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1 **A sharp incisor tool for predator house mice back to the wild**

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23 **Keywords**

24 geometric morphometrics, *Mus musculus domesticus*, adaptation, biting, functional morphology

25

26 **Abstract**

27 The house mouse (*Mus musculus domesticus*), as a successful invasive species worldwide, has to
28 forage a variety of resources. Sub-Antarctic mice display among the most notable diet shift from the
29 usual omnivorous-granivorous diet, relying on a larger proportion of terrestrial animal prey. In
30 agreement, a recent study of their mandible morphology evidenced an evolution of their mandible
31 shape to optimize incisor biting, and hence seize preys. Here, the incisors themselves are the focus of
32 a morphometric analysis combined with a 3D study of their internal structure, aiming at a
33 comparison between a Sub-Antarctic population (Guillou island, Kerguelen archipelago) with a range
34 of Western European continental, commensal mice. The predatory foraging behavior of Guillou mice
35 was indeed associated with a sharper bevel of the lower incisor, which appears as an efficient
36 morphology for piercing prey. The incisor of these mice also display a reduced pulp cavity, suggesting
37 slower eruption counter-balancing a reduced abrasion on such soft food material. The dynamics of
38 the ever-growing incisor may thus allow adaptive incisor sculpting and participate to the success of
39 mice in foraging diverse resources.

40

41

42

43 Introduction

44 Adaptations to successfully gather and process food are crucial for insuring survival and resources for
45 any function of an organism. They can involve many facets including digestive tract and behavior, and
46 in mammals, they further rely on a sophisticated dentition. Premolars and molars have evolved
47 complex morphologies for matching functional requirements related to various diets, and are
48 therefore the focus of many studies including morphofunctional and developmental aspects [e.g.
49 (Evans *et al.*, 2007; Jernvall *et al.*, 1996; Popowics & Fortelius, 1997)]. Incisors, in contrast, deserved
50 little interest, possibly due to their simple geometry, despite the fact that they often represent the
51 first tool to be in contact with food particles. In rodents and lagomorphs, incisors are ever-growing,
52 with a high growth rate that counterbalances continuous wear. Ever-growing incisors are the most
53 striking characteristic of the rodent order and they were probably a key component of the extensive
54 evolutionary radiation of rodents (Fabre *et al.*, 2012; Steppan & Schenk, 2017), associated with the
55 versatility of their feeding habits (Landry, 1970; Martin *et al.*, 2016). How the dynamics of eruption
56 varies through the animal's life, and in response to diet, has been investigated (Harari *et al.*, 2005;
57 Klevezal & Shchepotkin, 2012; Taylor & Butcher, 1951). Differences in terms of relative curvature,
58 coverage by the enamel and cross-sectional profile have been demonstrated between species of
59 murine rodents, with a possible link with habitats (Millien-Parra, 2000). Adaptations in terms of
60 curvature of the incisors have been further reported in chisel tooth digging rodents, in which
61 functional loads on the skull and incisors are extremely high (Samuels & Van Valkenburgh, 2009) and
62 in carnivorous rodents, in which low incisor curvature may improve the function of stabbing preys
63 (Fabre *et al.*, 2017; Rowe *et al.*, 2016). The geometry of the incisor's bevel, which constitutes its
64 cutting edge, may be of functional relevance for facing different diets but its role has never been
65 addressed directly in wild populations, especially at the intraspecific level. However, the incisor ever-
66 growing dynamics that can be modulated through the intensity of wear (Meredith *et al.*, 2015; Müller
67 *et al.*, 2014; Taylor & Butcher, 1951) may allow a rapid adjustment to varying food requirements.

68 Being commensal, the house mouse (*Mus musculus domesticus* Schwarz and Schwarz 1943) followed
69 the movement of people around the world, making it a highly successful global invader (Lowe *et al.*,
70 2000). It colonized even remote and inhospitable environments, such as Sub-Antarctic islands. On
71 these remote islands, the mice face conditions widely departing from their usual commensal habits.
72 Mice shifted their diet from their usual omnivorous-granivorous diet to a larger proportion of
73 terrestrial animal prey, mostly above ground and litter macroinvertebrates (Le Roux *et al.*, 2002; van
74 Aarde & Jackson, 2007). Such diet shifts triggered convergent evolution of mandible geometry in
75 various populations of Sub-Antarctic mice, all displaying an increased biomechanical functional
76 performance for incisor biting, constituting an adaptation to catch prey more efficiently (Renaud *et*

77 *al.*, 2018). If the incisor itself displayed adaptive change to increase prey catching was not
78 investigated.

79 The small Guillou Island (1.45 km²) is one of the Sub-Antarctic islands where house mice built
80 successful feral populations by relying on a large component of invertebrates in their diet (Le Roux *et*
81 *al.*, 2002). It is part of the Sub-Antarctic Kerguelen Archipelago, situated in the Indian Ocean about
82 4000 km away from the African and Australian coasts. Mice were introduced on the archipelago
83 during the 19th century (Kidder, 1876). Functional response to the diet shift had thus to evolve in less
84 than two centuries. This evolution occurred in isolation, even from the rest of the archipelago, since
85 Guillou mice all display the same mitochondrial haplotype, and evidence a strong differentiation
86 from mice from other Kerguelen islands (Hardouin *et al.*, 2010). Furthermore, the island experienced
87 in the last two decades a cortege of human-driven modifications: rabbit (*Oryctolagus cuniculus*)
88 eradication by poisoning in 1994 (Chapuis *et al.*, 2001); regression of the native vegetation cover
89 against invasive plants (Chapuis *et al.*, 2004) and increasing summer drought (Lebouvier *et al.*, 2011)
90 decreasing earthworms availability in the litter, both as a consequence of climate change; regression
91 of most native insects because of the spread of an invasive carabid predator (*Merizodus soledadenus*)
92 over the last 15 years (Laparie *et al.*, 2010). All may have changed access and composition of the
93 resources available to mice, as exemplified by a change in mandible shape and an increase in its
94 mineralization from 1993 to 2009, suggesting a higher investment in this trait and/or increased food
95 quality (Renaud, Gomes Rodrigues, *et al.*, 2015).

