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Phylogeny and adaptation shape the teeth of insular mice

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Abstract

By accompanying human travels since prehistorical times, the house mouse dispersed widely throughout the world, and colonized many islands. The origin of the travelers determined the phylogenetic source of the insular mice, which encountered on the various islands diverse ecological and environmental conditions. Insular mice are thus an exceptional model to assess the relative role of phylogeny, ecology and climate in evolution. Molar shape is known to vary according to phylogeny and to respond to adaptation. Using for the first time a 3D geometric morphometric approach, compared to a classical 2D quantification, the relative effects of size variation, phylogeny, climate and ecology were investigated on molar shape diversity across a variety of islands. Phylogeny emerged as the factor of prime importance in shaping the molar. Changes in competition level, mostly driven by the presence or absence of the wood mouse on the different islands, appeared as the second most important effect. Climate and size differences accounted for slight shape variation. This evidences a balance role of random differentiation related to history of colonization, and of adaptation possibly related to resource exploitation.

Keywords

Insular evolution, 3D geometric morphometrics, first upper molar, house mouse, *Mus musculus domesticus*

Introduction

The relative importance of chance, history and adaptation in evolution is a long-standing issue [1-4]. Stochastic processes are expected to play an important role in fragmented and isolated populations, because of founder effects and drift which are especially relevant in island populations and invasive species [3]. Adaptation is also expected in such contexts because species are encountering new environmental conditions that expose the immigrants to strong selective pressures [5, 6]. Accordingly, evolution on islands has been an emblematic model of adaptive evolution since Darwin's finches (e.g. [7]). Genetics and development can further constrain or facilitate evolution along certain directions, for instance due to genetically correlated traits [8]. Since these processes intermingle in shaping phenotypes, their respective role is difficult to tease apart [3], a fact that may lead to interpretations of differentiation being a collection of 'just-so stories' [9].

The house mouse (*Mus musculus*) has been accompanying human travels since prehistorical times [10], and as such is one of the 'best' world-wide invasives [11]. From the Western European continent, *Mus musculus domesticus* has colonized many remote areas, including islands [12]. Colonization of new environments, climatically and ecologically different from the source should promote adaptive changes especially when the mice meet their physiological limits [13].

As Western European populations display a complex genetic structure [14, 15], island populations will exhibit a variable genetic signature, depending on the colonization source and subsequent demography, itself related to human history [16]. Island house mice thus offer a remarkable model to investigate the relative importance of population history, adaptation and stochastic events on phenotypic evolution [9].

We tackled this issue by focusing on the differentiation of the first upper molar tooth. Molar teeth have been shown to be influenced by phylogenetic history [17, 18] as well as environmental conditions [19, 20]. Development may also constrain their evolution [21]. 3D geometric morphometrics was used for the first time to quantify tooth shape in several insular populations and continental reference groups. The results were compared to a 2D analysis including a larger sampling of the same groups. The phylogenetic relationships were assessed based on mtDNA data. The relative effect of allometry, phylogeny, climate and ecology on the morphological differentiation was then investigated.

Material and Methods

Material

Morphometric sampling. – 532 mice were part of the 2D morphometric analysis. This set was down-sampled to 90 mice for 3D morphometrics, including only animals with relatively unworn teeth (Table 1). The sampling included continental Western Europe and contrasted insular settings (Fig. 1A): Northern Atlantic (Orkney Archipelago), Macaronesian Islands in the sub-tropical mid-Atlantic region (Madeira and Canary Archipelago[14, 22]), and the Sub-Antarctic region (Marion Island; Guillou Island from the Kerguelen Archipelago[23]). All individuals were considered as adults and sub-adults based on the criterion that the third molars were fully erupted, which occurs at weaning. Males and females were pooled since no sexual dimorphism has been documented for tooth morphology in house mice [23, 24].

