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Sabrina Renaud, Emilie A. Hardouin, Jean-Pierre Quéré, Pascale Chevret

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

3

4 Sabrina Renaud <sup>1</sup>, Emilie A. Hardouin <sup>2</sup>, Jean-Pierre Quéré <sup>3</sup>, Pascale Chevret <sup>1</sup>

5 <sup>1</sup> Laboratoire de Biométrie et Biologie Evolutive, UMR 5558, CNRS, Université Lyon 1, Université de  
6 Lyon, F-69622 Villeurbanne, France

7 <sup>2</sup> Department of Life and Environmental Sciences, Faculty of Science and Technology, Bournemouth  
8 University, Christchurch House, Talbot Campus, Poole, Dorset BH12 5BB, United Kingdom

9 <sup>3</sup> Centre de Biologie et Gestion des Populations (INRA / IRD / Cirad / Montpellier SupAgro), Campus  
10 international de Baillarguet, CS 30016, F-34988, Montpellier-sur-Lez Cedex, France

11

12 **Abstract**

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

23 The feral population appeared as genetically highly diversified, suggesting a possible functioning as a  
24 sink in relation to the surrounding commensal populations. In contrast, commensal populations were  
25 highly homogeneous from a genetic point of view, suggesting each population to be isolated. This  
26 triggered morphological differentiation between neighboring farms. Seasonal differences in  
27 morphometric traits (mandible size and shape and molar size and shape) were significant in both  
28 settings, although seasonal variations were of larger amount in the feral than in the commensal  
29 population. Seasonal variations in molar size and shape could be attributed to differential wear in  
30 young or overwintered populations. Differences in mandible shape could be related to aging in

31 overwintered animals, but also possibly to differing growth conditions depending on the season. The  
32 impact of these ecological processes on morphometric traits is moderate compared to divergence  
33 over a large biogeographic scale, but their significance nevertheless underlines that even  
34 morphological characters may trace populations dynamics at small scale in time and space.

35

## 36 **Keywords**

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

39

## 40 **Introduction**

41 Issues of important current concern, such as adaptation of invasive species to their new  
42 environments, response of local species to invasions and/or climate change, deliver growing  
43 evidences that evolution can take place at time scales usually considered to be characteristic of  
44 ecological processes (Kinnison & Hairston, 2007; Lambrinos, 2004). Morphological characters were  
45 traditionally thought to vary at evolutionary timescales. Nevertheless, due to plasticity but also to  
46 microevolutionary changes, they can also vary at fast rates and be involved in contemporary  
47 evolution (Collyer, Stockwell, Adams, & Reiser, 2007; Ghalambor, McKay, Carroll, & Reznick, 2007).

48 **This growing body of evidences may also be due to the development of geometric morphometric**  
49 **methods, allowing to quantify such fine-scale variation. To provide a background for such fine-scale**  
50 **processes**, reference studies to assess the amount of variation expected for morphological traits due  
51 to background ecological processes, such as seasonal variations in populations, are often lacking  
52 because sampling designed for ecology, such as mark-recapture, are not designed for delivering  
53 osteological material for morphometric studies.

54 The aim of this study is to provide a hint into morphometric variations related to small-scale  
55 processes such as seasonal variation and local differentiation. The model is the Western house  
56 mouse (*Mus musculus domesticus*). It is known for its great adaptability and behavioral flexibility,  
57 thus exposing it to contrasted ecological conditions even at small scale in time and space. We  
58 investigated two features well studied in mice, having different developmental characteristics and  
59 hence potentially responding differently to processes at an ecological scale. The mandible is an  
60 osteological structure that grows postnatally, reaching ~95% of its final adult shape shortly after  
61 weaning (Swiderski & Zelditch, 2013). Bone remodeling occurs nevertheless during the whole

62 animal's life, and this can trigger plastic differences in response to food resources even during adult,  
63 post-weaning life (Anderson, Renaud, & Rayfield, 2014). In contrast, the first upper molar is  
64 mineralized during pre-natal life and once erupted, it is only affected by wear. This may make the  
65 mandible more prone to respond to short term processes.

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

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

85

## 86 **Material**

87 Commensal samples were sampled from two farms in Tourch (Brittany, France). Most mice were  
88 trapped in a large pigsty in Kerloyou, in August 2011, November 2011 and May 2012 (Table 1, Fig.  
89 1F). A farm in the nearby place Kerc'hoaler, 1.8 km away, was further sampled in June 2012.

