Morphometric variations at an ecological scale: Seasonal and local variations in feral and commensal house mice

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Abstract

The time scales of evolutionary and ecological studies tend to converge, due to increasing evidences of contemporary evolution occurring as fast as ecological processes. This ranges new questions regarding variation of characters usually considered to change mostly along the evolutionary time scale, such as morphometric traits, including osteological and dental features such as mandibles and teeth of mammals. Using two-dimensional geometric morphometric approach, we questioned whether such features can change on a seasonal and local basis, in relation with the ecological dynamics of the populations. Our model comprised populations of house mice (*Mus musculus domesticus*) in two contrasted situations in mainland Western Europe: a feral population vs. two close commensal populations. Mitochondrial DNA (D-loop) provided insight into the diversity and dynamics of the populations.

The feral population appeared as genetically highly diversified, suggesting a possible functioning as a sink in relation to the surrounding commensal populations. In contrast, commensal populations were highly homogeneous from a genetic point of view, suggesting each population to be isolated. This triggered morphological differentiation between neighboring farms. Seasonal differences in morphometric traits (mandible size and shape and molar size and shape) were significant in both settings, although seasonal variations were of larger amount in the feral than in the commensal population. Seasonal variations in molar size and shape could be attributed to differential wear in young or overwintered populations. Differences in mandible shape could be related to aging in overwintered animals, but also possibly to differing growth conditions depending on the season. The impact of these ecological processes on morphometric traits is moderate compared to divergence over a large biogeographic scale, but their significance nevertheless underlines that even morphological characters may trace populations dynamics at small scale in time and space.

Keywords

Mus musculus domesticus; Murinae; rodent; geometric morphometrics; mandible; molar shape; phylogeny; D-loop.

Introduction

Issues of important current concern, such as adaptation of invasive species to their new environments, response of local species to invasions and/or climate change, deliver growing evidences that evolution can take place at time scales usually considered to be characteristic of ecological processes (Kinnison & Hairston, 2007; Lambrinos, 2004). Morphological characters were traditionally thought to vary at evolutionary timescales. Nevertheless, due to plasticity but also to microevolutionary changes, they can also vary at fast rates and be involved in contemporary evolution (Collyer, Stockwell, Adams, & Reiser, 2007; Ghalambor, McKay, Carroll, & Reznick, 2007). This growing body of evidences may also be due to the development of geometric morphometric methods, allowing to quantify such fine-scale variation. To provide a background for such fine-scale processes, reference studies to assess the amount of variation expected for morphological traits due to background ecological processes, such as seasonal variations in populations, are often lacking because sampling designed for ecology, such as mark-recapture, are not designed for delivering osteological material for morphometric studies.

The aim of this study is to provide a hint into morphometric variations related to small-scale processes such as seasonal variation and local differentiation. The model is the Western house mouse (*Mus musculus domesticus*). It is known for its great adaptability and behavioral flexibility, thus exposing it to contrasted ecological conditions even at small scale in time and space. We investigated two features well studied in mice, having different developmental characteristics and hence potentially responding differently to processes at an ecological scale. The mandible is an osteological structure that grows postnatally, reaching ~95% of its final adult shape shortly after weaning (Swiderski & Zelditch, 2013). Bone remodeling occurs nevertheless during the whole animal's life, and this can trigger plastic differences in response to food resources even during adult, post-weaning life (Anderson, Renaud, & Rayfield, 2014). In contrast, the first upper molar is mineralized during pre-natal life and once erupted, it is only affected by wear. This may make the mandible more prone to respond to short term processes.

The size and shape of both features were quantified using a 2D outline analysis (Renaud, Hardouin, Pisanu, & Chapuis, 2013). Several small-scale sources of variations were investigated. (1) Seasonal variations in two populations sampled successively in time. (2) Way of life, by considering feral and commensal populations. Way of life may interfere with seasonal variation. Commensal mice may reproduce all year round when food of sufficient quality and quantity is available (Berry, 1981; Pocock, Searle, & White, 2004; Pryor & Bronson, 1981; Rowe, Swinney, & Quy, 1983). In contrast, feral populations are more exposed to seasonal variations in climate and food supply, and consequently, they should display seasonal breeding (Berry, 1981; Efford, Karl, & Moller, 1988; Matthewson, Van Aarde, & Skinner, 1994; Triggs, 1991). (3) Local differences between neighboring farms.

Wear stage based on the upper molar row has been proposed to be the best estimate of age for wild populations of mice (Lidicker, 1966). It was used to assess age structure in the different populations. Body size measurements were further used to evaluate body condition of the animals, and to compare the overall growth to the size of the osteological trait (mandible). Genetics (mitochondrial DNA) was assessed for a subset of mice, to describe the phylogenetic background of the investigated populations. Altogether, these informations provided the background to investigate morphometric variations of the mandible and molar through seasons and across populations. Finally, the variations due to seasonality and local differentiation were compared to a set of mainland localities, in order to assess the importance of the small-scale processes compared to larger scale differentiation.

Material

Commensal samples were sampled from two farms in Tourch (Brittany, France). Most mice were trapped in a large pigsty in Kerloyou, in August 2011, November 2011 and May 2012 (Table 1, Fig. 1F). A farm in the nearby place Kerc'hoaler, 1.8 km away, was further sampled in June 2012.

Mice from Frontignan corresponded to an outdoor, feral population found in the Aresquiers spit of land between the Mediterranean Sea and the Étang de Vic, close to Montpellier (France). Mice were trapped in April 2009, October 2009 and March 2010 over less than 1 km (Table 1). Despite the uneven sample size (7, 20 and 4 specimens respectively), the trapping effort was similar for the three seasons. One additional mouse was captured in March 2013.