Phylogenetic sampling. – Original samples from the Orkney archipelago were obtained from two field trips in 1992 (islands of Faray, Eday, Sanday and Papa Westray) and 2012 (island of Papa Westray). DNA was extracted from Ethanol-preserved tissue of Orkney mice, using the "DNeasy Blood and Tissue" kit (Qiagen, France). The D-loop was amplified using previously described primers and protocol [25]. The sequences generated were visualized using MEGA6 [26]. No new haplotypes were found in our samples of Orkney populations [27, 28]. We combined our sequences with sequences retrieved from Genbank into two datasets. (1) A general dataset to determine the phylogenetic origin of the groups used in the morphometric analysis (Supp. Table 1). (2) A dataset designed to include only sequences matching the morphometric sampling (Supp. Table 2). Haplotypes for each group were determined with DNAsp v5 [29] except when this information was already available: Marion Island [30] and Guillou Island [25].

Methods

Phylogenetic analyses. – The sequences were aligned with MUSCLE implemented in SeaView [31], the alignment was checked by eye and trimmed at both ends to remove portions with more than 50% of missing data. The final alignments comprised 173 sequences and 947 positions for the general dataset and 155 haplotypes and 874 positions for the morphometric-matching dataset. For this latter dataset, we determined the genetic diversity within each geographic group using MEGA6 [26]. The phylogenetic trees were reconstructed using maximum likelihood with PhyMl 3.0 [32] under the models (TN for the general tree and GTR for the morphometric-match, +I+G) selected with jModelTest [33] using the Akaike criterion (AIC) [34].

2D Morphometrics. Using a numerical camera mounted on a binocular, a picture was taken from each mouse molar, with the skull adjusted so that the occlusal surface of the first upper molar would be approximately flat. The molar shape was approximated by the 2D outline of the occlusal surface, towards the base of the crows, which is only affected by heavy wear. Each outline was defined by 64 points, which were analysed using a Fourier based approach [21]. Fourteen variables, corresponding to a set of Fourier coefficients (FCs) were deemed adequate to describe the molar shape [21]. An additional variable (A0) provided an estimate of the outline size.

Data acquisition for 3D morphometrics. – Skulls were scanned at a cubic voxel resolution of 18µm using a RX in-vivo Skyscan 1076 microtomograph (µCT) device at the Platform Montpellier RIO Imaging. The left first upper molar (UM1) (Fig. 2) was delimited on each slice using a threshold method in Avizo software (version 7.1 – Visualization Sciences Group, FEI Company) and connections with outer material (jaw bone and second upper molar) were manually closed and the surface generated. On a randomly chosen reference tooth, a template was prepared describing the outer surface of the tooth. Since age was not controled in wild-trapped populations, the template was designed not to take into account parts of the tooth most sensitive to wear: the top of the cusps were cut off the template (Supp. Fig.1). The template was defined by 1532 equally spaced sliding-landmarks anchored by eight landmarks. These eight landmarks were defined on all specimens and were used for a Procrustes superimposition to align all the specimens in space. Then, the original template was deformed in order to match the original surface of each tooth. Points were allowed to slide along tangent planes according to the minimum bending energy criterion, with an iterative procedure until convergence [35, 36]. Sliding-landmarks were adjusted for scaling, translation and rotation according to a Procrustes superimposition. All procedures were performed using the packages ‘Morpho’ [37] and ‘mesheR’ [38]. Procrustes coordinates, i.e. residual coordinates of the sliding-landmarks after Procrustes superimposition, constituted the shape variables describing tooth shape. Centroid size (square root of the sum of the squared distance from each sliding-landmark to the centroid of the configuration) estimated the size of the tooth.

Statistical analyses of tooth size and shape. – Based on 3D morphometric data, differences in tooth size were investigated by an analysis of variance. Allometric variation was assessed using a multivariate regression on the Procrustes coordinates. The residuals were considered as new allometry-free variables. A Principal Component Analysis (PCA) on the size-free variables provided a representation of morphometric variation.

Projection of the phylogeny on the morphometric space. – A matrix of genetic distances based on D-loop haplotypes of sequences from the same geographic areas as the morphometric samples, without outgroups, was designed (Supp. Table 2). It was analysed using a Principal Coordinate Analysis (PCOA) [39] using the R ‘ape’ package [40]. It converted the distance matrix into coordinates on principal axes. Mean values for each group were computed and was compared to morphometrics mean values. The phylogenetic relationships were projected on the morphometric space using ‘phytools’ R package [41], the ancestral states being calculated at each node using ‘fastAnc’ function.