90 Mice from Frontignan corresponded to an outdoor, feral population found in the Aresquiers spit of  
91 land between the Mediterranean Sea and the Étang de Vic, close to Montpellier (France). Mice were  
92 trapped in April 2009, October 2009 and March 2010 over less than 1 km (Table 1). Despite the

93 uneven sample size (7, 20 and 4 specimens respectively), the trapping effort was similar for the three  
94 seasons. One additional mouse was captured in March 2013.

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

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

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

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

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

118

### 119 *Phylogenetics*

120 The morphometrics study was complemented by a genetic analysis of the D-loop (mtDNA)  
121 sequences. Twenty-six mice were sequenced in Tournich, corresponding to six randomly sampled for  
122 each of the three seasons at the Kerloyou pigsty and eight at the Kerc’hoaler farm (Table 1). Twenty-

123 two mice were sequenced in Frontignan, most of them (14) trapped in October 2009 and the other  
124 ones trapped in April 2009 (5) March 2010 (2) and March 2013 (1) (Table 1).

125 For these samples, DNA was extracted from ethanol-preserved tissue, using the DNeasy Blood and  
126 Tissue kit (Qiagen, France). The D-loop was amplified using previously described primers and  
127 protocol (Hardouin et al., 2010). The sequences generated were visualized using MEGA6 (Tamura,  
128 Stecher, Peterson, Filipski, & Kumar, 2013). The new sequences were deposited in GenBank under  
129 accession numbers LT718851 to LT718898 (Supplementary Table 1).

130 This sampling was completed by sequences of various mainland Western Europe locations retrieved  
131 from GenBank (Supplementary Table 2).

132

## 133 **Methods**

### 134 *Phylogenetic analyses*

135 Haplotypes and genetic diversity indices for Frontignan and Tournay sequences were determined with  
136 DNAsp v 5 (Librado and Rozas, 2009). The haplotypes were combined with sequences retrieved from  
137 GenBank and aligned with MUSCLE implemented in SeaView (Gouy, Guindon, Gascuel, & Lyon,  
138 2010). The final alignments comprised 313 sequences and 833 base pairs. The phylogenetic tree was  
139 reconstructed using Bayesian inference (BI) with MrBayes v3.2 (Ronquist et al., 2012). The best  
140 model (TrN+I+G) was determined with jModelTest (Darriba, Taboada, Doallo, & Posada, 2012) using  
141 the Akaike criterion (AIC) (Akaike, 1973). As TrN model was not available in MrBayes we used  
142 Nst=mixed, which sampled across substitution models. Node robustness were estimated using  
143 posterior probabilities (PP) in BI analyses. For BI, two Markov chain Monte Carlo (MCMC) analyses  
144 were run independently for 20 000 000 generations. One tree was sampled every 500 generations.  
145 The burn-in was graphically determined with Tracer v1.6 (Rambaut, Suchard, Xie, & Drummond,  
146 2014). We also checked that the effective sample sizes (ESSs) were above 200 and that the average  
147 SD of split frequencies remained <0.05 after the burn-in threshold. We discarded 10% of the trees  
148 and visualized the resulting tree under Figtree v1.4 (Rambaut, 2012).

149

### 150 *Mandible and molar outline analysis*

151 Mandibles and tooth rows were pictured using a Leica MZ 9.5 binocular. The hemi-mandible was  
152 placed flat on its lingual side. The upper molar row was manually oriented so that the occlusal  
153 surface would match the horizontal plane. Based on these pictures, the shape of the mandible and  
154 the first upper molar (UM1) were described using 64 points sampled at equal curvilinear distance

155 along their 2D outline (Fig. 1G), sampled using the Optimas software. This sampling is sufficient to  
156 document even small structures such as the coronoid process. An outline-based method was chosen  
157 because reliable landmarks are difficult to position on the murine molar. The top of the cusps is  
158 abraded by wear and cannot be used for assessing the position of the cusps, and landmarks  
159 bracketing the cusps on the outline are difficult to position given the smooth undulation delineating  
160 the cusps along the outline (Fig. 1). Similarly, landmarks used to describe the murine mandible are all  
161 placed along the outline (e.g. (Klingenberg, Mebus, & Auffray, 2003)) and some may be difficult to  
162 position when the bone is of smooth shape.

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

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

177 The higher the rank of the harmonics, the more details they represent on the outline (Crampton,  
178 1995; Rohlf & Archie, 1984). This can be used to filter measurement error, by discarding high-order  
179 harmonics (Renaud, Michaux, Jaeger, & Auffray, 1996). This together reduces the number of  
180 variables required to describe the overall morphology of an object. The shape of a mouse mandible  
181 or molar tooth has been shown to be adequately described by the first seven harmonics, i.e. by 14  
182 variables (Renaud et al., 2013).