96 In the present study, the incisor morphology in the house mouse population inhabiting the Guillou
97 Island was investigated. First, the shape of the erupted part of the upper and lower incisors was
98 quantified using 2D geometric morphometrics. Guillou mice were compared to several populations
99 of continental Europe, documenting the usual commensal habitat where mice display an
100 omnivorous-granivorous diet. Four sampling years on Guillou, from 1993 to 2009, allowed
101 documenting the morphology of Guillou mice and its change through time. The objective was to
102 assess if incisor morphology differed between Guillou and continental mice, in relation with the more
103 predatory behavior of the former; and if it varied through years in Guillou, in response to the cohort
104 of human-driven environmental changes. In case of an adaptive response, sharper incisor tips were
105 expected in the predatory Guillou mice. On a subset of mice, 3D imaging of the incisors further
106 allowed to identify changes in the internal structure of the tooth, providing an insight into the
107 developmental processes involved in the incisor shape variations.

108

109 **Material and Methods**

110 *Material*

111 The sampling for Guillou Island included mice trapped on four years covering a 16 years' interval
112 (1993, 2001, 2008 and 2009) (Chapuis *et al.*, 2001; Renaud, Gomes Rodrigues, *et al.*, 2015). Four
113 Western European populations were used for comparison: Cologne-Bonn (Germany), Gardouch
114 (South-Western France), Tourn (Brittany, France) (Renaud *et al.*, 2017) and Balan (nearby Lyon,
115 France) (Fig. S1 and Table S1 in the Supporting Informations).

116 Almost all mice considered were sub-adults and adults, the criteria being the full eruption of the third
117 molars that occurs at weaning; for some mice in Balan, the eruption was ongoing. Sexual dimorphism
118 was not evidenced in continental nor in Guillou populations (Renaud *et al.*, 2013; Renaud *et al.*,
119 2017). Furthermore, sexual dimorphism in incisor size has been shown to be very limited in other
120 species of rodents (Millien-Parra, 2000). Hence, males and females were pooled for further analyses.
121 For all mice except those from Cologne-Bonn, body weight data were available. Mandible area
122 provided a further proxy of body size available for all populations (Renaud, Gomes Rodrigues, *et al.*,
123 2015; Renaud *et al.*, 2017).

124

125 *Morphometric analysis of the incisors*

126 The shape of the lower incisor was quantified based on 2D pictures of the mandible in labial view,
127 using a set of four landmarks and 8 sliding semi-landmarks (Fig. 1A) describing the erupted part of
128 the tooth. The upper incisor was quantified based on 2D pictures of the skull in lateral view, using a
129 set of three landmarks and 16 semi-landmarks. A total of 267 lower incisors and 209 upper incisors
130 were included in the final data set. For estimating measurement error, the population from Cologne-
131 Bonn was measured twice at several weeks of interval by the same operator (CD).

132 The configurations of landmarks and semi-landmarks were superimposed using a generalized
133 Procrustes analysis (GPA) standardizing size, position and orientation while retaining the geometric
134 relationships between specimens (Rohlf & Slice, 1990). During the superimposition, semi-landmarks
135 were allowed sliding along their tangent vectors until their positions minimize the shape difference
136 between specimens, the criterion being the bending energy. A Principal Component Analysis (PCA)
137 on the variance-covariance matrix of the aligned coordinates was used to summarize the shape
138 variance. Shape differences between groups (localities, and for Guillou the different years of
139 trapping) were tested using a permanova (non-parametric multivariate analysis of variance based on
140 9999 permutations) and associated pairwise post-hoc tests, using the PC axes explaining more than
141 5% of total variance. The pattern of differentiation between groups was further investigated using

142 Canonical Variate Analyses (CVA) on the aligned coordinates. This method aims at separating groups
143 by looking for linear combinations of variables that maximize the between-group to within-group
144 variance ratio. By standardizing within-group variance, it is efficient for evidencing relationships
145 between groups even in the case of important anisotropic within-group variance, as may be the case
146 when within-group allometry is important (Renaud, Dufour, *et al.*, 2015).

147 Differences in incisor size, estimated by the centroid size (CS: square root of the sum of squared
148 distances from the landmarks and semi-landmarks to the centroid), and its relationship with body
149 weight (BW) and mandible area were investigated using linear models. The continental vs Guillo
150 origin of the mice was included as a factor in some models and the associated size and shape
151 differences were assessed using t-tests. Allometric shape variations were assessed using linear
152 models between size and the first axis of the PCA, and in a multivariate way, using Procrustes ANOVA
153 comparing size and the aligned coordinates (tests based on 9999 permutations). A visualization of
154 the allometric pattern was provided using the regression score (Adams & Otarola-Castillo, 2013).

