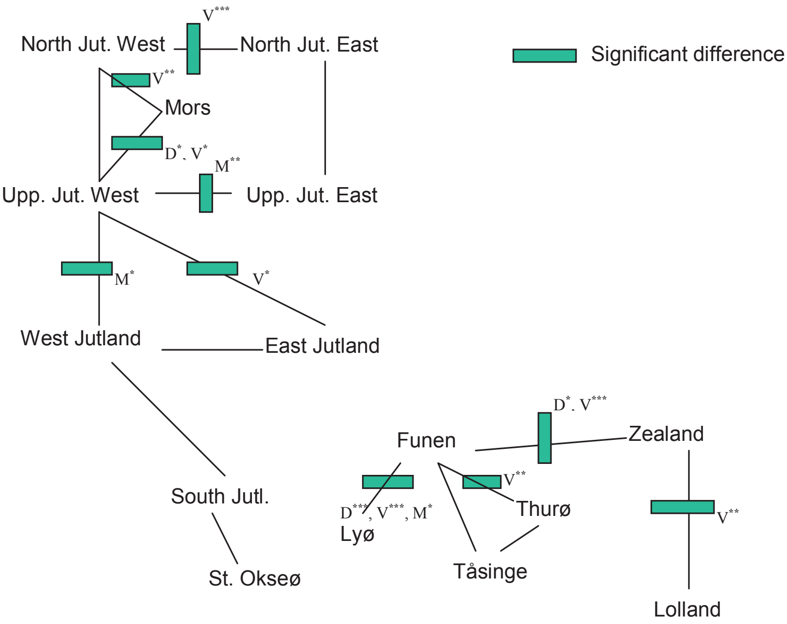


Figure 5 - Test for shape differences between neighbouring geographic samples in the mandible (M), the dorsal (D) and the ventral (V) side of the skull, see *Table 6*; $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$.

(see *Table 2*). The samples from the islands Funen and North Jutland were not different in PC2 from the neighbouring samples on the Jutland peninsula, but were different from more distant samples on the Jutland peninsula. The sample from Mors was different from the neighbouring geographic samples, but was not consistently different from other samples.

2c. Island area vs. skull size

On the dorsal side of the skull a significant but low correlation was found between the PC1 from the island samples and the area of the corresponding islands (Spearman's r = -0.181, p = 0.042). On the ventral side of the skull (Spearman's r = -0.109, p = 0.252) and the mandible (Spearman's r = 0.113, p = 0.317) the correlation between size and island area was low and not significant. If the islands with small sample sizes were excluded, the correlation on the dorsal side of the skull was still significant (Spearman's r = -0.258, p = 0.006), while the correlation on the ventral side was now significant (Spearman's r = -0.201, p = 0.037). The correlation on the mandible was still weak and not significant (Spearman's r = 0.026, p = 0.824).

DISCUSSION

1. Shape differences

The skull and the mandible consist of quantitative traits with a high heritability (Atchley et al. 1981; Sparks and Jantz 2003), and in general the shape of a morphological character involves several quantitative trait loci (Birdsall et al. 2000; Zimmerman et al. 2000; Klingenberg et al. 2001; Chase et al. 2002; Workman et al. 2002), which is a necessity for the skull differences found to be genetically manifested. Therefore, the shape differences found in this investigation suggest that the island field voles are genetically differentiated from each other and from the mainland relatives at the corresponding trait loci. On the Jutland peninsula the field voles seem to be more or less genetically homogeneous, because of few and not very significant differences in the shape of the skull detected. A combination of reduced gene-flow between field vole fragments due to increased distance between them and drift during population fluctuations could explain why significant shape differences were found within the Jutland peninsula (Palo et al. 2003; Wójcik et al. 2006). When subjected to ample fluctuations of population sizes (with periods of very small populations size), quantitative traits may be influenced by drift, since selective forces are distributed across the loci coding for the trait, and thereby making the selective forces sufficiently small to be overwhelmed by drift. Field vole populations do fluctuate in Denmark (Chitty and Phipps, 1966; Jensen, 1993) and the migration potential of the field vole is questionable (Manniche 1935; Ursin 1948), so drift may be the main factor which has created the shape differences found. Nevertheless, selection is often suggested to be the major force on shape, since the evolution of the shape of different characters could not be explained by drift alone (Marroig and Cheverud 2004; Polly 2004; Wójcik et al. 2006). Genotype and environmental interactions do occur (Turelli, 1988; Silva et al., 2006), so it is possible that field voles in this investigation have adapted to the specific environment on each island, since, especially, smaller islands have special climatic and floral composition (Christiansen and Jensen 2007).

