Original article: Geographic cline in the shape of the moose mandible: indications of an adaptive trend

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Abstract

Intra-specific geographic variation is probably one of the most common patterns studied in ungulate morphology. However, the shape of the mandible, a crucial feature with regard to feeding, has been greatly understudied in this context. Here, we utilized a museum collection of moose (*Alces alces*) mandibles to investigate whether we could detect significant variation in this species, and test for the existence of geographic patterns and associations with population genetic structure. We applied a landmark-based geometric morphometrics approach, analyzing shape data with principal component analysis and linear mixed models. A significant geographic shift in the shape of the moose mandible was revealed. The main pattern was similar in both sexes; however, there was a consistent difference in shape between males and females over the latitudinal scale. The main changes included an enlargement in the attachment surfaces of the muscles controlling biting and mastication, suggesting more effective mastication towards the north, plausibly as an adaptive response to a harder and tougher wintertime diet. Additionally, more subtle, yet statistically significant age-related shape variation was discovered. Interestingly, no or only a weak association between the morphometric variation and the genetic population structure was detected with neutral molecular markers.

Keyword: *Alces alces*; clinal variation; genetic structure; geometric morphometrics; mandible shape

Introduction

In morphological studies, the main focus has traditionally been on rather simply measured traits, such as size and mass. The development of geometric morphometrics methodology in the early 1990s, however, enabled a refined analysis of complex morphological shapes (Rohlf and Marcus 1993; Adams et al. 2004). One focal aspect in the context of shape analysis is the great variety of different biological processes that can result in morphologic differences between individuals and populations: e.g., long-term genetic divergence or environmentally driven plastic effects (Zelditch et al. 2012). In both cases, a further intriguing point is whether the outcome of the shape alteration is adaptive, i.e., whether it brings advantage in function, and/or moreover, an increase in fitness to the individual (Ghalambor et al. 2007).

The mammalian mandible, fundamentally important in food selection and comminution, has been investigated intensively as a general model for the development and evolution of complex morphological structures (e.g., Atchley and Hall 1991; Atchley 1993; Klingenberg et al. 2003, 2004). In addition, the relationship between the shape of the mandible, its biomechanical function and foraging ecology has recently been gaining increasing interest in evolutionary morphology (e.g., Monteiro et al. 2010; Young and Badyaev 2010; Prevosti et al. 2011; Anderson et al. 2014). In ungulates, the research has mostly concentrated on the inter-specific differences in the mandible shape in relation to the various feeding categories, such as browsers, grazers, or mixed feeders (Hofmann and Stewart 1972; Hofmann 1989; Pérez-Barbería and Gordon 1999; Raia et al. 2010; Fraser and Theodor 2011). On the contrary, the intra-specific level has received much less attention, despite the great geographic distribution and morphological variation in many ungulate species (Geist 1998). Yet, remarkable variation in the shape of the mandible related to differences in the habitat and diet has been reported in a few species of ungulates, such as the roe deer (*Capreolus capreolus*; Aragon et al. 1998) and the sika deer (*Cervus nippon*; Ozaki et al. 2007), as well as in studies on other mammalian taxa, including, e.g., the punaré rat (*Thrichomys apereoides*; Monteiro et al. 2003), the wood mouse (*Apodemus sylvaticus*; Renaud and Michaux 2003), and the raccoon dog (*Nyctereutes procyonoides*; Haba et al. 2008).

The moose (*Alces alces*) is a large, sexually size-dimorphic, boreal ungulate with a vast area of distribution throughout the Holarctic region (Garel et al. 2006). Currently, the species is divided into up to eight subspecies displaying differences in body size, coloration, and behavior, some of which are considered as ecological adaptations to the major habitat types within the moose range, i.e., tundra, boreal forests, and alpine areas (Peterson 1955; Geist 1987; Hundertmark and Bowyer 2004; Lister 2005). However, the masticatory system of the moose is globally regarded to show strict specialization (Breda 2010). Described as a concentrate selector and a browser herbivore (Hofmann et al. 1985; Hofmann 1989), the modern moose feeds mainly on various species of herbs, shrubs and sprigs during the

summer, whereas the shoots and twigs of trees and shrubs compose its more monotonous wintertime diet (Telfer 1984; Shipley et al. 1998; Mysterud 2000; Wam et al. 2010; Milligan and Koricheva 2013). However, fossil mandibles of ancient moose discovered in western Siberia from the late Pleistocene - Holocene epochs were indicative of adaptation to a steppe environment and mastication of dry and coarse food (Vasiliev 2011). So far, the question whether the modern moose exhibits any significant intra-specific variation in mandible shape on a geographic scale has remained unexplored. Yet, the remarkably varying environmental conditions within the present moose range would imply possible local adaptations.