The commensal population of Gardouch, near Toulouse, was further documented (Renaud, Dufour, Hardouin, Ledevin, & Auffray, 2015). It was trapped in a roe deer enclosure from November 2003 to April 2004 (68 specimens). This context of roe deer breeding provided the mice with food ad libitum, as in the Kerloyou pigsty.

In Frontignan, Tourch and Gardouch, animals were live trapped, weighted and measured for head + body length (HBL) by the same operator (JPQ). Wear stage was evaluated on the upper molar row for all animals, using classes from one (juvenile, third molar not erupted) up to seven (heavily worn molars with all cusps associated on the three molars of the row (Avenant & Smith, 2004). Each molar row was scored twice, and the average of the two scorings was used as proxy for the age of the animals. Although wear pattern may vary from one population to the other, depending on tooth morphology and food processed, it provided a proxy for assessing the age structure of the populations.

All these specimens are housed at the collection of the Centre de Biologie et Gestion des Populations (Baillarguet, France). The animals were sacrificed by cervical dislocation according to the 2010/63/UE directive with an official authorization to JPQ (permit 34–107 from the Préfecture de l'Hérault).

All mice were considered in the morphometric analysis, except fot the only mouse from March 2013 in Frontignan (Table 1; Fig. 1F). Adults and sub-adults animals with their third molars erupted were considered for morphometric analyses. This discarded one juvenile specimen from November 2011 in Kerloyou, Tourch. It was represented on the size plots but discarded from all tests.

Mandible and tooth shape in Tourch, Frontignan and Gardouch was compared to the geographic variation among a set of commensal populations in mainland Western Europe: Montpellier (Southern France), Cologne-Bonn (Germany), Reggiolo, San Bernardino and Lombardy (Northern Italy) (Renaud, Dufour, et al., 2015; Renaud, Gomes Rodrigues, et al., 2015).

Phylogenetics

The morphometrics study was complemented by a genetic analysis of the D-loop (mtDNA) sequences. Twenty-six mice were sequenced in Tourch, corresponding to six randomly sampled for each of the three seasons at the Kerloyou pigsty and eight at the Kerc'hoaler farm (Table 1). Twentytwo mice were sequenced in Frontignan, most of them (14) trapped in October 2009 and the other ones trapped in April 2009 (5) March 2010 (2) and March 2013 (1) (Table 1).

For these samples, DNA was extracted from ethanol-preserved tissue, using the DNeasy Blood and Tissue kit (Qiagen, France). The D-loop was amplified using previously described primers and protocol (Hardouin et al., 2010). The sequences generated were visualized using MEGA6 (Tamura, Stecher, Peterson, Filipski, & Kumar, 2013). The new sequences were deposited in GenBank under accession numbers LT718851 to LT718898 (Supplementary Table 1).
This sampling was completed by sequences of various mainland Western Europe locations retrieved from GenBank (Supplementary Table 2).

Methods

Phylogenenetic analyses

Haplotypes and genetic diversity indices for Frontignan and Tourch sequences were determined with DNAsp v 5 (Librado and Rozas, 2009). The haplotypes were combined with sequences retrieved from GenBank and aligned with MUSCLE implemented in SeaView (Gouy, Guindon, Gascuel, & Lyon, 2010). The final alignments comprised 313 sequences and 833 base pairs. The phylogenetic tree was reconstructed using Bayesian inference (BI) with MrBayes v3.2 (Ronquist et al., 2012). The best model (TrN+I+G) was determined with jModelTest (Darriba, Taboada, Doallo, & Posada, 2012) using the Akaike criterion (AIC) (Akaike, 1973). As TrN model was not available in Mrbayes we used Nst=mixed, which sampled across substitution models. Node robustness were estimated using posterior probabilities (PP) in BI analyses. For BI, two Markov chain Monte Carlo (MCMC) analyses were run independently for 20 000 000 generations. One tree was sampled every 500 generations. The burn-in was graphically determined with Tracer v1.6 (Rambaut, Suchard, Xie, & Drummond, 2014). We also checked that the effective sample sizes (ESSs) were above 200 and that the average SD of split frequencies remained <0.05 after the burn-in threshold. We discarded 10% of the trees and visualized the resulting tree under Figtree v1.4 (Rambaut, 2012).

Mandible and molar outline analysis

Mandibles and tooth rows were pictured using a Leica MZ 9.5 binocular. The hemi-mandible was placed flat on its lingual side. The upper molar row was manually oriented so that the occlusal surface would match the horizontal plane. Based on these pictures, the shape of the mandible and the first upper molar (UM1) were described using 64 points sampled at equal curvilinear distance along their 2D outline (Fig. 1G), sampled using the Optimas software. This sampling is sufficient to document even small structures such as the coronoid process. An outline-based method was chosen because reliable landmarks are difficult to position on the murine molar. The top of the cusps is abraded by wear and cannot be used for assessing the position of the cusps, and landmarks bracketing the cusps on the outline are difficult to position given the smooth undulation delineating the cusps along the outline (Fig. 1). Similarly, landmarks used to describe the murine mandible are all placed along the outline (e.g. (Klingenberg, Mebus, & Auffray, 2003)) and some may be difficult to position when the bone is of smooth shape.