This study showed that the mandible contained less inter-geographical shape variation than the ventral and the dorsal side of the skull (see *Table 2*). This is in accordance with Caumul and Polly (2005), who discovered that the mandibular variation in their study on marmots (*Marmota*, Rodentia) did not recover the phylogenic groupings of marmot, possibly because of environmental variation associated with individual life histories. Still, the shape of the mandible in mice is expressed by several QTL compared with control size (Atchley and Hall 1991; Klingenberg et al. 2001), which may explain why Kooij et al. (1997) found limited overlap in the shape of the mandible between *M. agrestis and M. arvalis*. Nonetheless, it is our recommendation that the mandible should only supplement the dorsal and the ventral side of the skull in investigations trying resolve geographic or phylogenetic skull variation.

2. Size differences

The principal component analyses and the pairwise t-tests indicated that the field voles from Mors, Lyø and Thurø were on average larger in the size of the skull than their mainland relatives, with East Jutland as an exception. The field voles from the larger islands, North Jutland, Funen and Zealand, were not consistently larger or smaller in skull size than con-specifics on the Jutland peninsula. The size of the skull is correlated with body size (Ellison et al., 1993), so it was therefore expected that the field voles from Mors, Lyø and Thurø were also larger in body size. The skull sizes on the islands in this study are well in accordance with the island rule, where small species experience gigantism on smaller islands (Lomolino 1985; Adler and Levins 1994; Lomolino 2005; White and Searle 2007). This relationship is supposed to evolve from a lower predation pressure and a lower inter-specific competition, which are both inversely correlated with island area and island-mainland distance (Lomolino 1985; Adler and Levins 1994). The smaller islands Mors, Lyø and Thurø all have fewer predator and rodent species than the Jutland peninsula, while the fauna on the larger islands: North Jutland, Zealand and Funen is more like the fauna on the Jutland peninsula (Christiansen and Jensen 2007). The results for skull size related to island area did give further support to the island theory, since both dorsal and ventral side of the skull had a significant correlation between size and island area. Meiri et al. (2005, 2006) could not support the island rule with their study of Carnivora, which did not conform towards an “optimal” body size on islands. Neither the size of the island nor the distance to mainland had a general influence on the body size of Carnivora. Meiri et al. (2006) concluded that the island rule was not a general rule across all orders of Vertebrata, but a tendency that is well supported by Rodentia.

It is believed that primarily better survival due to lack of predators on islands allows rodents to invest less energy in the production of offspring, i.e. smaller litters and delayed maturation, and to channel the energy towards larger body size to adapt to a larger intra-specific competition (Adler and Levins 1994). With time, adaptation towards larger size could create a genetically determined size difference between island populations and the source population on the mainland (Turelli 1988; Silva et al., 2006). Nevertheless, larger body size could also be the result of better habitat quality, e.g. improved food availability or quality. Size differences have been found in populations presumably not separated by natural barriers (Alcantara 1991; Ellison et al. 1993; Renaud 1999; Yom-Tov and Yom-Tov 2004). Yom-Tov et al. (2003) suggested that badgers in Denmark through the last century had increased in size because of improved diets. This kind of size difference between populations exchanging genetic material is a plastic response to food availability (habitat quality) or other environmental factors (Renaud 1999; Birdsall et al. 2000; Renaud and Michaux 2003; Wójcik et al. 2006). To find out whether size differences between geographical samples are genetically determined or caused by different environmental factors, a common-garden experiment may eventually be conducted.