The aim of the current study was to investigate whether we could detect geographic variation in mandible shape of the moose in Finland, northern Europe, residing between 60 and 70 degrees latitude with a clear bioclimatic south-north gradient (Fig. 1; Karlsen et al. 2006). Additionally, indications of possible local adaptations were inspected. The Finnish moose population is genetically divided into three latitudinal clusters, separated by admixture zones (Fig 1; Kangas et al. 2015). The emergence of these clusters was estimated to have occurred ~4,500 years ago, some thousands of years after the postglacial re-colonization of Finland. It is still unknown whether these particular clusters exhibit any phenotypic differences. Previous studies support a significant phylogenetic effect in mandible shape disparity in the ungulates at the inter-specific level (e.g., Pérez-Barbería and Gordon 1999; Raia et al. 2010). Furthermore, genetic structure was recently suggested to be reflected in the geographic variation of phenotypic traits in the moose (Herfindal et al. 2014). Therefore, we assessed whether the genetic background of the studied moose individuals plays a role in the formation of the mandible shape.

Material and methods

Data

We utilized a museum collection (maintained by the Zoological Museum of the University of Oulu) of the right side mandibles gathered from legally hunted moose across the whole of Finland in 1998 (Fig. 1). Altogether, 179 age-defined individuals, 93 males (mean age = 4.7 years) and 86 females (mean age = 7.0 years), with intact mandibles and fully developed incisors and molars were selected for the morphometric analyses. The age determination was performed in Matson's Laboratory (Montana, USA) from the root of the first or the second incisor according to the method of Sergeant and Pimlott (1959). The younger age of male moose in the data reflects the actual age structure of the Finnish moose population, where the sexually selective harvesting has caused the mean ages of males and females to diverge (Nygrén 2009).

All the sampled moose individuals have been previously genotyped at 15 microsatellite loci (see full details in Kangas et al. 2015) as part of a larger data set. These data were analyzed with the spatially explicit Bayesian clustering method implemented in the program TESS (Chen et al. 2007; Durand et al. 2009), where all the individuals were given an assignment probability into each of the three genetic clusters detected: southwestern, eastern, or northern (Fig. 1). Here, we divided the sampled individuals into these clusters according to the estimated probabilities (also called membership coefficients) using a threshold of 0.5. With this division, altogether 91 individuals were assigned to the southwestern cluster, 52 to the eastern cluster and 24 to the northern cluster. The remaining 12 admixed un-assigned individuals formed a fourth group.

Morphometric analyses

The shape of the mandibles was studied using landmark-based geometric morphometrics (Bookstein 1997; Zelditch et al. 2012). The labial view of each specimen was photographed in a standardized manner and altogether 28 points, composed of ten landmarks and 18 sliding semilandmarks, were digitized on the images to depict the mandible shape using the program TPSDIG2 (Rohlf 2013a;). The fixed landmarks (1, 6, 7, 8 12, 13, 15, 22, 26, and 27) were: 1) the tip of the coronoid process, 6) the posterior alveolar margin of the sixth molar (m6), 7) the border of the alveolar margins between m3 and m4, 8) the anterior alveolar margin of m1, 12) the posterior margin of the fourth incisor alveolus, 13) the anterior margin of the first incisor alveolus, 15) the posterior edge of the mental foramen, 22) the curve between the ramus and the body of the mandible, 26) the posterior tip of the condyle, and

27) the ventral edge of the mandibular notch, as illustrated with sample no. SK4671-98 in Fig. 2 (SK refers to Satakunta, a region in southwestern Finland where the specimen was collected; the sample is stored with this identification number in the mammalian mandible collection of the Zoological Museum of the University of Oulu). Generalized Procrustes superimposition of the landmark configurations was performed in TPSRELW (Bookstein 1997; Rohlf 2013b) to remove all the non-shape variation, i.e., variation due to size, translation, and rotation, from the data. At the same time, a consensus configuration of landmarks across all specimens was computed and partial warps scores (shape variables depicting localized shape change), two uniform components, and centroid size values were obtained.

Although the superimposition removes the effects of isometric growth, the effects of allometry remain (Zelditch et al. 2012). Therefore, all the partial warps and uniform components in the full data were regressed against the centroid size in order to remove the potential effects of allometric growth on the moose mandible (Sæther 1983, Stelkens et al. 2012). The residuals of each regression were then subjected to principal component (PCA) analysis in R (R Core Team 2013), after which the retained principal components (PCs, with a minimum proportion of variance of 0.075), were visualized by deformation grids (deformations of the consensus landmark configuration corresponding to the PC scores) in order to interpret the shape changes. These grids were obtained using the software TPSREG 1.3 (Rohlf 2011), performing multivariate regression of the shape (captured by the partial warp scores and two uniform shape components) onto the retained PCs.

In order to estimate the possible measurement error introduced during the photographing and landmark digitizing procedure, the mandibles of 30 randomly selected moose individuals were photographed twice. After landmark placement, superimposition, and PCA, the values were entered into one-way analysis of variance in R with PCs set as dependent variables and individual as a factor. The measurement error was then calculated as the percentage of within-individual variance of the total variance (Yezerinac et al. 1992).