For the mandible, the starting point was positioned at the junction between the incisor and the bone at the upper edge of the mandible. For the first upper molar, the starting point was positioned at the anteriormost part of the tooth, being aligned with the direction of the maximum elongation using the best-fitting ellipse to the outline (Renaud, Auffray, & Michaux, 2006).

From the 64 points, 64 radii (i.e. distance of each point to the center of gravity of the outline) were computed. Expressed as a function of the curvilinear distance along the outline, this set of radii constituted a function that was analyzed using a Fourier analysis (Rohlf & Archie, 1984). By comparing function(s) of a curve, and not the points collected on the outline, Fourier methods allow investigating shapes deprived of landmarks with clear homology (Bonhomme, Picq, Gaucherel, & Claude, 2014; Dujardin et al., 2014). According to Fourier method, the empirical function is decomposed into a sum of trigonometric functions of decreasing wavelength (the harmonics). Each is weighted by two Fourier coefficients (FCs), that constitute the shape variables to be compared among individuals. The zero harmonic (A0) is proportional to the size of the outline. It was used as size estimator, and to standardize all other FCs so that they represent shape only.

The higher the rank of the harmonics, the more details they represent on the outline (Crampton, 1995; Rohlf & Archie, 1984). This can be used to filter measurement error, by discarding high-order harmonics (Renaud, Michaux, Jaeger, & Auffray, 1996). This together reduces the number of variables required to describe the overall morphology of an object. The shape of a mouse mandible or molar tooth has been shown to be adequately described by the first seven harmonics, i.e. by 14 variables (Renaud et al., 2013).

Statistical analyses of morphometric data

Differences between groups (sex, season, or location) were tested using analyses of variance (ANOVA) and associated two-by-two Tukey tests for univariate parameters.

Shape of mandible and molar were each described by a multivariate dataset (14 FCs). A reduction of dimensionality (Sheets, Covino, Panasiewicz, & Morris, 2006) was performed prior to statistical tests using a Principal Component Analysis (PCA) on the variance-covariance matrix of the FCs. The axes explaining more than 5% of the total variance were kept for subsequent statistical tests. Differences between groups were tested by multivariate analyses of variance (MANOVA) and associated pairwise Hotelling's tests.

Associated to PCA, between-group principal component analyses (bgPCA) were also performed. Such analysis provides an estimate of the between- to total-variance ratio, and thus how much the grouping considered explains of the total variance, also including within-group variance. The bgPCA also provides a representation of the group means in a corresponding morphospace. The topology of the group means in this morphospace is close to the topology obtained based on a classical PCA (Renaud, Dufour, et al., 2015).

Relationships between morphological variables and explanatory variables (body size, seasons, and wear stage) were investigated using general linear models.

Euclidean distances in size (based on A0) and shape (based on the set of Fourier coefficients) were calculated between groups (seasons in feral Frontignan and commensal Kerloyou; local differences between Kerloyou and Kerc'hoaler; differences between Frontignan, Tourch, and the localities of comparison). These observed distances were compared to the distribution of distances between populations simulating random differentiation between mainland populations. For this purpose, 1000 populations of 20 and 15 specimens each were randomly sampled from the pool of mainland localities (Renaud et al., 2013).

Statistics were performed using Past (Hammer, Harper, & Ryan, 2001) and R (R_core_team, 2015). Multivariate analyses were performed using ade4 (Dray & Dufour, 2007).

Results

Phylogenetics

The phylogenetic tree (Fig. 2) shows that sequences from Tourch belong to the same clade whereas sequences from Frontignan are in three different ones. The genetic composition of the feral population was very different from that of the two farms from Tourch, Brittany. The feral population of Frontignan was highly diversified, sampling the whole genetic diversity present in this area. It included 11 haplotypes, distributed in 3 different clades of our house mouse phylogeography (Fig. 2).

Half of the haplotypes occurred only once. Haplotypes sampled several times often occurred at different seasons (Table 1; Supplementary Table 1).

In contrast, commensal populations were extremely homogeneous. Only two haplotypes, differing by one insertion, were present at Kerloyou. They occurred in the three seasons. One haplotype only was documented at Kerc'hoaler. It belongs to the same clade as the Kerloyou haplotypes (Fig. 2).

Reduction of dimensionality

Principal component analyses were performed to summarize the shape information on few synthetic axes. Separate PCA were performed for Tourch, Frontignan and Gardouch. Axes representing more than 5% of variance were retained for subsequent statistical tests.

Regarding mandible shape, shape variation was summarized by four PC axes in the three populations (Tourch: PC1 = 42.5%, PC2 = 25.9%, PC3 = 12.9%, PC4 = 7.8%; Frontignan: PC1 = 50.7%, PC2 = 18.0%, PC3 = 14.5%, PC4 = 7.6%; Gardouch: PC1 = 50.6%, PC2 = 22.0%, PC3 = 9.1%, PC4 = 6.9%).

For molar shape, five axes represented more than 5% of variance in Tourch (PC1 = 38.7%, PC2 = 22.3%, PC3 = 8.6%, PC4 = 6.9%, PC5 = 6.2%) and Gardouch (PC1 = 38.9%, PC2 = 19.9%, PC3 = 12.7%, PC4 = 8.4%, PC5 = 6.0%). Four axes explained more than 5% of variance in Frontignan (PC1 = 31.7%, PC2 = 20.3%, PC3 = 18.4%, PC4 = 9.7%).