183

#### 184 *Statistical analyses of morphometric data*

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

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

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

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

201 Euclidean distances in size (based on A0) and shape (based on the set of Fourier coefficients) were  
202 calculated between groups (seasons in feral Frontignan and commensal Kerloyou; local differences  
203 between Kerloyou and Kerc'hoaler; differences between Frontignan, Tournich, and the localities of  
204 comparison). These observed distances were compared to the distribution of distances between  
205 populations simulating random differentiation between mainland populations. For this purpose,  
206 1000 populations of 20 and 15 specimens each were randomly sampled from the pool of mainland  
207 localities (Renaud et al., 2013).

208 Statistics were performed using Past (Hammer, Harper, & Ryan, 2001) and R (R\_core\_team, 2015).  
209 Multivariate analyses were performed using ade4 (Dray & Dufour, 2007).

210

## 211 **Results**

### 212 *Phylogenetics*

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



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

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

223

#### 224 *Reduction of dimensionality*

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

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

231 For molar shape, five axes represented more than 5% of variance in Tournich (PC1 = 38.7%, PC2 =  
232 22.3%, PC3 = 8.6%, PC4 = 6.9%, PC5 = 6.2%) and Gardouch (PC1 = 38.9%, PC2 = 19.9%, PC3 = 12.7%,  
233 PC4 = 8.4%, PC5 = 6.0%). Four axes explained more than 5% of variance in Frontignan (PC1 = 31.7%,  
234 PC2 = 20.3%, PC3 = 18.4%, PC4 = 9.7%).

235

#### 236 *Sexual dimorphism*

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

243

#### 244 *Wear pattern*

245 Wear pattern can be considered as a proxy for the age of the animals. Its distribution differed  
246 between seasons **in the feral population from** Frontignan ( $P < 0.001$ ). This corresponds to a

247 difference between the autumn population of October, dominated by relatively young animals, and  
248 the spring populations composed of animals with teeth worn down, suggesting overwintered animals  
249 (Fig. 3).

250 The difference between season was not significant in the commensal population from Kerloyou ( $P =$   
251 0.068). The summer and autumn populations were mostly composed of young animals, as in  
252 Frontignan, including a juvenile without the third molar erupted in August 2011. The spring  
253 population included young animals together with overwintered ones.

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

256

#### 257 *Morphometric variations in the commensal populations from Tourch*

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

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

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

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

275

#### 276 *Morphometric variations in the feral population from Frontignan*

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

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

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

291

#### 292 *Allometric relationships in different localities*

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

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

306

#### 307 *Patterns of shape differentiation*

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

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

321 Regarding molar shape, seasonal differences did not emerge on the first principal plane (Fig. 6B).  
322 Seasonal samples from Frontignan were clustered on one side of bgPC1 whereas seasonal samples  
323 from Kerloyou were clustered together on the other side of bgPC1, close to one Northern Italian  
324 locality (San Bernardino, IT-SBER). Molars from Kerc'hoaler were further divergent along bgPC1.  
325 Other mainland localities represented a cloud of variation towards the center of the first principal  
326 plane. Here again, correcting for molar size did not change the pattern of differentiation (Supp. Fig.  
327 1B).

328

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

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

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

336 Distances between localities reached higher values than those due to seasonality. For the four cases  
337 considered (mandible size and shape, molar size and shape), some distances between the localities  
338 of the present studies were in the upper range of the distances expected between random European

339 localities (Fig. 7). For molar size and shape, some observed distances outreached the simulated ones.  
340 The neighboring farms of Kerloyou and Kerc'hoaler were differentiated by morphological distances  
341 as high as observed between remote places such as Gardouch and Frontignan or Tournich, but except  
342 for molar size, they were within the range of the simulated distances, and hence congruent with a  
343 random differentiation.

344

## 345 **Discussion**

### 346 *Contrasted genetic diversity in feral and commensal populations*

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

359 In contrast, the feral population in Frontignan was diversified (11 haplotypes, haplotype diversity  $H_d$   
360 = 0.90; nucleotide diversity  $\pi$  = 0.0078), sampling the variety of distant haplogroups present in the  
361 surrounding region. This suggests that this feral population may be a sink population regularly fueled  
362 by immigration of the surrounding areas. Such gene flow may be a factor counterbalancing local  
363 adaption to this peculiar environment (Lenormand, 2002).

364

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

366 Tooth wear has been proposed as the most reliable morphological trait to estimate age in the house  
367 mouse (Lidicker 1966). It provided a rough proxy for the age structure of the populations. Seasonality  
368 in age structure was more pronounced in the feral population of Frontignan than in the commensal  
369 population of Kerloyou. This is in agreement with the fact that feral populations are known to display

370 seasonal breeding (Berry, 1981; Efford et al., 1988; Matthewson et al., 1994; Triggs, 1991) whereas  
371 mice breed all year round on commensal habitats (Berry, 1981; Pocock et al., 2004; Rowe et al.,  
372 1983).

