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1 **Phylogeny and adaptation shape the **teeth** of insular mice**

2

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26

27 **Abstract**

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

41

42 **Keywords**

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

45

46 **Introduction**

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

57 The house mouse (*Mus musculus*) has been accompanying human travels since prehistorical times
58 [10], and as such is one of the 'best' world-wide invasives [11]. From the Western European
59 continent, *Mus musculus domesticus* has colonized many remote areas, including islands [12].
60 Colonization of new environments, climatically and ecologically different from the source should
61 promote adaptive changes especially when the mice meet their physiological limits [13].

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

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

75

76 **Material and Methods**

77 *Material*

78 *Morphometric sampling.* – **532 mice were part of the 2D morphometric analysis. This set was down-**
79 **sampled to 90 mice for 3D morphometrics, including only animals with relatively unworn teeth**
80 (Table 1). The sampling included continental Western Europe and contrasted insular settings (Fig.
81 1A): Northern Atlantic (Orkney Archipelago), Macaronesian Islands in the sub-tropical mid-Atlantic
82 region (Madeira and Canary Archipelago[14, 22]), and the Sub-Antarctic region (Marion Island;
83 Guillou Island from the Kerguelen Archipelago[23]). All individuals were considered as adults and
84 sub-adults based on the criterion that the third molars were fully erupted, which occurs at weaning.
85 Males and females were pooled since no sexual dimorphism has been documented for tooth
86 morphology in house mice [23, 24].

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

98 *Methods*

99 *Phylogenetic analyses.* – The sequences were aligned with MUSCLE implemented in [SeaView](#) [31],
100 the alignment was checked by eye and trimmed at both ends to remove portions with more than
101 50% of missing data. The final alignments comprised 173 sequences and 947 positions for the general
102 dataset and 155 haplotypes and 874 positions for the morphometric-matching dataset. For this latter
103 dataset, we determined the genetic diversity within each geographic group using MEGA6 [26]. The
104 phylogenetic trees were reconstructed using maximum likelihood with PhyML 3.0 [32] under the
105 models (TN for the general tree and GTR for the morphometric-match, +I+G) selected with
106 jModelTest [33] using the Akaike criterion (AIC) [34].

107 *2D Morphometrics.* Using a numerical camera mounted on a binocular, a picture was taken from
108 each mouse molar, with the skull adjusted so that the occlusal surface of the first upper molar would
109 be approximately flat. The molar shape was approximated by the 2D outline of the occlusal surface,
110 towards the base of the crows, which is only affected by heavy wear. Each outline was defined by 64
111 points, which were analysed using a Fourier based approach [21]. Fourteen variables, corresponding
112 to a set of Fourier coefficients (FCs) were deemed adequate to describe the molar shape [21]. An
113 additional variable (A0) provided an estimate of the outline size.

114 *Data acquisition for 3D morphometrics.* – Skulls were scanned at a cubic voxel resolution of 18µm
115 using a RX in-vivo Skyscan 1076 microtomograph (µCT) device at the Platform Montpellier RIO
116 Imaging. The left first upper molar (UM1) (Fig. 2) was delimited on each slice using a threshold
117 method in Avizo software (version 7.1 – Visualization Sciences Group, FEI Company) and connections
118 with outer material (jaw bone and second upper molar) were manually closed and the surface

119 generated. On a randomly chosen reference tooth, a template was prepared describing the outer
120 surface of the tooth. Since age was not controlled in wild-trapped populations, the template was
121 designed not to take into account parts of the tooth most sensitive to wear: the top of the cusps
122 were cut off the template (Supp. Fig.1). The template was defined by 1532 equally spaced sliding-
123 landmarks anchored by eight landmarks. These eight landmarks were defined on all specimens and
124 were used for a Procrustes superimposition to align all the specimens in space. Then, the original
125 template was deformed in order to match the original surface of each tooth. Points were allowed to
126 slide along tangent planes according to the minimum bending energy criterion, with an iterative
127 procedure until convergence [35, 36]. Sliding-landmarks were adjusted for scaling, translation and
128 rotation according to a Procrustes superimposition. All procedures were performed using the
129 packages 'Morpho' [37] and 'mesheR' [38]. Procrustes coordinates, i.e. residual coordinates of the
130 sliding-landmarks after Procrustes superimposition, constituted the shape variables describing tooth
131 shape. Centroid size (square root of the sum of the squared distance from each sliding-landmark to
132 the centroid of the configuration) estimated the size of the tooth.