Statistical analyses

We first ran a multivariate linear model on the partial warps and uniform components to analyze general patterns of shape variation in the moose mandible with sex, age, latitude, and longitude as well as their interactions set as fixed factors. Subsequently, we applied separate linear mixed models (function 'lmer' in the package lme4 in R, Bates et al. 2014) on the retained PCs. One after another, each of them was set as a dependent variable with all possible combinations of fixed variables (sex, age, latitude, longitude) and their interactions, while including the genetic cluster as a constant random factor (function 'dredge' in the package MuMIn; Bartoń 2012). The residuals of the models were

visually examined to assure acceptable fit. All the models were ranked on the basis of the Akaike information criterion (AIC) for goodness-of-fit (Burnham and Anderson 2002). In case the AIC was not able to rank the models unambiguously (ΔAIC < 2), we utilized an information criterion-based model averaging for the top models (function 'model.avg' in the package MuMIn) to acquire the definitive models and then to assess the relative importance of the fixed effects. All the explanatory variables were centered and standardized in the model averaging analyses to enable the correct interpretation of the main effects in case of significant interactions (see Schielzeth 2010). The model averaging approach was mainly used for finding the most important factors explaining the shape variation. In further analyses and graphics, we considered the factors that were significant and relatively important (the sum of the Akaike weights over all of the models in which the term appears being > 0.75) in the model averaging results (without shrinkage, i.e., averaging over only models in which the variable is present; Burnham and Anderson 2002). Excluding the random factor (see Results), the effects of the significant fixed factors on the shape were graphically described by applying the 'predict' function in R; the predicted response was plotted on the minimum to maximum scale of the explanatory variable while keeping the other explanatory variables constant, either at their mean values, or for categorical factors (e.g., sex) at a specific value. As we had a constant random effects structure in the lmer-analyses, we used maximum likelihood (ML) in model selection for the fixed effects structure (e.g., Bolker et al. 2008).

The relationship between the morphological and genetic variation among moose was investigated further with partial Mantel test (Mantel 1967; Smouse et al. 1986) implemented by the software PASSAGE2 (Rosenberg and Anderson 2011) while controlling for the geographic distances among the studied individuals. As a morphological trait, we used pairwise Procrustes distances calculated from the landmark configurations in TPSSMALL 1.02 (Rohlf 1997), whereas the pairwise genetic distances were computed from the microsatellite data in GENALEX 6.5 (Peakall and Smouse 2012). The analysis was repeated separately for males and females and statistical significances were tested with 10,000 permutations.

The existence and direction of possible geographic gradients in the mandible shape was further examined with the bearing analysis (Falsetti and Sokal 1993; Fruciano et al. 2011), also implemented by PASSAGE2. Put simply, this method determines the geographic direction of greatest correlation between morphometric distances (calculated as Procrustes distances) and geographic distances, and the statistical significance is tested with a standard Mantel permutation test (10,000 permutations).

Results

The multivariate general linear model revealed significant overall variation in the moose mandible shape; all the four fixed factors had a significant effect (age: Wilk's $\lambda = 0.456$, $P = 3.309^{-6}$; sex: Wilk's $\lambda = 0.385$, $P = 2.710^{-9}$; latitude: Wilk's $\lambda = 0.472$, $P = 1.205^{-5}$; longitude: Wilk's $\lambda = 0.607$, $P = 0.039$) in addition to two significant interactions (age x sex: Wilk's $\lambda = 0.596$, $P = 0.024$; age x latitude: Wilk's $\lambda = 0.559$, $P = 0.004$).

After the PCA, altogether three variable components were retained with the following proportions of variance: 0.324, 0.131, and 0.076. The corresponding measurement error values calculated with the subset of 30 individuals for these variables were: 1.01%, 3.38%, and 4.60%. The measurement error was considered not to have a notable effect on the obtained results.

The existence of geographic trends in the moose mandible shape was supported by the most fit linear mixed model explaining the variable PC1, which included the significant effects of sex, latitude, and longitude (Table 1); this variable was positively related to latitude but negatively to longitude, and females had a higher intercept than males (Fig. 3). However, the addition of longitude alongside with sex and latitude did not improve the final model according to an ANOVA comparison ($F= 3.239$, $P = 0.073$); thus its effect was considered to be minor. The deformation grids showed PC1 to be associated with a change in the width of the mandibular angle, orientation of the ascending ramus, the length and orientation of the coronoid process, and the angularity of the corpus (Fig. 3). Moreover, as the PC1 value increases with increasing latitude the mandibular angle widens and the ascending ramus becomes less posteriorly inclined. For PC2, the only statistically significant effect was that of age (Table 1); the decreasing values of PC2 along the increasing age included a shift in the horizontal position of the mental foramen, a decrease in the width of the coronoid process, and thickness and angularity of the anterior part of the corpus as well as a change in the roundness of the mandibular angle (Fig. 4). Respectively, the decreasing values of PC3 were associated with a shortening of the coronoid process and widening of the mandibular angle (Fig. 1 Online Resource 1). However, neither the applied explanatory variables nor their interactions were able to explain the changes described by PC3 (Table 1).