Size, phylogeny, climate and ecology as explanatory variables of morphometric variation. – A linear model was used to investigate effects of size, phylogeny, ecology, and climate on tooth shape. (1) Size was evaluated as the centroid size of the tooth. (2) Phylogeny was included as the first four axes of the PCOA on the D-loop distance matrix, including the set of axes with > 90% of variance. (3) Climatic data were extracted from the WorldClim database with a resolution of 2.5 arc-minutes using the ‘raster’ package [42]. The data included Annual Mean Temperature, Temperature Seasonality, Mean Warmest Quarter, Mean Coldest Quarter, Annual Precipitation, Precipitation of the Wettest Quarter, Precipitation of the Driest Quarter. A PCA was performed to summarize these partly redundant data. The first three PCs explaining > 90% of the total variance were retained as explanatory variables in the model. (4) Ecological coding included presence/absence of competitors and of predators according to the literature (Supp. Table 3) and coded these data as 0/1 (Table 1; Supp. Table 4). Since house mice strongly rely on human populations for resources and transport, human population density was also included as an explanatory variable.

Finally, the residuals of this model were analysed using a between-group and within-group PCA using the ade4 package [43]. This procedure allowed us to assess the percentage of variance attributed to between vs within group variance in the residual shape variation.

Comparison between 3D and 2D morphometrics. – A PCA was performed on the FCs of the 2D outline. The scores of the group means on PC axes provided a configuration that were compared to the configuration of group means obtained by the 3D approach using a Procrustes superimposition procedure (Protest [44]). The significance of the association were tested using permutations. Distances between the two configurations were further compared using a Mantel test. The linear model of shape (PC axes) vs. size (A0), phylogeny, ecology, and climate (same variables as for the 3D analysis) was further used on the 2D data in order to investigate the stability of the results to method and sampling.

Visualisations of shape changes were performed using the 'Morpho' package [37]. PCOA and Protests were performed using the vegan package [45].

Results

Phylogeny

The continental Western European groups displayed a large haplotypic diversity. Each island represented a subsampling of this diversity (Table 1). The founding of the insular populations appear to be so recent regarding the evolutionary rate of the genetic marker that no island displayed private haplotype, hindering the estimate of a divergence date. Four independent instances of insular colonisation could be identified, in agreement with previous studies (Fig. 1B). (1) Orkney nested into a mostly Scandinavian and British haplogroup [16] which has been interpreted as the signature of a Norvegian Viking colonization. (2) Guillou shared its only haplotype with other mice from Kerguen

Archipelago and Western European specimens from England, Germany and France, and from harbours on the way to the Southern Oceans as in Cameroon [25]. (3) Madeira, the Canary islands of La Palma and El Hierro, and Marion island were mostly nested within a Northern European haplogroup [14]. This genetic assignation has been interpreted as related to a possible introduction by Danish Vikings onto Madeira [46], mice being later translocated to the Canaries by Portuguese travels. (4) Tenerife appeared more related to a Southern European haplogroup, a signature of exchanges between the Canaries and the Spanish realms [14]. Evidences of mixing exist on Madeira and all three Canary islands investigated (Fig. 1; Supp. Fig. 2): typical Tenerife haplotypes seldom occur on Madeira, El Hierro and La Palma, and vice-versa.

Three-dimensional tooth morphology

Tooth size varied significantly across populations ($P < 0.001$; Supp. Fig. 3). Insular mice tended to display larger molars than their continental relatives.

The size-shape allometric relationship was significant ($P < 0.001$). The analysis of allometry-free residuals provided two axes of almost equal importance (19% and 18%) along which a geographic structure emerged (Fig. 2). Western European continental teeth clustered together whereas insular teeth by far exceeded this continental range of variation. Changes along PC1, mostly corresponding to the transition from continental Western European to Guillou – Marion – El Hierro morphologies, involved a pinching at the labial forepart and deepening of the lingual gutter between the central and lingual rows of cusps. Along PC2, characterizing the Macaronesian Madeira – La Palma group, the tooth mostly shortened in its forepart and broadened laterally.