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

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

145 Size, phylogeny, climate and ecology as explanatory variables of morphometric variation. – A linear
146 model was used to investigate effects of size, phylogeny, ecology, and climate on tooth shape. (1)
147 Size was evaluated as the centroid size of the tooth. (2) Phylogeny was included as the first four axes
148 of the PCOA on the D-loop distance matrix, including the set of axes with > 90% of variance. (3)
149 Climatic data were extracted from the WorldClim database with a resolution of 2.5 arc-minutes using
150 the 'raster' package [42]. The data included Annual Mean Temperature, Temperature Seasonality,

151 Mean Warmest Quarter, Mean Coldest Quarter, Annual Precipitation, Precipitation of the Wettest
152 Quarter, Precipitation of the Driest Quarter. A PCA was performed to summarize these partly
153 redundant data. The first three PCs explaining > 90% of the total variance were retained as
154 explanatory variables in the model. (4) Ecological coding included presence/absence of competitors
155 and of predators according to the literature (Supp. Table 3) and coded these data as 0/1 (Table 1;
156 Supp. Table 4). Since house mice strongly rely on human populations for resources and transport,
157 human population density was also included as an explanatory variable.

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

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

169 Visualisations of shape changes were performed using the ‘Morpho’ package [37]. PCOA and Protests
170 were performed using the vegan package [45].

171

172 **Results**

173 *Phylogeny*

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

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

190 *Three-dimensional tooth morphology*

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

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

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

208 **The 2D analysis (Supp. Fig. 4) provided a correlated configuration of between-group differentiation**
209 **(comparison between PCs $>5\%$ [5 in 3D and 5 in 2D]: Protest $P = 0.006$, Mantel $P = 0.008$). As in 3D,**
210 **Western European samples appeared as well clustered. The convergence between Tenerife, La Palma**
211 **and Madeira on the one hand, and from Guillou Island and El Hierro on the other hand, were further**

212 supported. The idiosyncrasy of Orkney, making all island to group together in the 3D analysis, was
213 not captured by the 2D outline. Orkney islands appeared as widely dispersed in the corresponding
214 morphospace.

215 *Tooth shape vs size, phylogeny, climate and ecology*

216 The total shape variation of the tooth could be summarized along five axes, totalling more than 60%
217 of variance (19.1%, 18.6%, 10.9%, 6.1% and 5.7%). Climatic data were summarized on three PC axes
218 (66.4%, 19.3%, and 13.1% of variance). Phylogenetic data were summarized on four PCOA axes
219 (58.0%, 20.7%, 9.9% and 3.7%). Ecological data (competition, predation, human density) were further
220 included in the linear model.

221 The model indicated a weak contribution of size (3.4%), a balanced influence of ecology (7.3%) and
222 climate (6.0%), and the strongest influence of phylogeny (12.1%) (all $P < 0.01$, competition and
223 phylogeny $P < 0.001$) (Fig. 4). Effects on tooth shape were the following. (1) Size: larger molars were
224 longer at their forepart and thinner in their labial region. (2) Phylogeny: the first axis, roughly
225 opposing continental Western Europe to Orkney and Macaronesian islands, corresponded to an
226 anterior elongation and a reduction of the protocone and neighboring lingual cusp. (3) Ecology:
227 among competition, predation and human density, only competition had a significant effect.
228 Decrease in competition involved a forepart expansion together with an overall thinning of the cusps.
229 (4) Climate: With a temperature decrease and a precipitation increase (from Macaronesian to Sub-
230 Antarctic islands), central cusps moved forward and the anterior lingual fringe expanded. From
231 seasonal (continental) to less seasonal (more or less all islands) environments, the tooth lengthened
232 in its forepart and most cusps shortened. **A similar hierarchy of factors was found when including**
233 **shape axes totalling more than 80% of variance (11 axes): phylogeny (9.4%), ecology (7.5%), climate**
234 **(5.7%) and size (2.4%). The same hierarchie was further found based on the 2D outline analysis, with**
235 **even less variance explained (shape described by all 5 axes > 5%, totalling 87% of variance):**
236 **phylogeny (7.2%), ecology (3.5%), climate (2.7%) and size (1.0%). The 2D sampling include teeth of all**
237 **wear stage and possible uncertainty in orientation of the occlusal plane, possibly explaining the larger**
238 **percentage of unexplained variance.**