155 For assessing measurement error, separate Procrustes superimpositions focused on datasets
156 including only the two sessions of measurements of the Cologne-Bonn (CB) population were
157 performed. The difference between the two sessions were assessed using t-tests for centroid size,
158 and Procrustes ANOVA for the aligned coordinates.

159 Procrustes superimposition, PCA on the aligned coordinates and Procrustes ANOVA were performed
160 using the R package geomorph (Adams & Otarola-Castillo, 2013). Canonical Variate Analyses were
161 computed using the package Morpho (Schlager, 2017). Permanova were performed using Past 3
162 (Hammer *et al.*, 2001). The data are available as Supplementary Data (Supp. Data 1 and 2 for the
163 lower and upper incisors respectively).

164

165 *3D incisor structure*

166 A subset of Guillo (4 from 1993 and 4 from 2009) and continental mice (4 from Cologne-Bonn, CB)
167 were CT-scanned at a cubic voxel resolution of 18 μm using a RX-Skyscan 1076 device at the Platform
168 Montpellier RIO Imaging. The protocol used during the scanning and the reconstruction of the
169 radiographic data (software NRecon v1.6.6.0) was identical for all specimens, allowing direct
170 comparisons of the data (Renaud, Gomes Rodrigues, *et al.*, 2015). Reconstructed data consist of a
171 stack of cross-sectional greyscale images, the grey value (GV) in each pixel being associated to a
172 density value. The higher the grey level, the higher is the density in the concerned pixel. Based on
173 these scans, the structure of the lower incisors was investigated.

174 First, the scans were reoriented using Avizo in order to get a cross-section of the lower incisor
175 between the basis of the bevel and the mandibular bone (Fig. 1B). These slices were transformed in
176 8-bits grey levels (GV ranging from 1 to 256) and analyzed using the image analyzing software
177 Optimas 6.5. Area of the cross-section and its mean and minimal grey values were estimated for each
178 incisor. Differences between continental and Guillole mice were tested using t-tests.

179 Regarding 3D volumes, isosurfaces were constructed based on several thresholds in order to
180 delineate the mandible itself (right hemi-mandible including bone and teeth), the dentine and the
181 enamel of each lower incisor. The mandible was reconstructed by including all material with a grey
182 value > 9000 . The dentine surface of the lower incisor was estimated by including all material with
183 $20000 < GV < 40000$; connections with the mandibular bone were manually removed. The enamel
184 surface was estimated by including all material with $GV > 40000$.

185 For each object (mandible, dentine and enamel of the lower incisor), the volume and mean GV was
186 calculated. Based on a visualization of the mandible, dentine and enamel in lingual view, a set of five
187 landmarks was used to assess geometric differences of the internal structure related to the
188 mandibular bone (Fig. 1B). The longitudinal structure of the mandible was described by the tip of the
189 incisor, the anteriormost point of the mandibular bone along the incisor, and the posterior extremity
190 of the condyle. The anterior tip of the pulp cavity and the posterior basis of the enamel layer
191 described internal structures of the mandible. The configurations of landmarks were superimposed
192 using a Procrustes procedure, providing aligned coordinates that were analyzed using a PCA.
193 Geometric differences between the three groups (CB, Guillole 1993 and Guillole 2009) were assessed
194 using a permanova on the PC axes explaining more than 5% of variance.

195 The Procrustes superimposition and PCA were performed using geomorph, and the permanova was
196 done using Past 3.

197

198 **Results**

199 *Measurement error*

200 The two repeated measurements for the Cologne-Bonn population were not different for lower
201 incisor ($P = 0.4571$) nor for upper incisor shape ($P = 0.9997$). Differences in incisor size between the
202 two measurement sessions were not significant either (lower incisor: $P = 0.535$; upper incisor: $P =$
203 0.925).

204

205 *Lower incisor 2D size and shape*

206 The configuration of landmarks and sliding semi-landmarks on the incisors describes only the visible
207 part of the tooth erupted outside the bone. For the lower incisors, the centroid size of this
208 configuration was highly correlated with the size of the mandible bearing it (CS ~ Mandible Area: $R^2 =$
209 0.327 , $P < 2.2e-16$) and even more tightly related with the body size (CS ~ Body Weight: $R^2 = 0.455$, P
210 $< 2.2e-16$). Guillole mice tended to display slightly larger erupted lower incisors than continental
211 relatives (t-test: $P = 0.0004$), especially for small-size animals (Fig. 2A). The difference between the
212 two groups (here, continent vs Guillole) was however reduced compared with the size-related
213 variation; the slopes of the relationship with body weight appeared to be slightly different between
214 the two groups (CS ~ BW * group: BW = 45.5% of the total variance, $P < 2.20E-16$; group = 5.1%, $P =$
215 $6.44E-07$; interaction = 2.2%, $P = 0.0008$).

216 Allometry appeared as a major factor driving incisor shape variation. Incisor size was correlated with
217 the first axis of the PCA on the aligned coordinates (PC1, 53.7% of total variance ~ CS: $R^2 = 0.414$; $P <$
218 $2.2e-16$; data not shown). Investigating allometric variations on the aligned coordinates confirmed
219 the importance of size-related shape variation. The difference between Guillole and the continent
220 was however significant and the allometric slope was different between the two groups, but this
221 difference was of reduced importance compared to the overall allometric trend (Fig. 2B) (Procrustes
222 ANOVA: shape ~ CS * group: CS = 23.3% of the total variance, $P = 0.0001$; group = 6.2%, $P = 0.0001$;
223 interaction = 1.2%, $P = 0.001$). With increasing incisor size (and hence increasing age of the animals),
224 the bevel tended to become longer relative to the erupted part of the incisor (Fig. 2C).