The increasingly larger skull size from south to north of Jutland could partly be explained by Bergman's rule (Mayr 1963), where the body size of conspecies becomes larger with higher latitudes, because of the advantage of larger body volume to surface in colder climates. Although possible, it is unlikely that Bergman's rule could be the major explanation for the 5% change in skull length over a distance of roughly 230 km. Another possibility for the size difference in Jutland may be found in the level of habitat fragmentation in the different regions. Schmidt and Jensen (2003) suggested that mammals and birds had conformed towards an ”optimal” size (Lomolino 2005) due to an increasingly fragmented landscape in Denmark through the last 175 years. This could indicate that the north and east of Jutland have a more fragmented habitat with larger field voles. But Schmidt and Jensen (2003, 2005) did not take into consideration fluctuating populations of predators through the last 200 years which may influence the size of prey (Adler and Levins 1994) or possible fluctuations in habitat quality because of changing practices in agriculture (Yom-Tov et al. 2003). Instead, the cause of this size cline may be found in better habitat quality, i.e. food quality and availability, for the field voles in the east and north of Jutland.

3. Morphometric differentiations

Most of the tests of our investigation consists of pairwise tests for morphometrical differentiation between populations, where some populations consisted of few individuals and where the number of landmarks was considerably higher than the number of individuals compared in the tests. A Procustes analysis which analyse the landmarks rather than the Euclidean distances between the coordinates could therefore not be utilised for our data-set as sample size in a morphometric study should equal at least twice the number of landmarks (for two-dimensional data) to have fewer variables than observations (Caldecutt and Adams 1998).

The results from the principal component analyses revealed that the samples from Funen, North Jutland and Zealand were not differentiated from other samples to the same extent. While the sample from Zealand was different in shape from every other sample, the Funen and the two North Jutland samples were not different from neighbouring samples on the Jutland peninsula. From the size of these three islands, it is expected that they contain large populations of field voles, so drift becomes a minor force on the morphology. Nevertheless, large fluctuations in population size could possibly create enough genetic drift on all three islands to differentiate the populations in shape. The "unique" skull shape found on Zealand may partly be explained from the dimensions of the barrier formed by the Storebælt, which separates Funen and Zealand. The Storebælt is a much more extreme barrier than Lillebælt and Limfjorden, which separates Funen and North Jutland respectively from the Jutland peninsula. Zealand was connected to Sweden until approx. 9,000 years ago and thus the field voles on Zealand may instead resemble the South Swedish field voles in skull shape.

The comparisons from the multivariate analyses including Okseø ($n = 3$), Tåsinge ($n = 2$) and to some extent also Lolland ($n = 5$) and South Jutland ($6 \leq n \leq 8$) were influenced by small sample sizes, which makes it difficult to reach firm conclusions, except for South Jutland.

The specimens in this study were not from the same year or same period of time. The specimens from North Jutland West (1954), Thurø (1944), Zealand (1965) and four specimens from Lyø (1944) were roughly 50 years older than the rest of the samples. Pergrams and Ashley (2001) and Millien (2006) both found rapid morphological changes on islands after founding events. Pergrams and Ashley's (2001) study on *Mus musculus, Rattus rattus* and *Peromyscus maniculatus* showed that 60% of the traits had changed with a rate of 600 darwins[1] or higher after the rodents arrived to the islands off the shore of California about 100 years ago. Even on the mainland, morphological shape changes may occur through time. Wójcik et al. (2006) discovered changes in skull shape of *A. flavicollis* over a 7 year period because of fluctuations in population density. Size may also change through time because of changes in the habitat (Yom-Tov et al. 2003). Therefore, conclusions based on comparisons of samples from two different periods have to be made with care.