The significance of genetic clustering on the mandible shape variation as a random effect was very low, as revealed by the marginal variance component in the lmer-analyses (Table 1 in Online Resource 1). Therefore, the random factor was left out from the predicted response estimations. In comparison, the partial Mantel test performed between the morphometric and genetic distance matrices suggested no correlation for males $r = 0.036$, two-tailed $p = 0.477$, whereas a weak but statistically significant positive relationship was detected for females ($r = 0.140$, two tailed $p = 0.020$).

The bearing analysis found a significant relationship between the mandible shape and geography: the strongest positive correlation ($r = 0.083$, $p = 0.014$; Fig. 2 in Online Resource 1) between the morphometric and geographic distances at 145° clockwise from the north, which resides between directions of southeast to northwest and south-southwest to north-northwest, respectively (Fig. 1). In contrast, the strongest negative correlations were discovered at 25-30° ($r = -0.066$, $p = 0.030$; Fig 2. in Online Resource 1) corresponding close to direction of south-southwest to North-northeast.

Discussion

With the help of geometric morphometrics, we revealed a significant geographic cline in the shape of the moose mandible in a northern population. The main pattern was similar in both sexes; however, there was a consistent sexual difference in the shape over the spatial scale. Depending on the applied method, no or only a weak association was found between the morphometric variation and the population genetic structure detected with neutral molecular markers (Kangas et al. 2015). Additionally, more subtle, yet statistically significant age-related shape variation was discovered.

Latitudinal variation in morphology is probably one of the most common patterns found in ungulates. For example, in northern Europe, moose have been shown to follow Bergmann's rule (Bergmann 1848 in Mayr 1963) of increasing body size towards the north, a phenomenon considered an adaptation to the colder environment (Herfindal et al. 2006; Nygrén et al. 2007; Lundmark 2008). In a similar fashion, latitude was the main geographic factor in explaining the mandible shape variation of moose in the present data set. The lesser effect of longitude was shown as a slight deviation from the strict north-to-south direction in the bearing analysis, which brought further evidence for the existence of a significant spatial trend.

In Finland, latitude correlates strongly with a number of environmental factors, such as the mean annual temperature and snow depth during the winter. These factors in turn affect the annual growing season, which is significantly shorter in the north of the country compared with the south (Karlsen et al. 2006). As a consequence, the northern moose are dependent on dormant woody plants for notably longer periods of time during the year (Swihart and Bryant 2001). In fact, the latitudinal gradient in the hardness and toughness of diet does provide a feasible explanation for the observed main pattern in the moose mandible shape. The most substantial changes occur in the coronoid process and the mandibular angle (Fig. 3), which are the attachment points of the temporalis and the masseter/pterygoid group muscles, respectively. These muscles control biting and mastication and therefore play an important role in grinding and chewing of food (Pérez-Barbería and Gordon 1999). Moreover, the widening of the mandibular angle implies enlarging masseter and pterygoid muscles (Greaves 1991) and thus more effective mastication better suited for harder and tougher food towards the north. This, in turn, would suggest that the shape shift is adaptive. Additionally, the more vertically oriented ascending ramus and coronoid process (Fig. 3) can possibly enhance bite force of the northern moose by increasing the moment arm of the temporalis muscle as well as reducing its resistance moment arm, according to the lever model by Pérez-Barbería and Gordon (1998). Moreover, the change in the orientation of the coronoid can also change the muscle line of action of the temporalis, and thus its mechanical advantage and efficiency. However, the

question whether the shift in shape provides actual evolutionary adaptive advantage and hence an increase in fitness is well beyond the scope of this study to answer.

The mammalian mandible is a developmentally complex structure resulting from a number of different factors. According to the quantitative genetic model by Atchley and Hall (1991), the development of the mandible is affected by intrinsic genetic, epigenetic, postnatal nursing, and non-heritable environmental factors, as well as their interactions. Interestingly, the observed geographic trend in the moose mandible shape was not influenced by the genetic background of the studied individuals, resembling the previous findings on other taxa by Renaud and Michaux (2003) and Caumul and Polly (2005), for example. In addition, only a weak relationship between the pairwise morphological and genetic distances was discovered in female moose with the partial Mantel test. These results contradict the suggestion of Herfindal et al. (2014) according to which the genetic structure might play an important role in the distribution of phenotypic variation in the moose. Consequently, the questions of whether the mandible shape presents only an exception to a general pattern, and whether the three detected genetic clusters of the Finnish moose exhibit any distinct phenotypic differences, require further attention.