Sexual dimorphism

Sexual dimorphism was absent for most morphological parameters considered (Table 2). It was weakly significant for mandible size in Tourch, molar size in Frontignan, and molar shape in Gardouch. Because of the absence of a consistent signal of sexual dimorphism among the populations, and because sexual dimorphism was repeatedly shown to be of minor importance in house mice (Renaud et al., 2013; Valenzuela-Lamas, Baylac, Cucchi, & Vigne, 2011), males and females were pooled in subsequent analyses.

Wear pattern

Wear pattern can be considered as a proxy for the age of the animals. Its distribution differed between seasons in the feral population from Frontignan ($P < 0.001$). This corresponds to a difference between the autumn population of October, dominated by relatively young animals, and the spring populations composed of animals with teeth worn down, suggesting overwintered animals (Fig. 3).

The difference between season was not significant in the commensal population from Kerloyou ($P = 0.068$). The summer and autumn populations were mostly composed of young animals, as in Frontignan, including a juvenile without the third molar erupted in August 2011. The spring population included young animals together with overwintered ones.

The autumn to spring population of the commensal population from Gardouch appeared as dominated by young animals with relatively unworn teeth.

Morphometric variations in the commensal populations from Tourch

Mice from the two neighboring farms of Kerloyou and Kerc'hoaler did not differ in body weight or body length (Table 3; Fig. 4). In contrast, they differed in mandible and molar size and shape. This corresponded to a between- to total-variance ratio of 9.0% for the mandible and 10.9% for the UM1 (Table 4).

In Kerloyou, seasons had no impact on body weight, body length, mandible and molar size (Table 3; Fig. 4). Shape differences were however significant, corresponding to differences between the two well sampled populations from summer and autumn 2011 (Table 3). The difference in shape between seasons was smaller, however, than the difference between Kerloyou and Kerc'hoaler: the between- to total-variance ratio associated with seasons was 4.3% for mandible shape and 5.0% for molar shape.

More than a direct effect of the season of trapping, differences between samples in Kerloyou were mostly due to the size and age of the animals, as estimated by wear stage (Table 5). Body size was a significant explanatory variable for body weight, mandible size and mandible shape. Wear stage was significant in explaining body weight, mandible size, mandible shape, and molar shape. Season per se was only marginally significant for mandible shape.

Body size was by far more important than wear stage in explaining mandible size; for other variables however, the effect of both body size and wear stage appeared to be balanced.

Morphometric variations in the feral population from Frontignan

Mice differed between seasons in the feral population from Frontignan for body length but not for body weight or mandible size (Table 3; Fig. 4). They further differed between seasons for molar size, mandible shape and molar shape. This corresponded to a significant difference between samples from autumn (October) and spring (April) 2009, except for molar size which differed between the two spring samples (April 2009 and March 2010).

This between-group shape variation represented 9.4% and 8.9% of the total variance for mandible and molar shape respectively (Table 4). This range of value is thus twice what was observed for the seasonal variations in Kerloyou.

When considering the respective role of body size, season and wear stage as explanatory variables (Table 5), wear stage appeared less important than in Kerloyou. It was significant only for body weight and for molar size. Body size had a significant effect only on body weight and mandible size, for which it was the most important explanatory variable. Season per se had a significant effect on body weight, on which it has the most important effect, mandible size, molar size, and mandible shape.

Allometric relationships in different localities

The allometric relationship between body length and other size-related variables (body weight, mandible and molar size) was further investigated, by comparing the relations observed in the commensal population from Tourch and the feral population from Frontignan to another commensal population, Gardouch.

Body weight and mandible size varied primarily with body length, whereas molar size was only differing according to the locality (Table 6). Regarding body weight, the significant interaction term between the factors body length and locality showed that the allometric relationship differed in the different populations. This is due to the fact that in Frontignan, some spring specimens tended to exhibit a body weight much less than expected based on their large body length (Fig. 5). This likely corresponded to overwintered animals, possibly in poor body conditions after winter. This did not impact notably their mandible size, though. Hence, for mandible and molar size, the relationship in the feral mice of Frontignan was similar to what is observed in commensal mice at Tourch and Gardouch (Fig. 5).

Patterns of shape differentiation

The shape variation related to seasonal differences in the commensal Tourch and feral Frontignan populations was compared to variation occurring between several mainland localities (Fig. 6). By including a larger geographic sampling, the between-group to total-variance ratio reached a higher value (17.3% for mandible shape and 22.1% for molar shape) than considering seasonal variation in Frontignan or local variation in Tourch, both twice as important as the seasonal variation in Tourch (Table 4).

For the mandible (Fig. 6A), the different samples from Tourch were clustered on the first principal plane defined by bgPC1 and bgPC2, and slightly divergent from the other mainland localities. The autumn sample of Frontignan (October 2009) was close to the other mainland groups, and especially from the neighboring population of Montpellier, whereas the two spring samples of Frontignan diverged along mostly bgPC2. Considering the residuals of the shape variables vs. mandible size, thus correcting for allometric variation, did not change this pattern and even increased the percentage of bgPC2 along which the seasons of Frontignan are differentiated (Supp. Fig. 1A).

Regarding molar shape, seasonal differences did not emerge on the first principal plane (Fig. 6B). Seasonal samples from Frontignan were clustered on one side of bgPC1 whereas seasonal samples from Kerloyou were clustered together on the other side of bgPC1, close to one Northern Italian locality (San Bernardino, IT-SBER). Molars from Kerc'hoaler were further divergent along bgPC1. Other mainland localities represented a cloud of variation towards the center of the first principal plane. Here again, correcting for molar size did not change the pattern of differentiation (Supp. Fig. 1B).