Studies using morphometrics have so far tried to find single trait differences, and/or used a rather limited number of traits for a multivariate analysis (Yom-Tov et al. 1999; Simonsen et al. 2003; Yom-Tov et al. 2003; Yom-Tov and Yom-Tov 2004). Geometric morphometrics techniques are often used for testing morphological differentiation among samples and age groups using the relative position of homologous landmarks placed on skulls (e.g., Bookstein 1996). In contrast to ratios of

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[1] $d = |(\ln x_2 - \ln x_1) / (t_2 - t_1)|$

x = sample means of measurements at times t_1 and t_2. The time difference is measured in millions of years.

linear measurements, geometric morphometrics allow for statistical analyses of overall shape variation and the generation of graphical illustrations of shape differences (Bookstein 1996). The method also has the advantage that it captures all aspects of shape change among a set of landmarks without *a priori* knowing which specific anatomical change to search for. However it has the disadvantage that a large sample size of specimens is necessary for the analysis (Adams and Rohlf 2000). In the light of the different kinds of information obtained in this investigation using univariate and multivariate techniques, we encourage such an approach which could not have been undertaken with geometric morphometric techniques because of the limited sample. Furthermore we encourage the measurements on several traits on part of the skulls which are not developmentally linked to each other (for example dorsal side of the skull and mandible) and which could be under different selective regimes.

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Table 1 - Summary of the pairwise t-tests. The total number of significant differences ($p < 0.05$) in the pairwise comparisons are shown. The numbers in bold show the number of traits where the sample in the left column is significantly larger than the sample in the upper row. The number below the diagonal shows the number of traits where the sample in the left column is significantly smaller.

a) Dorsal side – 28 traits		Upp. Jut. East	Funen	Lyø	Mors	North Jut. West	North Jut. East	Zealand	Thurø	West Jut.	East Jut.	Upp. Jut. West
Upper Jutland East	>	**XX**	**2**	**2**	**0**	**0**	**0**	**0**	**0**	**5**	**0**	**0**
$7 \leq n \leq 10$	<	XX	0	14	15	6	2	0	12	0	4	1
Funen	>	**0**	**XX**	**1**	**0**	**0**	**0**	**0**	**0**	**3**	**0**	**0**
$15 \leq n \leq 16$	<	2	XX	19	19	11	9	3	14	0	10	1
Lyø	>	**14**	**19**	**XX**	**4**	**8**	**9**	**14**	**4**	**19**	**9**	**19**
$13 \leq n \leq 15$	<	2	1	XX	4	5	2	3	2	1	1	1
Mors	>	**15**	**19**	**4**	**XX**	**4**	**9**	**13**	**2**	**22**	**6**	**19**
$11 \leq n \leq 11$	<	0	0	4	XX	1	1	0	1	0	1	1
North Jutland West	>	**6**	**11**	**5**	**1**	**XX**	**6**	**6**	**0**	**17**	**0**	**11**
$14 \leq n \leq 18$	<	0	0	8	4	XX	2	0	2	0	3	0
North Jutland East	>	**2**	**9**	**2**	**1**	**2**	**XX**	**8**	**1**	**13**	**1**	**11**
$15 \leq n \leq 18$	<	0	0	9	9	6	XX	2	3	0	3	0
Zealand	>	**0**	**3**	**3**	**0**	**0**	**2**	**XX**	**0**	**13**	**1**	**5**
$19 \leq n \leq 20$	<	0	0	14	13	6	8	XX	11	0	8	1
Thurø	>	**12**	**14**	**2**	**1**	**2**	**3**	**11**	**XX**	**17**	**0**	**15**
$12 \leq n \leq 16$	<	0	0	4	2	0	1	0	XX	0	0	0
West Jutland	>	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**XX**	**0**	**0**
$10 \leq n \leq 11$	<	5	3	19	22	17	13	13	17	XX	13	6
East Jutland	>	**4**	**10**	**1**	**1**	**3**	**8**	**8**	**0**	**13**	**XX**	**8**
$19 \leq n \leq 21$	<	0	0	9	6	0	1	1	0	0	XX	0
Upper Jutland West	>	**1**	**1**	**1**	**1**	**0**	**1**	**1**	**0**	**6**	**0**	**XX**
$15 \leq n \leq 19$	<	0	0	19	19	11	5	5	15	0	8	XX