The morphometric structure partly reflected the phylogenetic relationships, with obvious discrepancies. The different Orkney islands clustered together but they displayed an important variation constrasting with their genetic homogeneity. Macaronesian islands from Madeira, Tenerife and La Palma were grouped together, a geographical cluster contradicting the distinct haplotypic dominant signature of Tenerife. Marion and El Hierro, genetically close to La Palma – Madeira group, were morphologically well differentiated. Guillou Island displayed a convergence in molar shape with Marion and El Hierro, despite a different genetic/geographic origin.

The 2D analysis (Supp. Fig. 4) provided a correlated configuration of between-group differentiation (comparison between PCs >5% [5 in 3D and 5 in 2D]: Protest $P = 0.006$, Mantel $P = 0.008$). As in 3D, Western European samples appeared as well clustered. The convergence between Tenerife, La Palma and Madeira on the one hand, and from Guillou Island and El Hierro on the other hand, were further supported. The idiosyncrasy of Orkney, making all island to group together in the 3D analysis, was not captured by the 2D outline. Orkney islands appeared as widely dispersed in the corresponding morphospace.

Tooth shape vs size, phylogeny, climate and ecology

The total shape variation of the tooth could be summarized along five axes, totalling more than 60% of variance (19.1%, 18.6%, 10.9%, 6.1% and 5.7%). Climatic data were summarized on three PC axes (66.4%, 19.3%, and 13.1% of variance). Phylogenetic data were summarized on four PCOA axes (58.0%, 20.7%, 9.9% and 3.7%). Ecological data (competition, predation, human density) were further included in the linear model.

The model indicated a weak contribution of size (3.4%), a balanced influence of ecology (7.3%) and climate (6.0%), and the strongest influence of phylogeny (12.1%) (all $P < 0.01$, competition and phylogeny $P < 0.001$) (Fig. 4). Effects on tooth shape were the following. (1) Size: larger molars were longer at their forepart and thinner in their labial region. (2) Phylogeny: the first axis, roughly opposing continental Western Europe to Orkney and Macaronesian islands, corresponded to an anterior elongation and a reduction of the protocone and neighboring lingual cusp. (3) Ecology: among competition, predation and human density, only competition had a significant effect. Decrease in competition involved a forepart expansion together with an overal thinning of the cusps. (4) Climate: With a temperature decrease and a precipitation increase (from Macaronesian to Sub-Antarctic islands), central cusps moved forward and the anterior lingual fringe expanded. From seasonal (continental) to less seasonal (more or less all islands) environments, the tooth lenghthened in its forepart and most cusps shortened. A similar hierarchy of factors was found when including shape axes totalling more than 80% of variance (11 axes): phylogeny (9.4%), ecology (7.5%), climate (5.7%) and size (2.4%). The same hierarchie was further found based on the 2D outline analysis, with even less variance explained (shape described by all 5 axes > 5%, totalling 87% of variance): phylogeny (7.2%), ecology (3.5%), climate (2.7%) and size (1.0%). The 2D sampling include teeth of all wear stage and possible incertainty in orientation of the occlusal plane, possibly explaining the larger percentage of unexplained variance.

Removing size, phylogeny, climate and ecology provided 64.4% residual variance. This residual variation corresponded mostly (80.1%) to within-group variance. The remaining within group-variance corresponded to a trend of anterior expansion combined with a backward movement of the main cusps and their overall thinning. A similar percentage of remaining within-group variance was found in 2D (79.1%).

Discussion

Methodological originality: tooth evolution in 3D

This study is the first one to document tooth shape variation of house mice in its three-dimensional complexity. It largely corroborated results obtained by 2D analyses, evidencing the importance of anterior tooth elongation in the evolution of the mouse upper molar [21, 47]. It further illustrated complex changes in the shape of the cusps and their relative position, up to the deepening of gutters (sulci) between rows of cusps. By describing such features, the 3D description appeared as more performant than the 2D in assessing the idiosyncrasy of the Orkney archipelago. The possible functional significance of such changes is unexplored, since such shape changes have not been described so far.