Instead of genetic structure, our discovery suggests that the shift in the shape of the mandible results more likely from other factors, such as differential selection or environmentally induced plastic response. Interestingly, previous studies on mice and shrews have linked plasticity in the mandible development to formation of shape variation and local ecological adaptations. More particularly, foraging and diet consistency have been shown to have a significant impact on the growth and shape remodelling of the mandible as bones respond to their functional environment (Renaud et al. 2010; Young and Badyaev 2010). Furthermore, the shift in the shape caused by hard food, requiring a higher bite force, was recently proven to enhance the biomechanical abilities of the mouse jaw and increase its adaptation to its function, i.e., more powerful mastication (Anderson et al. 2014). In this sense, it could be that the latitudinal shape trend observed here in the moose reflects a plastic response to a gradient in diet consistency. A similar type of geographic variation in mandible morphology has been reported, e.g., in the roe deer (Aragon et al. 1998), the wood mouse (Renaud and Michaux 2003), and the punaré rat (Monteiro et al. 2003). In all three cases, the cause for the shape change was considered to be environment and diet-induced. Unfortunately, detailed data on the composition and consistency of the annual diet of the moose in a geographic scale is not available to address the issue further. On the whole, more extensive studies are needed in order to separate the total contribution of the genetic and environmental components to the mandible shape variation in this species. One possible and rather simple approach would be to investigate whether a similar trend can be observed in the mandibles of calves, i.e., individuals that have not been subjected to a winter diet.

Moose exhibit clear sexual size dimorphism as well as behavioral differences in habitat selection, use, and foraging (Nikula et al. 2004; Garel et al. 2006; Bjørneraas et al. 2011; Bjørneraas et al. 2012). Barboza and Bowyer (2000) hypothesized that the sexual segregation in dimorphic ungulates is a consequence of the differing life-histories and energetic needs among males (body and antler growth) and females (gestation and lactation). As the differences in the diet can induce phenotypic changes in the mandible through biomechanical function (Anderson et al. 2014), it is possible that the clear differences in the mandible shape between the two sexes reflect their different dietary optimum and foraging behavior (Barboza and Bowyer 2000). An alternative (or additional) process is brought about by the seasonal growth and presence of antlers in males, plausibly affecting the structure of the cranium (Bartosiewicz 1987; Nygrén et al. 1992a, 1992b). This effect could be mediated further to the shape of the mandible, causing structural dimorphism between the sexes. In addition to sexual difference, subtle age-related changes in the shape of the coronoid and the position of the mental foramen were revealed (Table 1, Fig. 1 and 2 in Online Resource 1). The peculiar reduction in the size of the coronoid with increasing age might be explained by a relatively stronger growth of the rest of the mandible (Sæther 1983), i.e., allometry, which still remains despite the size correction performed.

The present study provided the first evidence of a significant geographic mandibular shape shift in the modern moose. Thus, it seems that the mandible shape is more flexible than previously anticipated. We likely have managed only to scratch the surface of the true quantity of the morphological variation and plausible adaptive potential possessed by this species. These features together with the very flexible behavior (e.g., Bjørneraas et al. 2011, 2012; Eldegard et al. 2012; Melin et al. 2014) would give at least a partial explanation for the success and extensive range of the moose.

References

Adams DC, Rohlf FJ, Slice DE (2004) Geometric morphometrics: ten years of progress following the 'revolution'. Ital J Zool 71: 5–16

Anderson PS, Renaud S, Rayfield EJ (2014) Adaptive plasticity in the mouse mandible. BMC Evol Biol 14: 85

Aragon S, Braza F, San Jose C, Fandos P (1998) Variation in skull morphology of roe deer (*Capreolus capreolu*s) in Western and Central Europe. J Mammal 79: 131–140

Atchley WR (1993) Genetic and developmental aspects of variability in the mammalian mandible. In: Hanken J, Hall BK (eds) The Skull. The University of Chicago Press, Chicago, pp 207–247

Atchley WR, Hall BK (1991) A model for development and evolution of complex morphological structures. Biol Rev 66: 101–157

Barboza PS, Bowyer RT (2000) Sexual segregation in dimorphic deer: a new gastrocentric hypothesis. J Mammal 81: 473–489.