b) Ventral side – 33 traits		Upp. Jut. East	Funen	Lyø	Mors	North Jut. West	North Jut. East	Zealand	Thurø	West Jut.	East Jut.	Upp. Jut. West
Upper Jutland East	>	**XX**	**1**	**1**	**0**	**1**	**0**	**2**	**2**	**5**	**0**	**2**
7≤n≤10	<	XX	0	10	9	9	1	7	9	0	3	0
Funen	>	**0**	**XX**	**0**	**0**	**2**	**0**	**2**	**1**	**3**	**0**	**4**
9≤n≤16	<	1	XX	15	17	13	7	12	13	0	7	2
Lyø	>	**10**	**15**	**XX**	**2**	**8**	**5**	**9**	**5**	**15**	**8**	**18**
7≤n≤15	<	1	0	XX	4	2	2	3	4	0	1	1
Mors	>	**9**	**17**	**4**	**XX**	**9**	**6**	**12**	**10**	**19**	**9**	**19**
10≤n≤11	<	0	0	2	XX	0	0	1	2	0	0	0
North Jutland West	>	**9**	**13**	**2**	**0**	**XX**	**5**	**5**	**3**	**12**	**6**	**11**
13≤n≤18	<	1	2	8	9	XX	6	3	4	0	3	2
North Jutland East	>	**1**	**7**	**2**	**0**	**6**	**XX**	**6**	**6**	**10**	**4**	**8**
13≤n≤18	<	0	0	5	6	5	XX	4	5	0	1	0
Zealand	>	**7**	**12**	**3**	**1**	**3**	**4**	**XX**	**4**	**15**	**8**	**13**
19≤n≤20	<	2	2	9	12	5	6	XX	7	0	5	2
Thurø	>	**9**	**13**	**4**	**2**	**4**	**5**	**7**	**XX**	**9**	**5**	**10**
11≤n≤16	<	2	1	5	10	3	6	4	XX	0	2	1
West Jutland	>	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**XX**	**0**	**0**
8≤n≤10	<	5	3	15	19	12	10	15	9	XX	6	0
East Jutland	>	**3**	**7**	**1**	**0**	**3**	**1**	**5**	**2**	**6**	**XX**	**7**
15≤n≤21	>	0	0	8	9	6	4	8	5	0	XX	0
Upper Jutland West	<	**0**	**2**	**1**	**0**	**2**	**0**	**2**	**1**	**0**	**0**	**XX**
12≤n≤19	>	2	4	18	19	11	8	13	10	0	7	XX

c) Mandible – 30 traits		Upp. Jut. East	Funen	Lyø	Mors	North Jut. West	North Jut. East	Zealand	Thurø	West Jut.	East Jut.	Upp. Jut. West
Upper Jutland East	>	**XX**	**5**	**0**	**1**	**0**	**0**	**1**	**2**	**4**	**1**	**7**
15≤n≤15	<	XX	0	5	9	0	1	10	7	0	1	0
Funen	>	**0**	**XX**	**0**	**0**	**0**	**1**	**0**	**0**	**5**	**0**	**6**
12≤n≤17	<	5	XX	11	14	4	5	17	13	0	5	3
Lyø	>	**5**	**11**	**XX**	**0**	**2**	**7**	**2**	**2**	**17**	**8**	**17**
8≤n≤15	<	0	0	XX	3	0	1	5	8	0	0	0
Mors	>	**9**	**14**	**3**	**XX**	**8**	**11**	**2**	**6**	**21**	**10**	**21**
10≤n≤11	<	1	0	0	XX	1	1	3	1	0	0	0
North Jutland West	>	**0**	**4**	**0**	**1**	**XX**	**2**	**0**	**3**	**6**	**1**	**12**
1≤n≤18	<	0	0	2	8	XX	2	5	6	0	1	1
North Jutland East	>	**1**	**5**	**1**	**1**	**2**	**XX**	**1**	**1**	**8**	**1**	**10**
15≤n≤19	<	0	1	7	11	2	XX	7	8	0	2	2
Zealand	>	**10**	**17**	**5**	**3**	**5**	**7**	**XX**	**3**	**19**	**10**	**20**
18≤n≤20	<	1	0	2	2	0	1	XX	3	0	1	2
Thurø	>	**7**	**13**	**8**	**1**	**6**	**8**	**3**	**XX**	**18**	**8**	**15**
7≤n≤16	<	2	0	2	6	3	1	3	XX	0	0	1
West Jutland	>	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**XX**	**0**	**2**
9≤n≤12	<	4	5	17	21	6	8	19	18	XX	5	2
East Jutland	>	**1**	**5**	**0**	**0**	**1**	**2**	**1**	**0**	**5**	**XX**	**8**
21≤n≤21	<	1	0	8	10	1	1	10	8	0	XX	1
Upper Jutland West	>	**0**	**3**	**0**	**0**	**1**	**2**	**2**	**1**	**2**	**1**	**XX**
17≤n≤21	<	7	6	17	21	12	10	20	15	2	8	XX