Phylogenetic history as a key factor in tooth shape evolution

The phylogenetic signal appeared of primary importance in explaining first upper molar shape. We focused on this molar because of its high taxonomic value within murine rodents [48]. Within *Mus musculus*, a strong imprint of historical factors is well documented on the first lower molar shape, which is used as a valuable proxy for identifying subspecies [18] and even for tracing the geographic origin within a subspecies [17, 18, 24]. As co-variation of the occluding lower and upper molars is under functional constraints [49], a phylogenetic signal may be expected on the upper molar as well. However, the first upper molar was shown to be more evolvable than its lower counterpart [21]. Our present results demonstrate that high evolvability of the upper molar do not fully override the signature of the history of colonization on molar shape.

Discrepancies between the morphological and phylogenetic signature raised questions regarding population history. Madeira, La Palma and Tenerife shared a similar tooth shape. The morphological similarity of Madeira and La Palma was expected given their phylogenetic relatedness [14], possibly reflecting early trading routes between the Madeira and Canaries archipelagoes by the Portuguese during the XVth century. The morphological similarity between Tenerife and La Palma was not surprising given their geographic proximity, but was in conflict with the difference in the dominant mitochondrial haplogroup in both islands. The sporadic occurrence of Tenerife-like haplotypes on La Palma, and La Palma-like ones on Tenerife, suggested that gene flow occurred between neighboring islands and that the resilience of local populations to later invaders [25] may break down when human exchanges are important. The similar tooth morphology on Tenerife and La Palma may be due to a genetic homogenization on the Canary archipelago that would not be traced by mtDNA, argued to be a signature of the initial colonization [16, 25]. Alternatively, it could be a convergence in similar ecological and climatic environments. The persistence of this tooth shape despite multiple colonizations anyway suggests a resilience of this morphology and/or strong selective pressure maintaining it in the insular populations.

The morphological cluster associating Guillou, Marion and El Hierro teeth constituted another discrepancy with phylogeny. (1) Marion and El Hierro were genetically associated with La Palma – Madeira based on their dominant haplotypes. (2) The Guillou population derived from an independent colonization event with a different phylogenetic signature. This demonstrated that factors other than phylogenetic history (as traced by mitochondrial markers) contributed to the divergence in molar shape.

Competition as a driving evolutionary force on molar shape

Ecological factors, predominantly inter-specific competition, emerged as driving forces almost as important as phylogeny in explaining molar shape divergence. Differences in competition levels are mostly related to the occurrence of the wood mouse *Apodemus sylvaticus* on the Western European continent and most Orkney islands, and its absence on Macaronesian archipelagoes and in Sub-Antarctic islands (Table 1; Supp. Data 3). The wood mouse is a competitor of the house mouse, limiting its presence in non-commensal habitats where it occurs – and vice versa [50]. In the absence of the wood mouse, the house mouse may exploit more outdoor resources instead of remaining strictly commensal [51]. The tooth shape changes associated with the absence of the wood mouse corresponds to the anterior elongation. Narrow, elongated teeth have been associated in murine rodents with a rather faunivorous diet [52]. Considering this diet /tooth shape trend, non-commensal house mice may be relying more on invertebrates in the absence of the wood mouse, especially when resources are scarce as on Sub-Antarctic islands [53, 54].

Secondary importance of climate

In addition to phylogeny and ecology, climate further impacted tooth shape. Temperature and precipitations mainly opposed warm, dry Macaronesian islands to cold, wet Orkney and Sub-Antarctic islands. The climatic regime changes the available resources and thus constitutes an indirect selective pressure on tooth shape. For instance, mice on the barren Sub-Antarctic Marion and Guillou islands are known to increase the invertebrate component in their diet [53, 55]. However, the exploited resources also depend on variations of the commensal way of life. On

Madeira and Canary islands, mice still rely on resources of anthropic origin but tend to forage outdoor [56] whereas they were trapped indoor in most continental and Orkney locations. Such effects are difficult to quantify and may indirectly impact our results through climate or ecology, since mice mostly adopt outdoor behavior in the absence of the wood mouse competitor.