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

244

245 **Discussion**

246 *Methodological originality: tooth evolution in 3D*

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

255 *Phylogenetic history as a key factor in tooth shape evolution*

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

265 Discrepancies between the morphological and phylogenetic signature raised questions regarding
266 population history. Madeira, La Palma and Tenerife shared a similar tooth shape. The morphological
267 similarity of Madeira and La Palma was expected given their phylogenetic relatedness [14], possibly
268 reflecting early trading routes between the Madeira and Canaries archipelagoes by the Portuguese
269 during the XVth century. The morphological similarity between Tenerife and La Palma was not
270 surprising given their geographic proximity, but was in conflict with the difference in the dominant
271 mitochondrial haplogroup in both islands. The sporadic occurrence of Tenerife-like haplotypes on La
272 Palma, and La Palma-like ones on Tenerife, suggested that gene flow occurred between neighboring
273 islands and that the resilience of local populations to later invaders [25] may break down when
274 human exchanges are important. **The similar tooth morphology on Tenerife and La Palma may be**

275 due to a genetic homogenization on the Canary archipelago that would not be traced by mtDNA,
276 argued to be a signature of the initial colonization [16, 25]. Alternatively, it could be a convergence in
277 similar ecological and climatic environments. The persistence of this tooth shape despite multiple
278 colonizations anyway suggests a resilience of this morphology and/or strong selective pressure
279 maintaining it in the insular populations.

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

286 *Competition as a driving evolutionary force on molar shape*

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

299 *Secondary importance of climate*

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

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

310 *Insularity magnifying phylogenetic and adaptive differences*

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

327 *Repeated tooth elongation: a line of least evolutionary resistance?*

328 Anterior tooth elongation appeared as a recurrent feature of shape variation, involved in the
329 response to size increase, to competition and to seasonality. It also corresponded to the residual
330 within-group variation, matching previous 2D observations [21, 62]. The main direction of within-
331 group variance has been suggested to constitute a 'line of least resistance to evolution' [63]
332 producing variants to be screened by selection. The recurrent mobilization of the anterior tooth
333 shape elongation may document the existence of a standing variation for this trait, explaining its
334 potential for fast and convergent evolution. In contrast, phylogenetic signatures seemed to involve
335 much more localized and discrete morphological features, suggesting that such changes may simply
336 accumulate at a low pace [64]. Our 3D results evidence that despite predictions [65], substantial
337 evolution and adaptation can occur even when accounting for the whole complexity of a phenotype

338 although signals of convergent evolution and fast divergence appear as even stronger when using a
339 'degenerate' description of the tooth, namely its 2D quantification describing only the overall
340 arrangement of the cusps. These are challenging results suggesting that as one of the oldest
341 passengers of human travels, one of the best world-wide invasives, and one of the most studied
342 laboratory model in developmental biology, the house mouse offers a unique opportunity to unravel
343 the complexity of the responses to new environments.