Table 2 - Significant differences in PC1 and PC2. Samples have been tested pairwise. From the variance-covariance matrix, PC1 and PC2 have been subjected to a t-test or a Mann-Whitney U-test. *p<0.05, **p<0.01, ***p<0.001. Fields marked with **bold** are significant after Bonferroni correction (tables a-d, K=105, tables e-f, K=91).

a) Size differences in the dorsal side of skull (*italic* – region in left column is largest)

PC1	Funen	Lolland	Lyø	Mors	N. Jut. W	N. Jut. E	St. Okseø	South Jut.	Zealand	Thurø	Tåsinge	West Jut.	East Jut.	Upp. Jut. W	Upp. Jut. E
Funen n=16	XXX XXX	t=-2.691 *	U= 37 **	t=-3.684 **	t=-2.711 *	t=-2.657 *		*t=2.855* **		t=-2.833 **			t=-2.114 *		
Lolland n=5		XXX XXX					*t=2.706* *	***t=5.361*** ***				*t=3.126* **		*t=2.869* **	
Lyø n=15			XXX XXX				*U=3* *	***U=0*** ***	*U=74* *			***U=13*** ***		***U=32*** ***	*U=49* *
Mors n=10				XXX XXX			*t=2.893* *	***t=5.972*** ***	*t=2.519* *			*t=4.142* **		***t=4.220*** ***	*t=2.709* *
North Jut. West n=16					XXX XXX			***t=4.748*** ***				*t=3.608* **		*t=3.114* **	
North Jut. East n=16						XXX XXX		***t=4.809*** ***				*t=3.472* **		*t=2.652* *	
St. Okseø n=3							XXX XXX								
South Jutland n=8								XXX XXX	t=-3.686 **	**t=-4.389** ***	t=-3.135 *		t=-3.846 **	t=-2.918 **	t=-3.743 **
Zealand n=19									XXX XXX			*t=2.218* *			
Thurø n=14										XXX XXX		*t=3.532* **		*t=3.119* **	
Tåsinge n=2											XXX XXX				
West Jutland n=11												XXX XXX	t=-3.013 **		t=-2.143 *
East Jutland n=20													XXX XXX	*t=2.310* *	
Upper Jut. West n=17														XXX XXX	
Upper Jut. East n=13															XXX XXX