Insularity magnifying phylogenetic and adaptive differences

The pattern of genetic diversity, high on the continent (within group p-distances: 0.5-0.9%) and low on islands (0-0.3%) contrasted with the pattern of tooth shape differentiation, showing a low variance among continental specimens but a large differentiation for insular samples. This differentiation occurred quite rapidly, from ~1200 years for Orkney, most probably colonized following Viking routes [16] to less than 200 years for Sub-Antarctic islands where mice were brought by sealers during the 19th century [57, 58]. Stochastic events are reputed important on islands, with founder events and drift in populations of reduced effective size. Such factors likely promoted the important and rapid divergence from the continental stock, matching a general observation of fast initial divergence upon arrival on an island [59, 60]. The importance of the geographic origin [9] is underlined here by the phylogenetic imprint on tooth morphology. Subsequent divergence occurred under constraints related to the local environment: when phylogeny, ecology and climate are taken into account, only ~20% of between-group variance remains unexplained. Note that a rather similar hierarchy of factors and percentages of variance were found in the divergence between species of marmots [61] suggesting both a generality of the trend (phylogeny explaining slightly more than climate in tooth divergence) and the important divergence occurring at the intra-specific level in insular house mice.

Repeated tooth elongation: a line of least evolutionary resistance?

Anterior tooth elongation appeared as a recurrent feature of shape variation, involved in the response to size increase, to competition and to seasonality. It also corresponded to the residual within-group variation, matching previous 2D observations [21, 62]. The main direction of within-group variance has been suggested to constitute a ‘line of least resistance to evolution’ [63] producing variants to be screened by selection. The recurrent mobilization of the anterior tooth shape elongation may document the existence of a standing variation for this trait, explaining its potential for fast and convergent evolution. In contrast, phylogenetic signatures seemed to involve much more localized and discrete morphological features, suggesting that such changes may simply accumulate at a low pace [64]. Our 3D results evidence that despite predictions [65], substantial evolution and adaptation can occur even when accounting for the whole complexity of a phenotype although signals of convergent evolution and fast divergence appear as even stronger when using a 'degenerate' description of the tooth, namely its 2D quantification describing only the overall arrangement of the cusps. These are challenging results suggesting that as one of the oldest passengers of human travels, one of the best world-wide invasives, and one of the most studied laboratory model in developmental biology, the house mouse offers a unique opportunity to unravel the complexity of the responses to new environments.

Ethics

Orkney mice were obtained with authorization n° CEEA-LR-12162 from the Languedoc-Roussillon Comité d'Ethique pour l'Expérimentation Animale to JCA. Other samples come from preexisting collections and were previously published. The sacrifice of wild animals for the purpose of taking samples, when performed according to authorized protocols, is not considered as an experiment (Journal Officiel de la République Française, Décret n° 2013-118 du 1er février 2013, Section 6, Sous-section 1). As such, agreement of the ethical committees is not required.

Data accessibility

No new sequence was deposited, since all original sequences corresponded to a sequence already available in GenBank. The dataset including morphometric, climatic, ecologic and genetic data is available as a Supplementary file.

Competing interest

No competing interest.

Authors' contribution

SR, RL, PC, JBD, and JCA conceived and designed the experiments. JCA, PC, GG, JBD, JLC, BP, MLM, EAH and SR participated in the field trips and/or delivered samples. RL and StS performed the 3D morphometrics and associated statistics. PC did the phylogenetic analyses. RL, PC and SR prepared the illustrations. All authors contributed in writing the main text.