344

345 **Ethics**

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

352 **Data accessibility**

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

356 **Competing interest**

357 No competing interest.

358 **Authors' contribution**

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

363

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373 **References**

- 374 1. Pergams O.R.W., Ashley M.V. 2001 Microevolution in island rodents. *Genetica* **112-113**, 245-
375 256.
- 376 2. Travisano M., Mongold J.A., Bennet A.F., Lenski R.E. 1995 Experimental tests of the roles of
377 adaptation, chance, and history in evolution. *Science* **267**, 87-90.
- 378 3. Keller S.R., Taylor D.R. 2008 History, chance and adaptation during biological invasion:
379 separating stochastic phenotypic evolution from response to selection. *Ecology Letters* **11**(852-866).
- 380 4. Berry R.J. 1973 Chance and change in British long-tailed field mice (*Apodemus sylvaticus*).
381 *Journal of Zoology, London* **170**, 351-366.
- 382 5. Carroll S.P. 2008 Facing change: forms and foundations of contemporary adaptation to biotic
383 invasions. *Molecular Ecology* **17**, 361-372.
- 384 6. Losos J.B., Schoener T.W., Warheit K.I., Creer D. 2001 Experimental studies of adaptive
385 differentiation in Bahamian *Anolis* lizards. *Genetica* **112-113**, 399-415.
- 386 7. Losos J.B., Ricklefs R.E. 2009 Adaptation and diversification on islands. *Nature* **457**, 830-836.
- 387 8. Beldade P., Koops K., Brakefield P.M. 2002 Developmental constraints versus flexibility in
388 morphological evolution. *Nature* **416**, 844-847.
- 389 9. Berry R.J. 1996 Small mammal differentiation on islands. *Philosophical Transactions of the*
390 *Royal Society, London B* **351**(1341), 753-764.
- 391 10. Vigne J.-D., Zazzo A., Thomas C., Guilaine J. 2014 The transportation of mammals sheds light
392 on early voyaging and boats in the Mediterranean Sea. *Eurasian Prehistory* **10**, 157-176.
- 393 11. Lowe S., M. B., S. B., M. D.P. 2000 100 of the World's Worst Invasive Alien Species. A
394 selection from the Global Invasive Species Database. pp. 1-12, The Invasive Species Specialist Group.
- 395 12. Guénet J.-L., Bonhomme F. 2003 Wild mice: an ever-increasing contribution to a popular
396 mammalian model. *Trends in Genetics* **19**(1), 24-31.
- 397 13. Berry R.J., Peters J., Van Aarde R.J. 1978 Sub-antarctic House mice: colonization, survival and
398 selection. *Journal of Zoology, London* **184**, 127-141.
- 399 14. Bonhomme F., Orth A., Cucchi T., Rajabi-Maham H., Catalan J., Boursot P., Auffray J.-C.,
400 Britton-Davidian J. 2011 Genetic differentiation of the house mouse around the Mediterranean
401 basin: matrilineal footprints of early and late colonization. *Proceedings of the Royal Society of*
402 *London, Biological Sciences (serie B)* **278**, 1034-1043.
- 403 15. Hardouin E.A., Orth A., Teschke M., Darvish J., Tautz D., Bonhomme F. 2015 Eurasian house
404 mouse (*Mus musculus* L.) differentiation at microsatellite loci identifies the Iranian plateau as a
405 phylogeographic hotspot. *BMC Evolutionary Biology* **15**, 26. (doi:10.1186/s12862-015-0306-4).
- 406 16. Searle J.B., Jones C.S., Gündüz İ., Scascitelli M., Jones E.P., Herman J.S., Victor R.R., Noble L.R.,
407 Berry R.J., Giménez M.D., et al. 2009 Of mice and (Viking?) men: phylogeography of British and Irish

408 house mice. *Proceedings of the Royal Society of London, Biological Sciences (series B)* **276**, 201-207.
409 (doi:doi:10.1098/rspb.2008.0958).

410 17. Cucchi T. 2008 Uluburun shipwreck stowaway house mouse: molar shape analysis and
411 indirect clues about the vessel's last journey. *Journal of Archaeological Science* **35**, 2953-2959.
412 (doi:doi:10.1016/j.jas.2008.06.016).

413 18. Cucchi T., Bălăşescu A., Bem C., Radu V., Vigne J.-D., Tresset A. 2011 New insights into the
414 invasive process of the eastern house mouse (*Mus musculus musculus*): Evidence from the burnt
415 houses of Chalcolithic Romania. *The Holocene* **21**(8), 1195-1202.