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

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

393

#### 394 *Impact of seasonal variations in size on morphometric characters*

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

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

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

418

#### 419 *Seasonal shape variations*

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

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

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

442

#### 443 *Population dynamics in feral vs commensal populations*

444 Different and possibly conflicting effects may contribute to the dynamics of the populations and  
445 impact their morphometric differentiation. On the one hand, commensal populations have been  
446 shown to function as small demes at a very small geographic scale of few meters (Pocock et al.,  
447 2004). This should promote drift in highly sub-structured populations. In contrast, non-commensal  
448 mice have been reported to move further (Pocock et al., 2004). Dispersal being more important, this  
449 should lead to homogenization of the populations at a regional scale.

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

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



465 In contrast, the commensal populations of Touch displayed a reduced genetic diversity, as reduced  
466 as the one observed on remote islands (Hardouin et al., 2010). Commensal populations of mice are  
467 known to function as small demes of few mice, but there are also known to be transient (Singleton,  
468 1983). The commensal habitat is composed of patches of highly suitable habitats – for instance, farm  
469 buildings where livestock is housed – but these habitats are highly variable in space and time – for  
470 instance, during the clearance and sanitary emptying of poultries and pigsties. Our results suggest  
471 that despite this unpredictable environmental variability, mice managed to sustain an apparently  
472 homogeneous and stable population, at least at the timescale documented here, possibly because  
473 recolonization of the buildings after clearance occurred from related mice from the surrounding  
474 buildings.

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

483

## 484 **Conclusions**

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

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

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

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

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

515

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523

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639

640

641

642 **Tables**

643

Locality	Farm	Latitude	Longitude	Season	Code	Nb Mb	Nb UM1	N <sub>genet</sub>	N <sub>haplo</sub>
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

644

645 **Table 1.** Sampling of the study: locality of trapping and eventually farm, with latitude and longitude,  
646 date of trapping, code, number of mandibles (Nb Md) and first upper molars (Nb UM1) measured,  
647 number of sequenced specimens (N<sub>genet</sub>) and number of haplotypes documented (N<sub>haplo</sub>).

648

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

649

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

655

656

	Kerloyou vs. Kerc'hoaler	Kerloyou Seasons				Frontignan Seasons			
	P	P	P <sub>pairwise</sub>	P <sub>pairwise</sub>	P <sub>pairwise</sub>	P	P <sub>pairwise</sub>	P <sub>pairwise</sub>	P <sub>pairwise</sub>
			Aug11/ Nov11	Aug11/ May12	Nov11/ May12		Oct09/ Apr09	Oct09/ Mar10	Apr09/ Mar10
HBL	0.388	0.399	0.931	0.297	0.493	<b>&lt; 0.001</b>	<b>0.002</b>	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	<i>0.025</i>	0.581	0.955	0.637	0.458	0.260	0.443	0.559	0.979
UM1 size	<b>&lt; 0.001</b>	0.923	0.964	0.999	0.961	<i>0.019</i>	0.785	<i>0.030</i>	<b>0.006</b>
Md shape	<b>&lt; 0.001</b>	<b>0.007</b>	<b>0.002</b>	0.535	0.090	<b>0.007</b>	<b>0.008</b>	<i>0.016</i>	0.620
UM1 shape	<b>0.002</b>	<i>0.020</i>	<b>0.007</b>	0.482	0.197	<i>0.029</i>	<b>0.009</b>	0.556	0.256

659 **Table 3.** Size and shape differences in Touch between the two farms (Kerloyou vs Kerc'hoaler),  
660 between the three seasons in Kerloyou, and between the three seasons in Frontignan. Variables  
661 investigated are body length (head + body length, HBL), body weight, mandible (Md) and first upper  
662 molar (UM1) size, and mandible and molar shape (summarized by the PC axes explaining more than  
663 5% of variance). Differences were investigated using an ANOVA for univariate variables, and a  
664 MANOVA for multivariate data sets (tests performed on the PCs representing more than 5% of  
665 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%

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

672

673



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

674

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

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

690

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

697

698 **Figure Captions**

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

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

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

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

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

724

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

731 mean shape was visualized using inverse Fourier transform for Gardouch, IT-REG, Frontignan October  
732 2009 and March 2010, Kerloyou November 2011 and Kerc'hoaler. Visualization is based on an inverse  
733 elliptic Fourier transform for the mandible, and on the radial Fourier transform for the molar.

734

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

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

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