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Tables

Group	Geographic area	N_{3D}	N_{2D}	N_{genet}	N_{haplo}	Div_{genet}
El Hierro	Canary	8	37	55	8	0.1
La Palma	Canary	3	38	38	10	0.1
Tenerife	Canary	4	35	48	8	0.2
Eday	Orkney	8	18	11	3	0
Faray	Orkney	9	12	5	1	0
Papa Westray	Orkney	7	10	9	2	0
Sanday	Orkney	6	8	7	1	0
Guillou 1993 and 2009	Sub-Antartic	13	44	79	1	0
Marion	Sub-Antartic	9	92	10	2	0.1
Madeira	Madeira	12	103	112	32	0.3
Col-Bonn	Western Europe	4	14	57	29	0.9
Southern France	Western Europe	3	81	71	32	0.5
Northern Italy	Western Europe	4	40	30	26	0.9

Table 1. Sampling of the study: Group (zone of trapping) and corresponding geographic area, number of first upper molars in the 3D morphometric analysis (N_{3D}) and in the 2D comparison (N_{2D}), number of D-loop sequences (N_{genet}), numbers of haplotypes documented (N_{haplo}), genetic diversity (Div_{genet}, within-group p-distance in %).

Figure captions

Figure 1. (A) Map of the localities sampled for morphometrics. (B) Phylogenetic tree based on D-loop sequences. Genetic sampling was designed to encompass at best the diversity of Western European mice, as well as the islands studied. Haplogroups defined by Bonhomme et al. (2011) are provided.

Figure 2. First upper molar differentiation in the morphospace based on 3D morphometrics. Symbols are group means linked by the phylogenetic relationship based on D-loop distances. Envelopes depict range of variation of the geographic groups. Depicted shape changes: along PC1 (from -0.06 to 0.04) and PC2 (from -0.04 to 0.06).

Figure 3. Summary of a model of 3D tooth shape vs. explanatory variables: size, phylogeny, ecology and climate and visualization of the various effects. Allometry: Shape change with a size increase from 5% to 95% of the distribution. The following representations were computed based on the 29 first PCs (totaling more than 95% of variance) on the size-free variables. Phylogeny: Changes along

the first phylogenetic axis, roughly corresponding to changes from Western Europe to the Macaronesian cluster. Ecology: Change from presence to absence of interspecific competition. Climate: Changes along the first climatic axis (opposing warm, dry to cold, wet environments) and along the second climatic axis (opposing seasonal, continental environments to non-seasonal, insular environments). In all cases, the shape change between 5% and 95% of the distribution is visualized. The residuals of the model including size, phylogeny, climate and ecology were decomposed into between and within-group variance. Shape changes along the first axis of within-group variance are depicted (+/-0.4 along wgPC1).

Supplementary File captions

Supplementary Figure 1. Design of 3D quantification of first upper molar shape. From top to bottom: (1) original shape of the tooth obtained by semi-automatic segmentation of the CT-scan. (2) Design of a template, with top of the cusps cut off to minimize the impact of wear. (3) Template on the tooth on which it has been designed. (4) Adjustment of the template to another tooth. In blue landmarks used for preliminary superimposition of the templates. In red sliding-landmarks that will be used afterward: front crown-root junction; inflexion between front and lingual root; inflexion between lingual and posterior root; lateral maximum of curvature on the anterior lingual cusp, and on the anterior and median labial cusps; base of the protocone and of the hypocone.

Supplementary Figure 2. Phylogenetic tree constructed using a geographic subsample matching the morphometric sampling (see Supp. Table 2).

Supplementary Figure 3. Centroid size differences of the upper molar between geographic groups.

Supplementary Figure 4. First upper molar differentiation in the morphospace based on 2D morphometrics. Axes are the first and second principal components of a PCA on descriptors (FCs) of the molar 2D outline. A. Group means linked by the phylogenetic relationship based on D-loop distances. B. Range of variation of the geographic groups in the same morphospace.

Supplementary Table 1. D-loop accession numbers of sequences used for the global phylogeographic tree (Figure 1).

Supplementary Table 2. List of haplotypes and number of samples (when available) for the localities matching the morphometric sampling.

Supplementary Table 3. Ecological data with references for the different localities.

Supplementary Table 4. Detailed dataset including morphometric, climatic, ecological, and transformed genetic data.