225 Despite this important source of within-group variation, continental populations tended to be
226 opposed to Guillole samples along the first axis (CVA1 = 52.0%) of a CVA on the aligned coordinates
227 (Fig. 2D), whereas the second axis (CVA2 = 17.7%) corresponded to variation among continental
228 samples. Guillole incisors, compared to continental ones, displayed a bevel of more or less the same
229 length, but with a sharper profile, especially at the tip (Fig. 2E; Fig. S2 in Supporting Informations).
230 The differences between groups (localities, and for Guillole the different years of trapping) was a
231 significant (permanova on PC1 = 53.8%, PC2 = 28.4%, and PC3= 10.7%: $P = 0.0001$). Associated
232 pairwise tests showed that continental populations were overall well differentiated from Guillole
233 samples (Table 1). In contrast, the different years in Guillole were not or only weakly differentiated;
234 the same pattern occurred among continental localities (Table 1).

235

236 *Upper incisor 2D size and shape*

237 Contrary to the lower incisors for which incisor growth seemed more or less constant with increasing
 238 body size, the increase in size of the upper incisors tended to progressively reach a plateau (Fig. 3A).
 239 Tests were thus performed on log transformed size variables. As for the lower incisor, the centroid
 240 size of the upper incisor was highly correlated with the size of the mandible ($\log(\text{CS}) \sim \log(\text{Mandible}$
 241 $\text{Area})$: $R^2 = 0.0505$, $P < 2.2e-16$) and with body size ($\log(\text{CS}) \sim \log(\text{Body Weight})$): $R^2 = 0.542$, $P < 2.2e-$
 242 16). The size of the upper incisor was highly correlated with the size of its lower counterpart ($R^2 =$
 243 0.361 , $P < 2.2e-16$) but increased twice less fast (slope of upper incisor CS \sim lower incisor CS: 0.458
 244 ± 0.042).

245 Guillole and continental mice displayed upper incisors of similar size (t-test: $P = 0.1477$). This was
 246 confirmed in a model including body weight and group as explanatory variables ($\log(\text{CS}) \sim \log(\text{BW}) +$
 247 group : $\log(\text{BW}) = 46.9\%$ of the total variance, $P < 2.20E-16$; $\text{group} = 0.3\%$, $P = 0.4220$).

248 Allometry was not a major factor driving upper incisor shape variation. Incisor size was not correlated
 249 with the first axis of the PCA on the aligned coordinates (PC1, 60.1% of total variance $\sim \log(\text{CS})$: $R^2 =$
 250 0.0160 ; $P = 0.0676$; data not shown). Investigating allometric variations on the aligned coordinates
 251 however showed some size-related incisor shape variation, but it was not so marked as the
 252 difference between continental and Guillole incisors (Procrustes ANOVA: $\text{shape} \sim \log(\text{CS}) * \text{group}$:
 253 $\log(\text{CS}) = 3.5\%$ of the total variance, $P = 0.0378$; $\text{group} = 10.2\%$, $P = 0.0002$; $\text{interaction} = 2.7\%$, $P =$
 254 0.0479). With increasing incisor size, the bevel tended to get indented by a small notch (Fig. 3B).

255 Continental populations tended to be opposed to Guillole samples along the first axis of a CVA on the
 256 aligned coordinates (CVA1 = 45.8%), but the pattern was less clear than for the lower incisor. Only
 257 the oldest samples in Guillole (1993 and to a lesser extent 2001) markedly diverged along PC1
 258 (Fig. 3C). The second axis (CVA2 = 22.1%) corresponded to variation among continental samples.
 259 Guillole incisors, compared to continental ones, displayed a bevel indented by a pronounced notch
 260 (Fig. 3D; Fig. S2).

261 Shape differences between groups (localities, and different years in Guillole) were significant
 262 (permanova on PC1 = 60.1%, PC2 = 16.0%, PC3 = 11.3%, and PC4 = 6.5%: $P = 0.0001$), but associated
 263 pairwise tests showed only few highly significant differences (Table 1). The sample from Guillole 1993
 264 was the only one consistently different from all continental populations.

265

266 *Internal structure of the lower incisors*

267 Considering cross-sections of the incisors at the basis of the bevel (Fig. 1B), continental and Guillole
 268 incisors did not differ in their mean grey value (GV) ($P = 0.2041$) and thus in their average density.

269 Similarly, there were not statistically significant differences in maximal GV ($P = 0.4068$). Continental
270 and Guillou incisors differed, however, in the minimal GV observed in this cross-section ($P = 0.0261$)
271 (Fig. 4A). This difference is due to the occurrence in continental incisors of a dark area at the center
272 of the cross-section (Fig. 4A, Fig. S3 in Supporting Informations), corresponding to the pulp cavity
273 which thus extends beyond the contact with the mandibular bone into the erupted part of the
274 incisor.

275 When considering the whole 3D structure, continental and Guillou mice did not differ in the volume
276 of dentine relative to the mandible volume (t-test: $P = 0.0704$) nor in the mean GV of the dentine ($P =$
277 0.2517). The same was true for the enamel (relative volume: $P = 0.1768$; mean GV: $P = 0.3659$). This
278 suggests that continental and Guillou mice did not differ substantially in the material properties of
279 the dentine and enamel.