416 19. Piras P., Marcolini F., Raia P., Curcio M.T., Kotsakis T. 2009 Testing evolutionary stasis and
417 trends in first lower molar shape of extinct Italian populations of *Terricola savii* (Arvicolidae,
418 Rodentia) by means of geometric morphometrics. *Journal of Evolutionary Biology* **22**(1), 179-191.

419 20. Wolf M., Friggens M., Salazar-Bravo J. 2009 Does weather shape rodents? Climate related
420 changes in morphology of two heteromyid species. *Naturwissenschaften* **96**, 93-101.

421 21. Renaud S., Pantalacci S., Auffray J.-C. 2011 Differential evolvability along lines of least
422 resistance of upper and lower molars in island house mice. *PLoS One* **6**(5), e18951.
423 (doi:10.1371/journal.pone.0018951).

424 22. Britton-Davidian J., Catalan J., Ramalhinho M.d.G., Auffray J.-C., Nunes A.C., Gazave E., Searle
425 J.B., Mathias M.d.L. 2005 Chromosomal phylogeny of Robertsonian races of the house mouse on the
426 island of Madeira: testing between alternative mutational processes. *Genetics Research* **86**(03), 171-
427 183.

428 23. Renaud S., Hardouin E.A., Pisanu B., Chapuis J.-L. 2013 Invasive house mice facing a changing
429 environment on the Sub-Antarctic Guillou Island (Kerguelen Archipelago). *Journal of Evolutionary*
430 *Biology* **26**, 612-624.

431 24. Valenzuela-Lamas S., Baylac M., Cucchi T., Vigne J.-D. 2011 House mouse dispersal in Iron Age
432 Spain: a geometric morphometrics appraisal. *Biological Journal of the Linnean Society* **102**, 483-497.

433 25. Hardouin E., Chapuis J.-L., Stevens M.I., van Vuuren J.B., Quillfeldt P., Scavetta R.J., Teschke
434 M., Tautz D. 2010 House mouse colonization patterns on the sub-Antarctic Kerguelen Archipelago
435 suggest singular primary invasions and resilience against re-invasion. *BMC Evolutionary Biology* **10**,
436 325. (doi:www.biomedcentral.com/1471-2148/10/325).

437 26. Tamura K., Stecher G., Peterson D., Filipski A., Kumar S. 2013 MEGA6: Molecular evolutionary
438 genetics analysis version 6.0. *Molecular Biology and Evolution* **30**, 2725-2729.

439 27. Nachman M.W., Boyer S.N., Searle J.B., Aquadro C.F. 1994 Mitochondrial DNA variation and
440 the evolution of Robertsonian chromosomal races of house mice, *Mus domesticus*. *Genetics* **136**,
441 1105-1120.

442 28. Searle J.B., Jones C.S., Gündüz I., Scascitelli M., Jones E.P., Herman J.S., Rambau R.V., Noble
443 L.R., Berry R.J., Giménez M.D., et al. 2009 Of mice and (Viking?) men: phylogeography of British and
444 Irish house mice. *Proceedings of the Royal Society, London B* **276**, 201-207.

445 29. Librado P., Rozas J. 2009 DnaSP v5: A software for comprehensive analysis of DNA
446 polymorphism data. *Bioinformatics* **25**, 1451-1452.

447 30. van Vuuren B.J., Chown S.L. 2007 Genetic evidence confirms the origin of the house mouse
448 on sub-Antarctic Marion Island. *Polar Biology* **30**, 327-332.

449 31. Gouy M., Guindon S., Gascuel O., Lyon D. 2010 SeaView version 4: A multiplatform graphical
450 user interface for sequence alignment and phylogenetic tree building. *Molecular Biology and*
451 *Evolution* **27**, 221-224.

452 32. Guindon S., Dufayard J.-F., Lefort V., Anisimova M., Hordijk W., Gascuel O. 2010 New
453 algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of
454 PhyML 3.0. *Systematic Biology* **59**, 307-321.

455 33. Darriba D., Taboada G.L., Doallo R., Posada D. 2012 jModelTest 2: more models, new
456 heuristics and parallel computing. *Nature Methods* **9**, 772. (doi:10.1038/nmeth.2109).