Seasonal and local differences compared to a random model of inter-locality variation

Euclidean distances in size and shape between seasons in Kerloyou and Frontignan, and between the localities Kerloyou, Kerc'hoaler, Frontignan and Gardouch, were compared to random populations generated from Western European populations (Renaud et al., 2013).

Seasonal variations in Frontignan overall produced higher morphological distances than seasonal variations in Kerloyou (Fig. 7). The distances related to seasonal variations remained in the range of distances expected between randomly simulated European localities.

Distances between localities reached higher values than those due to seasonality. For the four cases considered (mandible size and shape, molar size and shape), some distances between the localities of the present studies were in the upper range of the distances expected between random European localities (Fig. 7). For molar size and shape, some observed distances outreached the simulated ones. The neighboring farms of Kerloyou and Kerc'hoaler were differentiated by morphological distances as high as observed between remote places such as Gardouch and Frontignan or Tourch, but except for molar size, they were within the range of the simulated distances, and hence congruent with a random differentiation.

Discussion

Contrasted genetic diversity in feral and commensal populations

The commensal populations from Tourch displayed a low genetic diversity, with two related haplotypes in Kerloyou and only one haplotype in Kerc'hoaler. The D-loop being maternally inherited, it would be compatible with a foundation by one or few related females, and persistence thereafter as close system. The food ad libitum in the Kerloyou pigsty may indeed sustain a large and flourishing population. The result is a bit more surprising in Kerc'hoaler, with more traditional agricultural practices providing irregular food resources to mice. The absence of immigration may be explained by current agricultural practices, in which exchanges between farms are scarce. Reinforcing this barrier, the resident population may be resilient to immigration, as has been shown for insular populations (Hardouin et al., 2010). Dispersal may also be biased towards males (Pocock et al., 2004), a signal that mitochondrial DNA is not able to trace. Possibly, genetic diversity may be anyway reduced in the Brittany peninsula. Additional samples in the area would be required to better assess these different possible causes of reduced genetic diversity.

In contrast, the feral population in Frontignan was diversified (11 haplotypes, haplotype diversity Hd = 0.90; nucleotide diversity Pi = 0.0078), sampling the variety of distant haplogroups present in the surrounding region. This suggests that this feral population may be a sink population regularly fueled by immigration of the surrounding areas. Such gene flow may be a factor counterbalancing local adaption to this peculiar environment (Lenormand, 2002).

Wear stage and body size as hint about the ecology of feral vs commensal populations

Tooth wear has been proposed as the most reliable morphological trait to estimate age in the house mouse (Lidicker 1966). It provided a rough proxy for the age structure of the populations. Seasonality in age structure was more pronounced in the feral population of Frontignan than in the commensal population of Kerloyou. This is in agreement with the fact that feral populations are known to display seasonal breeding (Berry, 1981; Efford et al., 1988; Matthewson et al., 1994; Triggs, 1991) whereas mice breed all year round on commensal habitats (Berry, 1981; Pocock et al., 2004; Rowe et al., 1983).

Body length and body weight should tend to increase with age, the latter being also influenced by the condition of the animal. Neither varied with season in Kerloyou, in agreement with the absence of significant seasonal variation in age structure suggested by wear pattern distribution.

In the feral population from Frontignan, body length varied across seasons, in agreement with the variation in age structure suggested by wear pattern. Despite these variations in body length, body weight did not differ between seasons. As a consequence, mice in Frontignan displayed a different allometric relationship across seasons, differently from the commensal populations from Tourch and Gardouch. Another difference between the feral Frontignan population and the commensal population of Kerloyou was that season remained a significant explanatory variable of body weight together with body size and wear pattern in Frontignan. Altogether, this suggested that body weight in Frontignan mice was not only related to the age of the animal, but also to ecological conditions characteristic of the seasons. The spring populations in Frontignan were composed of specimens with heavily worn teeth, pointing to overwintered animals. Accordingly, they reached a high body length but they displayed relatively light weight (Fig. 4) compared to autumn animals. This explained the apparent paradox of season having a significant effect of season on the body length / body weight relationship, despite no difference in weight between seasons. This suggested that the animals were in poor conditions due to food shortage during winter (Efford et al., 1988). This is further supported by the low success of trapping for these spring populations, and previous observations of a winter decrease of house mouse populations in neighboring areas (Cassaing & Croset, 1985).

Impact of seasonal variations in size on morphometric characters

Variations in body size are likely the consequences of variations in the age structure of the populations, and possibly, their health status. Such factors may impact morphological characters as well. The first molar tooth, though, is patterned early during embryogenesis (Cho et al., 2007) and is erupted early in post-natal life. After weaning, it can only vary due to progressive wear. Accordingly, no clear variation of tooth size with either body size or seasonality was evidenced. A small effect could nevertheless be due to a more important frequency of old animals with worn teeth in spring than autumn, since advanced wear can affect the outline of the tooth (Renaud, 2005).

In contrast, mandible grows and remodels throughout the life of an animal. We evidenced a strong relationship between body size and mandible size, similar at all places (Fig. 4). This result apparently contradicts the observation that the growth of the mandible, and the associated changes in shape, are asymptotic. Mandible size has been shown to reach ~95% of its final size at an age close to weaning, and ~95% of its final shape at post-natal day 35 (Swiderski & Zelditch, 2013). Possibly, both body length and mandible grow at a similar pace, reaching at the same age the point when they slow down their growth.