Bartoń K (2012) MuMIn: multi–model inference. R package version 1.2. URL: http://CRAN.R-project.org/package=MuMIn

Bartosiewicz L (1987) Sexual dimorphism in the cranial development of Scandinavian moose (*Alces alces* (L.) *alces*). Can J Zool 65: 747–750

Bates D, Maechler M, Bolker B, Walker S (2015) lme4: Linear mixed-effects models using Eigen and S4. R package version 1.1–8. URL:http://CRAN.R–project.org/package=lme4

Bergmann C (1848) Über die Verhältnisse der Wärmeökonomie der Thiere zu ihrer Grösse. Gottingen studier 3: 595–708

Bjørneraas K, Herfindal I, Solberg EJ, Sæther B, van Moorter B, Rolandsen CM (2012) Habitat quality influences population distribution, individual space use and functional responses in habitat selection by a large herbivore. Oecologia 168: 231–243

Bjørneraas K, Solberg EJ, Herfindal I, Moorter BV, Rolandsen CM, Tremblay J, Skarpe C, Sæther B, Eriksen R, Astrup R (2011) Moose *Alces alces* habitat use at multiple temporal scales in a human-altered landscape. Wildl Biol 17: 44–54

Bookstein FL (1997) Landmark methods for forms without landmarks: morphometrics of group differences in outline shape. Med Image Anal 1: 225–243

Breda M (2010) Palaeoecology and palaeoethology of the Plio-Pleistocene genus *Cervalces* (Cervidae, Mammalia) in Eurasia. J Vertebr Paleontol 28: 886–899

Burnham KP, Anderson DR (2002) Model Selection and Multimodel Inference: A Practical Information–theoretic Approach. Springer Science & Business Media, USA

Caumul R, Polly PD (2005) Phylogenetic and environmental components of morphological variation: skull, mandible, and molar shape in marmots (Marmota, Rodentia). Evolution 59: 2460–2472

Chen C, Durand E, Forbes F, François O (2007) Bayesian clustering algorithms ascertaining spatial population structure: a new computer program and a comparison study. Mol Ecol Notes 7: 747–756

Durand E, Jay F, Gaggiotti OE, François O (2009) Spatial inference of admixture proportions and secondary contact zones. Mol Biol Evol 26: 1963–1973

Eldegard K, Lyngved JT, Hjeljord O (2012) Coping in a human–dominated landscape: trade–off between foraging and keeping away from roads by moose (*Alces alces*). Eur J Wildl Res 58: 969–979

Fraser D, Theodor JM (2011) Comparing ungulate dietary proxies using discriminant function analysis. J Morphol 272: 1513–1526

Fruciano C, Tigano C., Ferrito V (2011) Geographical and morphological variation within and between colour phases in *Coris julis* (L. 1758), a protogynous marine fish. Biol J Linn Soc 104: 148–162

Garel M, Solberg EJ, SÆther B, Herfindal I, Høgda K (2006) The length of growing season and adult sex ratio affect sexual size dimorphism in moose. Ecology 87: 745–758

Geist V (1987) On the evolution and adaptations of *Alces*. Swed Wildl Res Suppl 1: 11–23

Geist V (1998) Deer of the World: Their Evolution, Behaviour, and Ecology. Stackpole Books, Mechanicsburg

Ghalambor CK, McKay JK, Carroll SP, Reznick DN (2007) Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. Funct Ecol 21: 394–407

Greaves WS (1991) The orientation of the force of the jaw muscles and the length of the mandible in mammals. Zool J Linn Soc 102: 367–374

Haba C, Oshida T, Sasaki M, Endo H, Ichikawa H, Masuda Y (2008) Morphological variation of the Japanese raccoon dog: implications for geographical isolation and environmental adaptation. J Zool 274: 239–247

Herfindal I, Haanes H, Solberg EJ, Røed KH, Høgda KA, Sæther B (2014) Moose body mass variation revisited: disentangling effects of environmental conditions and genetics. Oecologia 174: 447–458

Herfindal I, Solberg EJ, Sæther B, Høgda KA, Andersen R (2006) Environmental phenology and geographical gradients in moose body mass. Oecologia 150: 213–224

Hofmann R (1989) Evolutionary steps of ecophysiological adaptation and diversification of ruminants: a comparative view of their digestive system. Oecologia 78: 443–457

Hofmann R, Fennessy P, Drew K (1985) Digestive physiology of the deer-their morphophysiological specialisation and adaptation. In: Fennessy PF, Drew KR (eds) Biology of Deer Production. Roy Soc of New Zealand Bull 22: 393–407

Hofmann R, Stewart D (1972) Grazer or browser: a classification based on the stomach-structure and feeding habits of East African ruminants. Mammalia 36(2): 226–240

Hundertmark KJ, Bowyer RT (2004) Genetics, evolution, and phylogeography of moose. Alces 40: 103–122

Kangas VM, Kvist L, Kholodova M, Nygrén T, Danilov P, Panchenko D, Fraimout F, Aspi J (2015) Evidence of post-glacial secondary contact and subsequent anthropogenic influence upon the genetic composition of Fennoscandian moose (*Alces alces*). J Biogeogr 42: 2197–2208

Karlsen SR, Elvebakk A, Høgda KA, Johansen B (2006) Satellite-based mapping of the growing season and bioclimatic zones in Fennoscandia. Global Ecol Biogeogr 15: 416–430

Klingenberg CP, Leamy LJ, Cheverud JM (2004) Integration and modularity of quantitative trait locus effects on geometric shape in the mouse mandible. Genetics 166: 1909–1921