280 In contrast, the geometry of the dentine and enamel was quite different between continental and
281 Guillou mice (Fig. 4B). Cologne-Bonn and Guillou mice strongly differed along the first axis of the
282 corresponding PCA, explaining more than 80% of the variance (Fig. 4C). The apex of the pulp cavity
283 was located differently in the different groups: close to the tip of the incisors in continental mice, and
284 much more posteriorly in Guillou mice. Differences between groups were significant (permanova on
285 $PC1 = 81.2\%$ and $PC2 = 15.6\%$: $P = 0.0019$). Pairwise tests showed no difference between years in
286 Guillou ($P = 0.2617$) but significant differences between Cologne-Bonn and the two years in Guillou
287 (CB vs G1993: $P = 0.0284$; CB vs G2009: $P = 0.0305$).

288

289 Discussion

290 *A functionally-relevant difference in lower incisor shape between omnivorous and predator house*
291 *mice*

292 So far rodent incisors have not received much attention, possibly because of their simple shape. Yet,
293 incisors assure initial food processing and acquisition; as such, they evolved in response to various
294 functions such as digging, cutting up food, piercing and capturing preys (Martin *et al.*, 2016).

295 Compared to omnivorous-granivorous rodents, genera adapted to carnivory tend to display thin,
296 narrow incisors with a straighter curvature improving their functioning for piercing preys (Fabre *et*
297 *al.*, 2017; Martin *et al.*, 2016; Rowe *et al.*, 2016). Compared to these cases of advanced specialization,
298 incisors of Guillou mice were only moderately modified. Only lower incisors were consistently
299 different from their continental commensal relatives, but they did not differ in incisor depth or
300 curvature. The geometric differences rather involved the bevel angle, sharper in Guillou mice, and

301 hence of adaptive value to stab preys. This limited amount of morphological differentiation may be
302 due to the different evolutionary scales considered: Mice were introduced on the Kerguelen
303 archipelago ~150 years ago. Compared to interspecific or even intergeneric evolution, this is a short
304 time span to adapt to local conditions, and even the carnivorous trend of Guillou mice, displaying an
305 increased component of invertebrates and especially earthworms (Le Roux *et al.*, 2002) is less
306 extreme than specialist carnivorous genera (Fabre *et al.*, 2017; Rowe *et al.*, 2016).

307

308 *Contrasted response of the upper and lower incisors to diet shift*

309 The upper incisors of Guillou mice did not display a sharper bevel, as their lower counterpart. Rather,
310 they were characterized by a more pronounced notch in the bevel than continental commensal
311 relatives. This difference, however, was only significant for the oldest sample in Guillou, trapped in
312 1993. This contrasts with the consistent differentiation of the lower incisors between Guillou and
313 continental mice.

314 The differential response of the lower and upper incisors to the dietary shift is probably related to
315 their different role during occlusion. While gnawing, the rodent anchor its head with the upper
316 incisors while the lowers work as chisels (Ness, 1956), which come into occlusion just behind the
317 upper incisors (Ness, 1956). If food particles do not fully prevent tooth-tooth contact (attrition), the
318 enamel of the lower incisor could thus get in contact with the dentine of the upper incisor, sculpting
319 a notch into it. During this movement, the lower incisor acts as the active pestle in a sort of “pestle-
320 and-mortar” system (Müller *et al.*, 2014), submitting it to higher functional demand than the upper
321 incisor. This differential role during occlusion could explain the clearer adaptive response to a diet
322 shift of the lower incisors compared to the upper ones. In contrast, the notch in the upper incisor
323 bevel would be a passive consequence of food comminution on the “mortar” of the system, more
324 marked if attrition is stronger. Based on tooth microwear analyses, mice before rabbit eradication in
325 1994 displayed a signature of animal-dominated feeders, switching thereafter towards a more
326 generalist diet (Renaud, Gomes Rodrigues, *et al.*, 2015) despite the persistence of animal preys in
327 their diet (Le Roux *et al.*, 2002). Invertebrates, and especially earthworms whose availability
328 decreased over the years in Guillou because of increasing summer drought (Lebouvier *et al.*, 2011),
329 offer little resistance to occlusion, leading to increased tooth-tooth contact while seizing prey. The
330 marked notch in the upper incisor of the oldest Guillou sample, in 1993 and hence before rabbit
331 eradication, probably testify of the abundance of such soft preys in the diet of the mice at that point.

332

333 *Incisor response within a complex masticatory apparatus*

334 In rodents, incisors and molars cannot come in occlusion at the same time (Cox & Jeffery, 2011) and
335 they are involved in different functions, biting at the incisors and chewing at the molars. The jaw is
336 moved by different masticatory muscles, the temporal and masseter muscles being mainly involved
337 during incision and chewing respectively. Depending on the diet, jaw shape is thus submitted to
338 different adaptive pressures to optimize incisor or molar biting. Guillole mice, and more generally
339 Sub-Antarctic mice which all display a shift towards a more predatory behaviour (Copson, 1986;
340 Smith *et al.*, 2002), have been shown to display biomechanical adaptation of jaw morphology to
341 optimize incisor biting (Renaud, Gomes Rodrigues, *et al.*, 2015; Renaud *et al.*, 2018). The sharp lower
342 incisor bevel evidenced by the present study is well adapted to piercing and capturing prey, thus
343 reflecting an adaptation of the incisor tool itself, and not only of the mandible moving it into
344 occlusion. Sharp blade tips are indeed advantageous to faunivorous mammals because they are
345 efficient to produce an initial tear in the tough foodstuff, such as insect cuticle (Popowics & Fortelius,
346 1997). The concerted changes of the incisor and jaw hence provides evidences of an integrated
347 adaptation of the masticatory apparatus in response to the diet shift towards a predatory behaviour.