The fact that the same relationship between body size and mandible size was observed in the feral and commensal populations suggests that the more severe conditions experienced by feral mice did not impact their investment in the growing bone. In contrast, Sub-Antarctic insular mice have been shown to display mandible smaller than expected based on the continental relationship, suggesting a decreased investment in the mandibular bone (Renaud, Gomes Rodrigues, et al., 2015). Explanations could be that the limitation in quality and/or quantity of the food is less dramatic in the Frontignan population, and/or that the food shortage occurred relatively late in life, when most of mandible growth was already achieved (Swiderski & Zelditch, 2013). This may be the case of immigrants from the surrounding areas, whose frequent occurrence is suggested by the high genetic diversity.

Seasonal shape variations

Molar shape varied with seasons in Frontignan and Kerloyou. Its relationship with wear stage in Kerloyou supports the facts that these variations are mostly due to an impact of wear on the shape of the outline. Even positioned relatively low down the crown, this outline is affected by heavy wear (Renaud, 2005), as mentioned for molar size. This contrasts with results found for arvicoline molars, for which seasonal variations were found as important, or even more important than large scale geographic variations (Guérécheau et al., 2010). This different sensitivity is related to differences in tooth shape and way to measure it: for arvicoline rodents, measurements of tooth shape are done on the occlusal plane, and thus at the top of the crown, a place heavily influenced by wear. In mice, the seasonal shape differences remained of small amount compared to observed and simulated differences between populations. Seasonal variations are thus unlikely to obscure geographic variation, in the case of biogeographic studies.

Seasonal variations also influenced mandible shape. This was the case in the feral population of Frontignan but also in the commensal population of Kerloyou. In Kerloyou, this difference was mostly related to body size and wear stage, suggesting that it may be related to aging in mandible shape. In

Frontignan, however, season was an important factor, and seasonal variations represented a higher proportion of the total variance than in Kerloyou. Possibly, in Frontignan, differences in mandible shape may trace a change in the resources exploited in the different season, leading to a differential plastic remodeling of the mandible (Anderson et al., 2014). These shape changes across season were of the same magnitude than observed difference between populations (Fig. 7). This corresponded to a clear shift between autumn and spring populations of Frontignan in the morphospace including the mainland localities of comparison (Fig. 6). This suggests that mandible shape may trace fine-scale ecological variations, and that such variations may interfere with evidences of geographic variations.

Population dynamics in feral vs commensal populations

Different and possibly conflicting effects may contribute to the dynamics of the populations and impact their morphometric differentiation. On the one hand, commensal populations have been shown to function as small demes at a very small geographic scale of few meters (Pocock et al., 2004). This should promote drift in highly sub-structured populations. In contrast, non-commensal mice have been reported to move further (Pocock et al., 2004). Dispersal being more important, this should lead to homogenization of the populations at a regional scale.

On the other hand, commensal mice always benefited from human activity (Cucchi, 2008) for traveling even over long distances, a key to their world-wide success in colonizing even remote environments. Exchanges between populations were thus expected to be favored by the commensal habits, fueling genetic diversity.

Our results are in agreement with the first view. The feral population of Frontignan displayed a high genetic diversity. It seemed to have sampled variation available in the surrounding commensal localities. It may function as a sink population persisting by the immigration of surrounding source populations. This would explain the little morphological divergence regarding its mandible shape, despite the ecological shift from commensal to feral way of life. The autumn population from Frontignan actually displays a mandible shape close to its closest relative from Montpellier. The divergence of the spring populations may be attributed to plastic remodeling in overwintered animals. In contrast, speaking for Frontignan being nowadays an isolated population, molar shape displayed a divergence from all other mainland populations, consistent throughout the different seasonal samples. Further genetic analyses would be required to better identify the dynamics of this feral population.

In contrast, the commensal populations of Tourch displayed a reduced genetic diversity, as reduced as the one observed on remote islands (Hardouin et al., 2010). Commensal populations of mice are known to function as small demes of few mice, but there are also known to be transient (Singleton, 1983). The commensal habitat is composed of patches of highly suitable habitats – for instance, farm buildings where livestock is housed – but these habitats are highly variable in space and time – for instance, during the clearance and sanitary emptying of poultries and pigsties. Our results suggest that despite this unpredictable environmental variability, mice managed to sustain an apparently homogeneous and stable population, at least at the timescale documented here, possibly because recolonization of the buildings after clearance occurred from related mice from the surrounding buildings.

Such a functioning may be prone to drift in a sub-structured population. This may be a trigger for morphological differentiation, as suggested by the divergence observed even between neighboring farms of Kerloyou and Kerc'hoaler. This differentiation is especially obvious for molar shape in the morphospace including mainland localities of comparison, with Kerc'hoaler mice displaying extremely elongated molar shape, opposed to short molars in Frontignan. This opposition between short and elongated molars is a recurrent pattern of variation in murine molars (Renaud, Dufour, et al., 2015) that may fuel rapid divergence due to drift in isolated populations, as happens on some islands (Renaud, Dufour, et al., 2015; Renaud, Pantalacci, & Auffray, 2011).

Conclusions

This study evidenced how differences at an ecological scale, such as seasonal variations, or feral vs. commensal way of life, may impact even morphometric traits in house mice populations.

Feral mice from Southern France appeared sensitive to seasonality, displaying evidences of seasonal reproduction and a winter decrease in the body conditions of the animals. Accordingly, mandible size and shape differed between autumn and spring populations, due to the more frequent occurrence of overwintered animals in spring but also possibly due to different conditions of growth.