Klingenberg CP, Mebus K, Auffray JC (2003) Developmental integration in a complex morphological structure: how distinct are the modules in the mouse mandible? Evol Dev 5: 522–531

Lister AM (2005) Subspecies differentiation among moose (*Alces alces* (L.)): geographical variation in cranial morphology. Quaternaire Suppl 2: 31–37

Lundmark C (2008) Morphological and behavioral adaptations of moose to climate, snow, and forage. Dissertation. Swedish University of Agricultural Sciences, Umeå

Mantel N (1967) The detection of disease clustering and a generalized regression approach. Cancer Res 27: 209–220

Mayr E (1963) Animal Species and Evolution. Harvard University Press, Cambridge

Melin M, Matala J, Mehtätalo L, Tiilikainen R, Tikkanen O, Maltamo M, Pusenius J, Packalen P (2014) Moose (*Alces alces*) reacts to high summer temperatures by utilizing thermal shelters in boreal forests–an analysis based on airborne laser scanning of the canopy structure at moose locations. Glob Change Biol 20: 1115–1125

Milligan HT, Koricheva J (2013) Effects of tree species richness and composition on moose winter browsing damage and foraging selectivity: an experimental study. J Anim Ecol 82: 739–748

Monteiro LR, Duarte LC, Reis SF (2003) Environmental correlates of geographical variation in skull and mandible shape of the punaré rat *Thrichomys apereoides* (Rodentia: Echimyidae). J Zool 261: 47–57

Monteiro LR, Nogueira MR (2010). Adaptive radiations, ecological specialization, and the evolutionary integration of complex morphological structures. Evolution 64: 724–744

Mysterud A (2000) Diet overlap among ruminants in Fennoscandia. Oecologia 124: 130–137

Nikula A, Heikkinen S, Helle E (2004) Habitat selection of adult moose *Alces alces* at two spatial scales in central Finland. Wildl Biol 10: 121–135

Nygrén K, Silvennoinen R, Kärnä M (1992a) Masticatory stress on cranial bones; a holographic study. Proc Soc Photo-Opt Ins 1647: 164–168

Nygrén K, Silvennoinen, R, Kärnä, M (1992b) Antler stress in the nasal bone region of moose. Alces Suppl 1: 84–90

Nygrén T (2009) Biology and policies in Finnish moose population regulation and management. Dissertation. University of Joensuu, Joensuu

Nygrén T, Pusenius J, Tiilikainen R, Korpelainen J (2007) Moose antler type polymorphism: age and weight dependent phenotypes and phenotype frequencies in space and time. Ann Zool Fenn 44: 445–461

Ozaki M, Suwa G, Kaji K, Ohba T, Hosoi E, Koizumi T, Takatsuki S (2007) Correlations between feeding type and mandibular morphology in the sika deer. J Zool 272: 244–257

Peakall R, Smouse PE (2012) GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research - an update. Bioinformatics 28: 2537-2539

Peterson RL (1955) North American Moose. University of Toronto Press, Toronto

Pérez-Barbería FJ, Gordon IJ (1999) The functional relationship between feeding type and jaw and cranial morphology in ungulates. Oecologia 118: 157–165

Prevosti FJ, Turazzini GF, Ercoli MD, Hings-Zaher E (2012). Mandible shape in marsupial and placental carnivorous mammals: a morphological comparative study using geometric morphometrics. Zool J Linn Soc-Lond 164: 836–855

R Core Team (2013) R: A language and environment for statistical computing, version 3.0.2. R Foundation for Statistical Computing, Vienna

Raia P, Carotenuto F, Meloro C, Piras P, Pushkina D (2010) The shape of contention: adaptation, history, and contingency in ungulate mandibles. Evolution 64: 1489–1503

Renaud S, Auffray JC, de la Porte S (2010) Epigenetic effects on the mouse mandible: common features and discrepancies in remodeling due to muscular dystrophy and response to food consistency. BMC Evol Biol 10: 28

Renaud S, Michaux JR (2003) Adaptive latitudinal trends in the mandible shape of *Apodemus* wood mice. J Biogeogr 30: 1617–1628

Rohlf JF (1997) tpsSmall, version 1.02. Department of Evolution and Ecology, State University of New York, Stony Brook

Rohlf JF (2011) tpsRegr, version 1.41. Department of Evolution and Ecology, State University of New York, Stony Brook

Rohlf JF (2013a) tpsDig2, version 2.17. Department of Evolution and Ecology, State University of New York, Stony Brook

Rohlf JF (2013b) tpsRelw, version 1.53. Department of Evolution and Ecology, State University of New York, Stony Brook

Rohlf JF, Marcus LF (1993) A revolution morphometrics. Trends Ecol Evol 8: 129–132

Rosenberg MS, Anderson CD (2011) PASSaGE: pattern analysis, spatial statistics and geographic exegesis. Version 2. Methods Ecol Evol 2: 229-232