348 Jaw shape was also shown to respond to the environmental changes over years in Guillole, with the
349 pronounced difference between Guillole and continental mice fading out through years (Renaud,
350 Gomes Rodrigues, *et al.*, 2015; Renaud *et al.*, 2013). The jaw shape change through years was
351 interpreted as result of an improved investment in the mineralization of the mandibular bone,
352 derived from the intake of better quality food. The signature observed on the upper incisor is very
353 similar and is likely due to the same resource adjustment despite retaining an overall predatory
354 behaviour.

355

356 *Incisor shape change through growth and interaction with response to diet*

357 The size of the incisor increases throughout animal's life (Harari *et al.*, 2005) but incisor growth
358 decelerate with age, due to a decrease in the width of the daily increments (Klevezal & Shchepotkin,
359 2012). In the present study, a deceleration of growth was obvious for the upper incisor but not for
360 the lower incisor. This suggests that wear related to occlusion and attrition was not enough to
361 perfectly counterbalance a growth occurring twice as fast as for the upper incisors. Allometric
362 change, as the response to diet, was discrete in the upper incisor, and both involved the sculpting of
363 a notch into the bevel. Being related to attrition during food comminution, this notch seems to
364 increase with age but more markedly in mice relying on soft-food items. Regarding the lower incisor,
365 both allometry and predator diet were associated with a sharper bevel. This shows that the

366 sharpening of the bevel occurs while functioning during the animal's life, providing mice with a
367 sharper tool with increasing age. Active attritional behavior may be at least partly responsible for this
368 tapering of the cutting edge of the lower incisor. The more pronounced allometric changes in
369 continental than Guillole mice may be related to two not mutually exclusive factors: the higher
370 prevalence of young individuals in the continental sample, and/or the existence of a higher variability
371 in diet consistency along the life of continental mice.

372

373 *Incisor continuous growth allowing for a dynamic sculpting as a response to diet*

374 Incisor growth can be considerably modulated by the use of the incisor itself. The absence of
375 occlusion has been shown to double their rate of eruption in rats. In contrast, soft food causing little
376 abrasion slowed down eruption rate by as much as 35% (Burn-Murdoch, 1993; Taylor & Butcher,
377 1951). Active attrition behavior probably participates to this modulation, contributing to maintain
378 the incisor to an adequate length for occlusion (Taylor & Butcher, 1951). The process of adjusting
379 eruption rate to a diet change occurs within few days (Ness, 1956; Taylor & Butcher, 1951).
380 Furthermore, difference in diet consistency were reported to cause differences in the bevel angle
381 that mimic those observed between continental mice, mostly relying on grains, and Guillole mice,
382 largely relying on invertebrate prey. Hard food particles fracture the cutting edge of the incisor, that
383 is sharpened again by attrition of the incisors against each other, but this leads to a greater bevel
384 angle than in unbroken teeth (Taylor & Butcher, 1951). Such pronounced abrasion requires increased
385 eruption so that the two processes counterbalance for an efficient occlusion.

386 Continuous growth of the incisors is achieved by a population of stem cells located at the cervical
387 end of the incisor (Sharpe, 2016). From these stem cell population, ameloblasts differentiate that
388 generate enamel in a centripetal direction towards the dentine; whereas odontoblasts, located at the
389 external border of the pulp cavity, generate the dentine in a centrifugal direction towards the
390 enamel. Stem cells are even present at the apex of the pulp cavity, being able to produce restorative
391 dentine in case of exposure of the pulp due to abrasion (Pang *et al.*, 2016). Increased eruption rate,
392 however, is not associated with an increased deposition of dentine and enamel, nor with a
393 displacement of the base of the incisor (Ness, 1956). As a consequence of a decreased quantity of
394 dentine and enamel material per unit of erupted tooth, tooth walls are thinner and the pulp cavity is
395 wider in rabbits experiencing accelerated incisor growth (Ness, 1956). Such a change in the dynamics
396 of eruption was traced here by the differences in internal structure of the lower incisors between
397 continental and Guillole mice. Continental mice, for which incisor eruption rate should be higher to
398 match a higher abrasion, displayed a pulp cavity going much further anteriorly than in Guillole mice.

399 In contrast, the pulp cavity hardly reached beyond the zone of molar insertion in Guillou mice. Even
400 the place where mineralized enamel could be detected tended to be located more posteriorly than in
401 continental mice. This can be interpreted as the consequence of more time for depositing enamel
402 and dentine material in the slower growing Guillou incisors. All changes observed between
403 continental and Guillou incisors are thus compatible with a purely plastic response allowed by the
404 modulation of incisor continuous growth. More profound geometric changes, involving incisor depth
405 and curvature were not observed in Guillou mice. The incisor enamel of rodents is usually iron-
406 enriched, leading to a harder enamel allowing to sustain important wear. This enamel-hardening is
407 lost in some specialized carnivorous rodents (Rowe *et al.*, 2016). There is no evidence for such a
408 trend in Guillou mice, since mean enamel density appeared to be similar than in continental mice.
409 Changes in such traits would involve the selection of heritable characters, requiring a longer time to
410 evolve in order to lead to specialized phenotypes as those observed in carnivorous taxa (Fabre *et al.*,
411 2017; Rowe *et al.*, 2016). The incisors of Guillou mice may thus exemplify the adaptive potential of
412 plastic changes to face environmental challenges at an ecological time-scale (Ghalambor *et al.*, 2007).