The commensal population from Brittany also displayed seasonal variations in morphometric traits, although the amount of difference between seasonal samples was overall less than what was observed in the feral population of Frontignan. The high genetic homogeneity of the Brittany farm suggests a functioning as a close system, with resilience to immigration, at least on a short time scale. This may promote drift and trigger local divergence in morphology, as observed between the neighboring Kerloyou and Kerc'hoaler farms in Tourch, Brittany.

Over a longer timescale, diffuse gene flow may buffer such local variations, as suggested by the relatedness of the haplotypes encountered in the two farms of Tourch, distant of only a few km, and by the relative homogeneity in shape at the scale of continental Western European house mice (Ledevin et al., 2016).

These results suggest that morphometric characters can trace ecological processes occurring over a small scale in time and space. Plastic variations may be involved, as in aging in mandible size and shape in overwintered populations. Microevolution may also occur, as for the divergence in molar shape between neighboring farms. Thus, morphometric traits may have the potential to trace small-scale processes of differentiation. Further small-scale studies would be required to better assess the processes involved in seasonal variations, but also in differences in way of life, such as feral vs. commensal populations. Such studies would provide a precious background for interpreting morphometric differences related to invasive processes and response to changing environment, as recently evidenced in insular populations (Renaud et al. 2015). On the other hand, differentiation related to seasonality remained overall of smaller amount than biogeographic differences. This is reinsuring for biogeographic studies, supporting the assumption of seasonal variations to be negligible with regards to geographic variations, an assumption commonly done in part due to the pragmatic fact that a broad geographical coverage cannot be designed with repeated sampling over seasons.

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Tables

Locality	Farm	Latitude	Longitude	Season	Code	Nb Mb	Nb UM1	N_{genet}	N_{haplo}
Tourch	Kerloyou	48° 00’ 41.22" N	3° 49’ 38.66" W	August 2011	TKY_Aug11	40	40	6	2
				November 2011	TKY_Nov11	28	27	6	2
				May 2012	TKY_May12	7	7	6	2
Tourch	Kerc’hoaler	47° 59’ 48.48" N	3° 50’ 02.30" W	June 2012	TKH_June12	13	12	8	1
Frontignan		43° 27’ 56.63" N	3° 49’ 27.30" E	April 2009	FRO_Apr09	19	19	5	5
				October 2009	FRO_Oct09	7	7	14	7
				March 2010	FRO_Mar10	4	4	2	2
				March 2013		-	-	1	1

Table 1. Sampling of the study: locality of trapping and eventually farm, with latitude and longitude, date of trapping, code, number of mandibles (Nb Md) and first upper molars (Nb UM1) measured, number of sequenced specimens (N_{genet}) and number of haplotypes documented (N_{haplo}).

	HBL	Weight	Md Size	UM1 Size	Md Shape	UM1 Shape
Tourch	0.168	0.120	*0.017*	0.120	0.258	0.095
Frontignan	0.301	0.975	0.566	*0.035*	0.306	0.542
Gardouch	0.111	0.077	0.076	0.336	0.981	*0.012*

Table 2. Sexual dimorphism in the populations of Tourch, Frontignan and Gardouch. Variables are head + body length (HBL), body weight, mandible (Md) size and shape, and first upper molar (UM1) size and shape. Probabilities of ANOVA for univariate size measurements and MANOVA for multivariate shape datasets are given (in italics $P < 0.05$, in bold $P < 0.01$). For shape, the tests were performed on the PCs representing more than 5% of variance.

	Kerloyou vs. Kerc’hoaler	Kerloyou Seasons				Frontignan Seasons			
	P	P	$P_{pairwise}$	$P_{pairwise}$	$P_{pairwise}$	P	$P_{pairwise}$	$P_{pairwise}$	$P_{pairwise}$
			Aug11/ Nov11	Aug11/ May12	Nov11/ May12		Oct09/ Apr09	Oct09/ Mar10	Apr09/ Mar10
HBL	0.388	0.399	0.931	0.297	0.493	**< 0.001**	**0.002**	0.305	0.076
Weight	0.518	0.477	0.967	0.368	0.510	0.758	0.941	0.762	0.926
Md size	*0.025*	0.581	0.955	0.637	0.458	0.260	0.443	0.559	0.979
UM1 size	**< 0.001**	0.923	0.964	0.999	0.961	*0.019*	0.785	*0.030*	**0.006**
Md shape	**< 0.001**	**0.007**	**0.002**	0.535	0.090	**0.007**	**0.008**	*0.016*	0.620
UM1 shape	**0.002**	*0.020*	**0.007**	0.482	0.197	*0.029*	**0.009**	0.556	0.256

Table 3. Size and shape differences in Tourch between the two farms (Kerloyou vs Kerc’hoaler), between the three seasons in Kerloyou, and between the three seasons in Frontignan. Variables investigated are body length (head + body length, HBL), body weight, mandible (Md) and first upper molar (UM1) size, and mandible and molar shape (summarized by the PC axes explaining more than 5% of variance). Differences were investigated using an ANOVA for univariate variables, and a MANOVA for multivariate data sets (tests performed on the PCs representing more than 5% of variance). Probabilities of pairwise differences are also given. In italics P < 0.05, in bold P < 0.01.

dataset	grouping	Mandible	UM1
Tourch	Kerloyou vs. Kerc’hoaler	9.0%	10.9%
Kerloyou	Seasons	4.3%	5.0%
Frontignan	Seasons	9.4%	8.9%
Geographic variation	Localities	17.3%	22.1%

Table 4. Percentage of between-group to total variance in between-group PCAs performed on the Fourier coefficients. The dataset is indicated together with the factor of grouping. Percentage is provided for the analysis of mandible and molar (UM1) shape.