- 457 34. Akaike H. 1973 Information theory as an extension of the maximum likelihood principle. In
458 *Second International Symposium on Information Theory* (ed. Kiado A.), pp. 267-281. Budapest,
459 Hungary.
- 460 35. Bookstein F.L. 1997 Landmark methods for forms without landmarks: morphometrics of
461 group differences in outline shape. *Medical Image Analysis* **1**, 225-243.
- 462 36. Gunz P., Mitteroecker P., Bookstein F.L. 2005 Semilandmarks in three dimensions. Modern
463 morphometrics in physical anthropology, pp. 73–98, Springer, In *Modern Morphometrics in Physical*
464 *Anthropology* (ed. Slice D.E.).
- 465 37. Schlager S. 2014 Morpho: Calculations and visualizations related to Geometric
466 Morphometrics. version (p. R package, 2.0.0.140402 ed.
- 467 38. Schlager S. 2013 mesheR: Meshing operations on triangular meshes. R package version 0.4-
468 00. . (<https://github.com/zarquon42b/mesheR>.
- 469 39. Gower J.C. 1966 Some distance properties of latent root and vector methods used in
470 multivariate analysis. *Biometrika* **53**, 325-338.
- 471 40. Paradis E., Claude J., Strimmer K. 2004 APE: analyses of phylogenetics and evolution in R
472 language. *Bioinformatics* **20**, 289-290.
- 473 41. Revell L.J. 2012 phytools: An R package for phylogenetic comparative biology (and other
474 things). *Methods in Ecology and Evolution* **3**, 217-223.
- 475 42. Hijmans R.J., van Etten J. 2014 raster: Geographic data analysis and modelling. . (p. R
476 package, 2.2-12 ed.
- 477 43. Dray S., Dufour A.-B. 2007 The ade4 package: implementing the duality diagram for
478 ecologists. *Journal of Statistical Software* **22**, 1-20.
- 479 44. Peres-Neto P.R., Jackson D.A. 2001 How well do multivariate data sets match? The
480 advantages of a Procrustean superimposition approach over the Mantel test. *Oecologia* **129**, 169-
481 178.
- 482 45. Oksanen J., Blanchet F.G., Kindt R., Legendre P., Minchin P.R., O'Hara R.B., Simpson G.L.,
483 Solymos P., Stevens M.H.H., Wagner H. 2013 vegan: Community Ecology Package. R package version
484 2.0-10. <http://CRAN.R-project.org/package=vegan>. (
- 485 46. Gündüz İ., Auffray J.-C., Britton-Davidian J., Catalan J., Ganem G., Ramalhinho M.G., Mathias
486 M.L., Searle J.B. 2001 Molecular studies on the colonization of the Madeiran archipelago by house
487 mice. *Molecular Ecology* **10**, 2023-2029.
- 488 47. Macholan M. 2006 A geometric morphometric analysis of the shape of the first upper molar
489 in mice of the genus *Mus* (Muridae, Rodentia). *Journal of Zoology* **270**(4), 672-681.
- 490 48. Misonne X. 1969 African and Indo-Australian Muridae. Evolutionary trends. Tervuren,
491 Belgique, Musée Royal de l'Afrique Centrale; 219 p.
- 492 49. Renaud S., Pantalacci S., Quéré J.-P., Laudet V., Auffray J.-C. 2009 Developmental constraints
493 revealed by co-variation within and among molar rows in two murine rodents. *Evolution and*
494 *Development* **11**(5), 590-602. (doi:DOI: 10.1111/j.1525-142X.2009.00365.x).
- 495 50. Granjon L., Cheylan G. 1988 Mécanismes de coexistence dans une guildé de muridés insulaire
496 (*Rattus rattus* L., *Apodemus sylvaticus* L. et *Mus musculus domesticus* Ruddy) en Corse: conséquences
497 évolutives. *Zeitschrift für Säugetierkunde* **53**, 301-316.
- 498 51. Nunes A.C., Britton-Davidian J., Catalan J., Ramalhinho M.G., Capela R., Mathias M.L., Ganem
499 G. 2005 Influence of physical environmental characteristics and anthropogenic factors on the
500 position and structure of a contact zone between two chromosomal races of the house mouse on the
501 island of Madeira (North Atlantic, Portugal). *Journal of Biogeography* **32**(12), 2123-2134.
- 502 52. Gómez Cano A.R., Fernández M.H., Álvarez-Sierra M.Á. 2013 Dietary ecology of Murinae
503 (Muridae, Rodentia): A geometric morphometric approach. *PLoS ONE* **8**(11), e79080.
504 (doi:doi:10.1371/journal.pone.0079080).
- 505 53. Le Roux V., Chapuis J.-L., Frenot Y., Vernon P. 2002 Diet of the house mouse (*Mus musculus*)
506 on Guillou Island, Kerguelen archipelago, Subantarctic. *Polar Biology* **25**, 49-57.