413

414 *Conclusion*

415 Consistent differences in the lower incisor bevel have been shown between continental mice,
416 generally relying on hard food stuffs such as grain, and Guillou mice, that switched their diet towards
417 an increased predatory component. According to functional expectations, the bevel of predatory
418 mice displays a sharper cutting edge allowing for an improved perforation of prey. This bevel shape
419 characteristic of Guillou mice may be largely, if not entirely, the product of a plastic modulation of
420 incisor growth, with a balance between active attritional behavior tapering the cutting edge of the
421 lower incisor, and decreased eruption rate modulated to match decreased abrasion. Such a role of
422 plasticity in tooth geometry and dynamics is largely underestimated, because teeth are usually not
423 prone to plastic variations. Being ever-growing, rodent incisors can however vary in response to
424 environmental differences within the time span of an animal's life, and even over few days. The
425 dynamic sharpening of the bevel would thus constitute another case of tooth sculpting, described so
426 far for particular molar morphology able to perform equally well when unworn and worn, and thus
427 adapted for safeguarding against dental senescence (Pampush *et al.*, 2016).

428 Investigating the internal structure of the incisor showed that such change in eruption dynamics lead
429 to profound differences in the pulp cavity and even on the place where enamel and dentine
430 mineralized. Because increased eruption rate is not associated with increased rate of enamel and
431 dentine deposition, the whole structure of mineralization is changed, being shifted towards the tip of

432 the incisor. Such internal signature enables to make a link between evolutionary and behavioral
433 aspects and developmental studies on the mouse incisor (Pang *et al.*, 2016; Sharpe, 2016) and may
434 open the way to investigate eco-evo-devo dynamics of incisor adaptation based on material available
435 in collections.

436

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446

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554

555 **Figure legends**

556

557 **Figure 1.** Data set for the quantification of the incisors shape and internal structure. (A) Example of
558 right upper and lower incisor on the mouse head, with the set of 2D landmarks and semi-landmarks
559 used for the 2D shape analysis in the yellow inserts. (B) Lingual profile of a right hemi-mandible,
560 based on μ CT-scans. The surface of the mandible including teeth, of the dentine and of the enamel
561 were segmented using constant grey value (GV) thresholds. The transparency allows visualizing the
562 location of the pulp cavity and of the enamel layer. Arrowheads point to the five landmarks used to
563 describe the internal structure of the incisor relative to the mandible: tip of the incisor, anteriormost
564 point of the bone along the incisor, posterior extremity of the condyle (in grey); apex of the pulp
565 cavity (in purple) and basis of the enamel layer (in blue).

566

567 **Figure 2.** Lower incisor size and shape variation. A. Incisor centroid size vs. body weight. B. Allometric
568 incisor shape variation, depicted as the variation of the allometric regression score vs. incisor
569 centroid size. Dotted lines correspond to the linear trends in the two groups (continent and Guillou).
570 C. Allometric shape difference between the smallest and the largest incisor (no magnification). D.
571 Differentiation in incisor shape between the different mouse populations, along the first two axes of
572 a Canonical Variate Analysis on the aligned coordinates. Guillou populations: light green, G1993;
573 green, G2001; light blue, G2008; dark blue: G2009. Continental populations: red: Balan; pink: Tourch;
574 violet: Gardouch; yellow: CB. E. Mean shape difference between continental and Guillou incisors
575 (magnification: x2).

576

577 **Figure 3.** Upper incisor size and shape variation. A. Incisor centroid size vs. body weight. B. Allometric
578 shape difference between the smallest and the largest incisor (no magnification). C. Differentiation in
579 incisor shape between the different mouse populations, along the first two axes of a Canonical
580 Variate Analysis on the aligned coordinates. Guillou populations: light green, G1993; green, G2001;
581 light blue, G2008; dark blue: G2009. Continental populations: red: Balan; pink: Tourch; violet:
582 Gardouch; yellow: CB. D. Mean shape difference between continental and Guillou incisors
583 (magnification: x2).

584

585

586 **Figure 4.** Internal structure of the lower incisor in a subset of continental (brown dots) and Guillou
 587 mice (green and blue dots), based on μ CT-scan data. A. Minimal grey value of a cross-section of the
 588 lower incisor between the bevel and the insertion in the mandibular bone. B. Example of a
 589 continental and a Guillou mandible, with mandible, dentine and enamel segmented with constant
 590 grey value thresholds. Arrowheads point to the five landmarks describing the internal structure of
 591 the incisor relative to the mandible. Grey arrowheads: tip of the incisor, anteriormost point of the
 592 bone along the incisor and coronoid posterior extremity. Purple arrowhead: apex of the pulp cavity;
 593 blue arrowhead: enamel basis. The pulp cavity extends much more anteriorly in continental mice. C.
 594 Plot of the PCA based on the aligned coordinates of the five landmarks. Brown: Cologne-Bonn (CB), in
 595 blue: Guillou 1993 and in darkblue: Guillou 2009. D. Visualization of the deformation along PC1.
 596 Right: configuration at the minimum value along PC1; left, configuration at the maximum value along
 597 PC1.

598

599 **List of Supporting Informations**

600 **Table S1.** Area, country, locality and code (abbreviation) for the different sampled populations.
 601 NLowInc: number of lower incisors measured in 2D. NUppInc: number of upper incisors measured in
 602 2D. N3D: number of lower incisors included in the 3D analysis. Collection: source and place of
 603 locations of the skulls. MPI Plön: Max Plank Institut of Evolutionary Biology, Plön, Germany; CBGP:
 604 Centre de Biologie et Gestion des Populations, Baillarguet, France; LBBE: Laboratoire de Biométrie et
 605 Biologie Evolutive, France.