		HBL		Season		Wear	
		%	P	%	P	%	P
Kerloyou	Weight	11.4%	**<0.001**	0.9%	0.410	12.1%	**<0.001**
	Md size	31.1%	**<0.001**	0.5%	0.417	3.4%	**0.001**
	UM1 size	3.6%	0.111	0.0%	0.992	4.3%	0.082
	Md shape	7.9%	**<0.001**	4.1%	*0.042*	5.8%	**<0.001**
	UM1 shape	0.7%	0.640	4.5%	0.116	6.6%	**<0.001**
Frontignan	Weight	11.6%	**0.003**	35.0%	**<0.001**	22.5%	**<0.001**
	Md size	35.9%	**<0.001**	12.7%	**0.007**	1.8%	0.195
	UM1 size	2.7%	0.307	37.7%	**0.003**	12.8%	*0.031*
	Md shape	4.1%	0.217	11.1%	*0.042*	6.4%	0.095
	UM1 shape	2.3%	0.644	8.7%	0.156	4.4%	0.194

Table 5. Linear models investigating relationships between size and shape of mandible and molars, and body size, season and wear stage in Kerloyou and Frontignan. Variables investigated are body weight, mandible (Md) and first upper molar (UM1) size, and mandible and molar shape (summarized by the PC axes explaining more than 5% of variance). The model investigated included as explanatory variables head + body length (HBL), season, and wear stage. Probabilities of pairwise differences are also given. Probabilities are given (P) as well as percentage of variance in the linear model (%). In italics P < 0.05, in bold P < 0.01.

	HBL		Locality		HBL * Locality	
	%	P	%	P	%	P
Weight	47.7%	**< 0.0001**	5.5%	**< 0.0001**	3.3%	**0.0003**
Md size	60.7%	**< 0.0001**	2.0%	**0.004**	0.4%	0.293
UM1 size	0.2%	0.561	3.5%	*0.042*	1.0%	0.397

Table 6. Allometric relationships between body length (head + body length, HBL) and weight, mandible (Md) and molar (UMA) size in the three populations of Tourch, Frontignan and Gardouch. A linear model including body length, locality and interaction was used to investigate differences in allometric relationships between the three populations. The percentage of variance (%) explained by body length, locality, and interaction is provided, together with the associated probability (P). In italics P < 0.05, in bold P < 0.01.

Figure Captions

Figure 1. Examples of mandibles (to the left) and first upper molars (to the right) of the house mouse *Mus musculus domesticus* from the feral population of Frontignan, Southern France (A, B) and the commensal populations from Brittany (France) Tourch Kerloyou (C, D) and the neighboring farm Tourch Kerc'hoaler (E). F. Map of the localities, including the Western European localities used for the morphometric comparison. G. The sixty-four points of the outline, positioned on an example of mandible and a molar.

Figure 2. Phylogenetic tree based on D-loop sequences. Genetic sampling was design to represent the diversity of Western European mice. The genetic composition of the feral population (Frontignan) and the two commensal populations from Tourch in Brittany (Kerloyou and Kerc'hoaler farms) were inserted in this background. Values at the nodes are posterior probabilities obtained with MrBayes.

Figure 3. Frequency distribution of the wear classes (based on the upper tooth row) in the different populations: three seasons in the Kerloyou farm, and neighboring Kerc'hoaler farm in Tourch (Brittany), three seasons in feral population of Frontignan, and the population of Gardouch.

Figure 4. Size variations in the commensal Tourch populations (upper panels) and the feral population of Frontignan (lower panels). From left to right: body size (head + body length), body weight, mandible size, upper molar size. Each dot corresponds to a specimen.

Figure 5. Relationship between body size (head + body length) and weight, mandible size and molar size (from left to right) in the commensal populations of Gardouch and Tourch, and the feral population of Frontignan.

Figure 6. Mandible shape (A) and molar shape (B) on the first two principal axes of the between-group PCA on the Fourier coefficients of the mandible or the molar. The mean of each group is represented by its abbreviation. Groups were various commensal Western European populations (Gardouch; FR-MONTP: Montpellier; GER-CB: Cologne-Bonn; IT-SBER: San Bernardino; IT-LOMB: Lombardy), and seasons in the feral population of Frontignan (FRO), and the commensal population of Tourch with the two neighboring farms Kerloyou (TKY) and Kerc'hoaler (TKH). Reconstruction of

mean shape was visualized using inverse Fourier transform for Gardouch, IT-REG, Frontignan October 2009 and March 2010, Kerloyou November 2011 and Kerc'hoaler. Visualization is based on an inverse elliptic Fourier transform for the mandible, and on the radial Fourier transform for the molar.

Figure 7. Morphological distances between seasonal samples in Frontignan (orange) and Tourch Kerloyou (dark blue), between the average values of Frontignan, Gardouch, Tourch Kerloyou and Tourch Kerc'hoaler (green; the arrow points to the distance between two neighboring farms Kerloyou and Kerc'hoaler), compared to the distribution (in grey) of morphological distances between simulated groups derived from a Western European pool. (A, C) Size distances. (B, D) Shape distances. (A, B) Mandible. (C, D) Molar.

Supplementary Figure 1. Size-free pattern of shape variation for the mandible (A) and molar (B). Axes are the first two principal axes of the between-group PCA on the residuals of a multivariate regression of the shape variables (Fourier coefficients) vs. the size of the feature (A0 of the mandible or the molar). The mean of each group is represented by its abbreviation. Groups as in Figure 6.