- 507 54. Smith V.R., Avenant N.L., Chown S.L. 2002 The diet and impact of house mice on a sub-
508 Antarctic island. *Polar Biology* **25**, 703-715. (doi:doi 10.1007/s00300-002-0405-8).
- 509 55. van Aarde R.J., Jackson T.P. 2007 Food, reproduction and survival in mice on sub-Antarctic
510 Marion Island. *Polar Biology* **30**, 503-511. (doi:doi 10.1007/s00300-006-0209-3).
- 511 56. Ganem G. 2012 Behavior, ecology, and speciation in the house mouse. In *Evolution of the*
512 *house mouse* (eds. Macholan M., Baird S.J.E., Munclinger P., Pialek J.), pp. 373-406. Cambridge,
513 Cambridge University Press.
- 514 57. Chapuis J.-L., Boussès P., Barnaud G. 1994 Alien mammals, impact and management in the
515 French subantarctic islands. *Biological Conservation* **67**, 97-104.
- 516 58. Reisinger R.R., De Bruyn P.J.N., Tosh C.A., Oosthuizen W.C., Mufanadzo N.T., Bester M.N.
517 2011 Prey and seasonal abundance of killer whales at sub-Antarctic Marion Island. *African Journal of*
518 *Marine Science* **33**, 99-105.
- 519 59. Millien V. 2006 Morphological evolution is accelerated among island mammals. *PLoS Biology*
520 **4**(10), e321. (doi:DOI: 10.1371/journal.pbio.0040321).
- 521 60. Cucchi T., Barnett R., Martinkova N., Renaud S., Renvoisé E., Evin A., Sheridan A., Mainland I.,
522 Wickham-Jones C., Tougaard C., et al. 2014 The changing pace of insular life: 5000 years of
523 microevolution in the Orkney vole (*Microtus arvalis orcadensis*). *Evolution* **68**(10), 2804-2820.
524 (doi:10.1111/evo.12476).
- 525 61. Caumul R., Polly P.D. 2005 Phylogenetic and environmental components of morphological
526 variation: skull, mandible, and molar shape in marmots (*Marmota*, Rodentia). *Evolution* **59**(11), 2460-
527 2472.
- 528 62. Renaud S., Auffray J.-C. 2013 The direction of main phenotypic variance as a channel to
529 morphological evolution: case studies in murine rodents. *Hystrix, The Italian Journal of Mammalogy*
530 **24**(1), 85-93. (doi:10.4404/hystrix-24.1-6296).
- 531 63. Schluter D. 1996 Adaptive radiation along genetic lines of least resistance. *Evolution* **50**(5),
532 1766-1774.
- 533 64. Polly P.D. 2001 On morphological clocks and paleophylogeography: towards a timescale for
534 *Sorex* hybrid zones. *Genetica* **112-113**, 339-357.
- 535 65. Salazar-Ciudad I., Marin-Riera M. 2013 Adaptive dynamics under development-based
536 genotype-phenotype maps. *Nature* **497**, 361-364.

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541 **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

542

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

547

548 **Figure captions**

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

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

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

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

568

569 **Supplementary File captions**

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

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

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

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

585

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

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

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

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

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