b) Shape differences in the dorsal side of skull

PC2	Funen	Lolland	Lyø	Mors	N. Jut. W	N. Jut. E	St. Okseø	South Jut.	Zealand	Thurø	Tåsinge	West Jut.	East Jut.	Upp. Jut. W	Upp. Jut. E
Funen	XXX		**U=23**				U=0		t=-2.593						
n=16	XXX		***				**		*						
Lolland		XXX	U=0	t=3.143		U=12									
n=5		XXX	**	**		*									
Lyø			XXX	**t=-7.127**	**t=-6.391**	**t=5.770**	U=1	t=-2.299	**t=-7.546**	**t=-5.442**		t=-2.276	t=2.709	**U=21**	**t=6.196**
n=15			XXX	***	***	***	*	*	***	***		*	*	***	***
Mors				XXX		t=2.542				t=-3.293	U=0		t=2.347	t=-2.470	
n=10				XXX		*				**	*		*	*	
North Jut. West					XXX				t=-2.492	t=-2.602					
n=16					XXX				*	*					
North Jut. East						XXX			**t=-4.292**	t=-2.295					
n=16						XXX			***	*					
St. Okseø							XXX		U=0			U=2		U=3	
n=3							XXX		**			*		*	
South Jutland								XXX							
n=8								XXX							
Zealand									XXX	t=-3.876			t=3.794	t=3.572	t=2.301
n=19									XXX	***			**	**	*
Thurø										XXX					
n=14										XXX					
Tåsinge											XXX				
n=2											XXX				
West Jutland												XXX			
n=11												XXX			
East Jutland													XXX		
n=20													XXX		
Upper Jut. West														XXX	
n=17														XXX	
Upper Jut. East															XXX
n=13															XXX

c) Size differences in the ventral side of the skull (*italic* – region in left column is largest)

PC1	Funen	Lolland	Lyø	Mors	N. Jut. W	N. Jut. E	St. Okseø	South Jut.	Zealand	Thurø	Tåsinge	West Jut.	East Jut.	Upp. Jut. W	Upp. Jut. E
Funen	XXX		U=40	t=-3.627	t=-3.412			*t=3.332*							
n=14	XXX		*	**	**			**							
Lolland		XXX					*t=3.408*	*t=4.349*						*t=2.379*	
n=5		XXX					*	**						*	
Lyø			XXX				*U=0*	*U=0*				*U=21*		*U=34*	
n=13			XXX				*	**				*		*	
Mors				XXX			*t=4.127*	***t=5.996***	*U=43*			*t=2.946*		*t=3.900*	*t=2.367*
n=10				XXX			**	***	*			**		**	*
North Jut. West					XXX		*t=3.832*	***t=6.014***	*U=112*			*t=3.011*		*t=3.810*	*t=2.594*
n=17					XXX		**	***	*			**		**	*
North Jut. East						XXX	*t=2.453*	***t=4.248***							
n=15						XXX	*	***							
St. Okseø							XXX		U=5	t=-2.217					
n=3							XXX		*	*					
South Jutland								XXX	U=5	t=-3.838			U=14	t=-3.410	t=-3.129
n=6								XXX	**	**			**	**	**
Zealand									XXX						
n=19									XXX						
Thurø										XXX					
n=14										XXX					
Tåsinge											XXX				
n=2											XXX				
West Jutland												XXX			
n=8												XXX			
East Jutland													XXX		
n=19													XXX		
Upper Jut. West														XXX	
n=16														XXX	
Upper Jut. East															XXX
n=12															XXX

d) Shape differences in the ventral side of the skull

PC2	Funen	Lolland	Lyø	Mors	N. Jut. W	N. Jut. E	St. Okseø	South Jut.	Zealand	Thurø	Tåsinge	West Jut.	East Jut.	Upp. Jut. W	Upp. Jut. E
Funen	XXX		t=-3.902		t=-2.453				**U=10**	t=3.522				**t=-4.863**	t=-2.787
n=14	XXX		**		*				***	**				***	*
Lolland		XXX	**t=-7.970**	t=2.989	**t=-4.874**	t=-3.855			t=3.112	t=4.103		t=3.615	U=15	t=-3.679	
n=5		XXX	***	*	***	**			**	**		**	*	**	
Lyø			XXX	**t=-7.528**	**t=8.017**	**t=8.796**			**t=6.310**	**t=8.212**				t=-2.971	
n=13			XXX	***	***	***			***	***				**	
Mors				XXX	t=-3.003					**t=7.250**		t=3.001		t=-2.16	
n=10				XXX	**					***		**		*	
North Jut. West					XXX	**U=33**			**t=4.225**	t=2.691			**U=47**		t=-3.347
n=17					XXX	***			***	*			***		**
North Jut. East						XXX			**t=5.056**	**t=7.403**		t=2.629		t=-3.519	
n=15						XXX			***	***		*		**	
St. Okseø							XXX		U=3						
n=3							XXX		*						
South Jutland								XXX	U=8						
n=6								XXX	**						
Zealand									XXX	**t=-8.500**		**t=-5.290**	**t=-8.218**	**t=-6.935**	U=30
n=19									XXX	***		***	***	***	**
Thurø										XXX			**t=4.201**	**t=-4.244**	**U=8**
n=14										XXX			***	***	***
Tåsinge											XXX				
n=2											XXX				
West Jutland												XXX			
n=8												XXX			
East Jutland													XXX	U=82	
n=19													XXX	*	
Upper Jut. West														XXX	
n=16														XXX	
Upper Jut. East															XXX
n=12															XXX