Schielzeth H (2010) Simple means to improve the regression coefficients. Methods Ecol Evol 2010: 103–113

Sergeant DE, Pimlott DH (1959) Age determination in moose from sectioned incisor teeth. J Wildl Manage 23: 315–321

Shipley L, Blomquist S, Danell K (1998) Diet choices made by free-ranging moose in northern Sweden in relation to plant distribution, chemistry, and morphology. Can J Zool 76: 1722–1733

Smouse PE, Long JC, Sokal RR (1986) Multiple regression and correlation extensions of the Mantel test of matrix correspondence. Syst Zool 35: 627–632

Stelkens RB, Jaffuel G, Escher M, Wedekind C (2012) Genetic and phenotypic population divergence on a microgeographic scale in brown trout. Mol Ecol 21: 2896–2915

Swihart RK, Bryant JP (2001) Importance of biogeography and ontogeny of woody plants in winter herbivory by mammals. J Mammal 82: 1–21

Telfer ES (1984) Circumpolar distribution and habitat requirements of moose (*Alces alces*). In: Olson R, Hastings R, Geddes F (eds) Northern Ecology and Recource Management. The University of Alberta Press, Edmonton, pp 154–182

Vasiliev S (2011) Morphological and ecological characteristics of moose, *Alces* cf. *alces* (Artiodactyla), from the southeast of Western Siberia in the late Neopleistocene and Holocene. Biol Bull 38: 754–764

Wam HK, Hjeljord O, Solberg EJ (2010) Differential forage use makes carrying capacity equivocal on ranges of Scandinavian moose (*Alces alces*). Can J Zool 88: 1179–1191

Yezerinac SM, Lougheed SC, Handford P (1992) Measurement error and morphometric studies: statistical power and observer experience. Syst Biol 41: 471–482

Young RL, Badyaev AV (2010) Developmental plasticity links local adaptation and evolutionary diversification in foraging morphology. J Exp Zool Part B 314: 434–444

Zelditch ML, Swiderski DL, Sheets HD (2012) Geometric Morphometrics for Biologists: A Primer. Elsevier, Waltham, Massachusetts

Tables

Table 1. Model-averaged fixed effects and their relative (Rel.) importance of linear models explaining variation in mandible shape variable principal components 1–3. Statistically significant effects are highlighted in bold.

Variable	Estimate	SE	*z*	*p*	Rel. importance
PC1					
(Intercept)	0.00011	0.00096	0.111	0.912	
Sex	**0.00556**	**0.00187**	**2.950**	**0.003**	**1.00**
Lat	**0.00482**	**0.00185**	**2.592**	**0.010**	**1.00**
Lon	**-0.00395**	**0.00196**	**2.007**	**0.045**	**0.94**
Lat x Lon	-0.00754	0.00452	1.655	0.098	0.65
Age	0.00156	0.00227	0.682	0.495	0.59
Age x Lon	0.00634	0.00387	1.628	0.104	0.37
Sex x Age	0.00604	0.00461	1.300	0.194	0.26
Sex x Lon	-0.00415	0.00394	1.044	0.296	0.21
PC2					
(Intercept)	-0.00009	0.00059	0.147	0.883	
Age	**-0.00584**	**0.00135**	**4.292**	**<0.001**	**1.00**
Lat	0.00162	0.00112	1.431	0.153	0.65
Age x Lat	-0.00283	0.00256	1.095	0.274	0.50
Sex	-0.00083	0.00119	0.697	0.486	0.32
Sex x Age	0.00445	0.00282	1.566	0.117	0.17
PC3					
(Intercept)	-0.00023	0.00066	0.345	0.730	
Lat	-0.00135	0.00091	1.476	0.140	0.18
Age	-0.00058	0.00091	0.628	0.530	0.16
Lon	0.00066	0.00101	0.650	0.516	0.16

Lat=latitude, Lon=longitude.

Figure legends

Fig. 1. The sampling localities of moose in Finland. Black circles represent individuals assigned to the southwestern, white to the eastern, and grey to the northern cluster, respectively, whereas black triangles stand for un-assigned individuals. The black two-headed arrow points in the direction of the strongest positive correlation between the morphometric and geographic distances, according to the bearing analysis.

Fig. 2. Mandible of a moose (museum specimen number: SK4671-98) with landmarks 1−28 (A) and points of the consensus plot (B). Points 1, 6, 7, 8 12, 13, 15, 22, 26, and 27 were defined as fixed landmarks and the rest as sliding landmarks. C.P = coronoid process, M.A. = mandibular angle.

Fig. 3. Relationship between the shape variable principal component 1 (PC1) and latitude together with mandible shape deformation grids at the positive and negative extreme values of the PC1.

Fig. 4. Relationship between the shape variable principal component 2 (PC2) and moose age together with mandible shape deformation grids at the positive and negative extreme values of the PC2.