e) Size differences in the mandible (*italic* – region in left column is largest)

PC1	Funen	Lolland	Lyø	Mors	N. Jut. E	St. Okseø	South Jut.	Zealand	Thurø	Tåsinge	West Jut.	East Jut.	Upp. Jut. W	Upp. Jut. E
Funen	XXX	t=-3.592	t=-2.117	t=-3.706	t=-2.121			t=-3.545	t=-2.976					
n=14	XXX	**	*	**	*			**	**					
Lolland		XXX				*t=4.631*	*t=4.423*			*t=2.653*	*t=3.058*	*t=20*	***t=4.175***	*t=2.804*
n=5		XXX				**	**			*	**	*	***	*
Lyø			XXX			*t=3.176*	*t=3.464*						*t=2.824*	
n=8			XXX			*	**						*	
Mors				XXX	*t=2.099*	*t=3.210*	***t=4.455***				*t=2.65*		***t=4.195***	*t=2.962*
n=10				XXX	*	**	***				*		***	**
North Jut. East					XXX		*t=3.319*						*t=2.702*	
n=15					XXX		**						*	
St. Okseø						XXX		t=-2.535						
n=3						XXX		*						
South Jutland							XXX	***t=-4.217***	t=-3.349			U=27		t=-3.123
n=7							XXX	***	**			*		**
Zealand								XXX			*t=2.968*	*U=104*	***t=4.107***	*t=2.745*
n=17								XXX			**	*	***	*
Thurø									XXX		*t=2.136*		*t=3.238*	*t=2.492*
n=6									XXX		*		**	*
Tåsinge										XXX				
n=2										XXX				
West Jutland											XXX			
n=11											XXX			
East Jutland												XXX		
n=20												XXX		
Upper Jut. West													XXX	
n=17													XXX	
Upper Jut. East														XXX
n=18														XXX

f) Shape differences in the mandible

PC2	Funen	Lolland	Lyø	Mors	N. Jut. E	St. Okseø	South Jut.	Zealand	Thurø	Tåsinge	West Jut.	East Jut.	Upp. Jut. W	Upp. Jut. E
Funen	XXX		U=26		U=57	U=4					U=35		**U=18**	
n=14	XXX		*		*	*					*		***	
Lolland		XXX												
n=5		XXX												
Lyø			XXX		**t=-4.246**		t=2.189	t=-3.469			t=-2.634			t=-3.594
n=8			XXX		***		*	**			*			**
Mors				XXX	t=-3.076			t=-2.791	t=-2.202					
n=10				XXX	**			*	*					
North Jut. East					XXX			t=-3.164	t=-2.49			**t=4.086**		
n=15					XXX			**	*			***		
St. Okseø						XXX								
n=3						XXX								
South Jutland							XXX							
n=7							XXX							
Zealand								XXX		U=1	t=2.224			t=3.831
n=17								XXX		*	*			**
Thurø									XXX					
n=6									XXX					
Tåsinge										XXX				
n=2										XXX				
West Jutland											XXX		t=-2.138	
n=11											XXX		*	
East Jutland												XXX		
n=20												XXX		
Upper Jut. West													XXX	t=2.859
n=17													XXX	**
Upper Jut. East														XXX
n=18														XXX