606 **Figure S1.** Map of the localities considered in this study.

607 **Figure S2.** Consensus shape of the upper and lower incisors for continental and Guillou mice.

608 **Figure S3.** Cross-section of lower incisor between the basis of the bevel and the insertion of the
 609 bone, for Cologne-Bonn (brown, upper row), Guillou 1993 (green, mid row) and Guillou 2009 (blue,
 610 lower row) mice. Right, location of the cross-section on a mandible (bone in violet, dentine in pink,
 611 enamel in orange).

612

613 **Supplementary data 1.** Raw data for the lower incisor geometric morphometrics.

614 **Supplementary data 2.** Raw data for the upper incisor geometric morphometrics.

615 **Tables**616 **Table 1.** Incisor shape differentiation between geographic groups.

Lower incisor	Locality	Group	N	Balan	CB	Gardouch	Tourch	G1993	G2001	G2008	G2009
	Balan	Continent	14	-							
	CB	Continent	14	<i>0.0083</i>	-						
	Gardouch	Continent	68	0.0277	0.0261	-					
	Tourch	Continent	88	0.1111	<i>0.0020</i>	0.0190	-				
	G1993	Guillou	22	0.0002	0.0804	0.0002	0.0001	-			
	G2001	Guillou	19	0.0007	0.0001	0.0002	0.0004	0.0001	-		
	G2008	Guillou	20	0.0005	0.0001	0.0001	0.0009	0.0001	0.7118	-	
	G2009	Guillou	22	<i>0.0065</i>	0.0007	<i>0.0026</i>	0.0006	0.0186	0.0105	0.0427	-
Upper incisor				Balan	CB	Gardouch	Tourch	G1993	G2001	G2008	G2009
	Balan	Continent	9	-							
	CB	Continent	14	0.0389	-						
	Gardouch	Continent	59	0.0008	0.3080	-					
	Tourch	Continent	59	0.0847	0.0645	0.0001	-				
	G1993	Guillou	16	<i>0.0026</i>	0.0010	0.0001	0.0001	-			
	G2001	Guillou	16	0.0181	0.0618	0.0174	<i>0.0014</i>	0.0801	-		
	G2008	Guillou	16	<i>0.0021</i>	0.0932	0.0897	0.0001	0.0113	0.2503	-	
	G2009	Guillou	20	<i>0.0033</i>	0.4857	0.0515	0.0108	<i>0.0011</i>	0.0200	0.0323	

617

618 Upper panel: lower incisor; shape variables: first three PC axes. Lower panel: upper incisor; shape
619 variables: first four PC axes. N: sample size. Probabilities of pairwise permanova are provided, based
620 on 9999 permutations. In italics: $P < 0.01$; in bold $P < 0.001$.

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623

Figure 1. Data set for the quantification of the incisors shape and internal structure. (A) Example of right upper and lower incisor on the mouse head, with the set of 2D landmarks and semi-landmarks used for the 2D shape analysis in the yellow inserts. (B) Lingual profile of a right hemi-mandible, based on μ CT-scans. The surface of the mandible including teeth, of the dentine and of the enamel were segmented using constant grey value (GV) thresholds. The transparency allows visualizing the location of the pulp cavity and of the enamel layer. Arrowheads point to the five landmarks used to describe the internal structure of the incisor relative to the mandible: tip of the incisor, anteriormost point of the bone along the incisor, posterior extremity of the condyle (in grey); apex of the pulp cavity (in purple) and basis of the enamel layer (in blue).

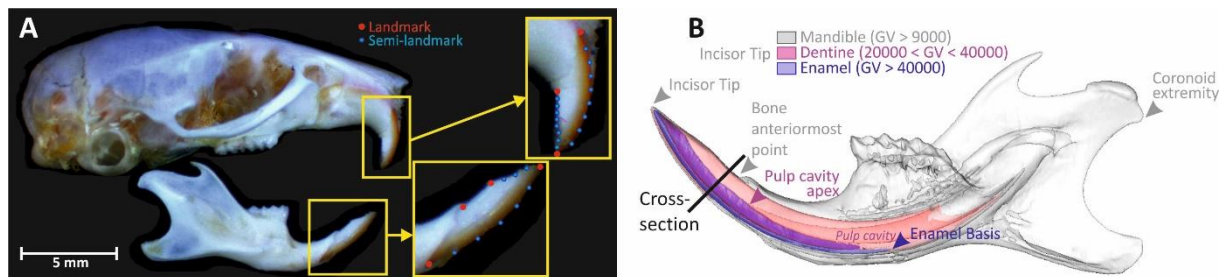


Figure 2. Lower incisor size and shape variation. A. Incisor centroid size vs. body weight. B. Allometric incisor shape variation, depicted as the variation of the allometric regression score vs. incisor centroid size. Dotted lines correspond to the linear trends in the two groups (continent and Guillou). C. Allometric shape difference between the smallest and the largest incisor (no magnification). D. Differentiation in incisor shape between the different mouse populations, along the first two axes of a Canonical Variate Analysis on the aligned coordinates. Guillou populations: light green, G1993; green, G2001; light blue, G2008; dark blue: G2009. Continental populations: red: Balan; pink: Tourn; violet: Gardouch; yellow: CB. E. Mean shape difference between continental and Guillou incisors (magnification: x2).

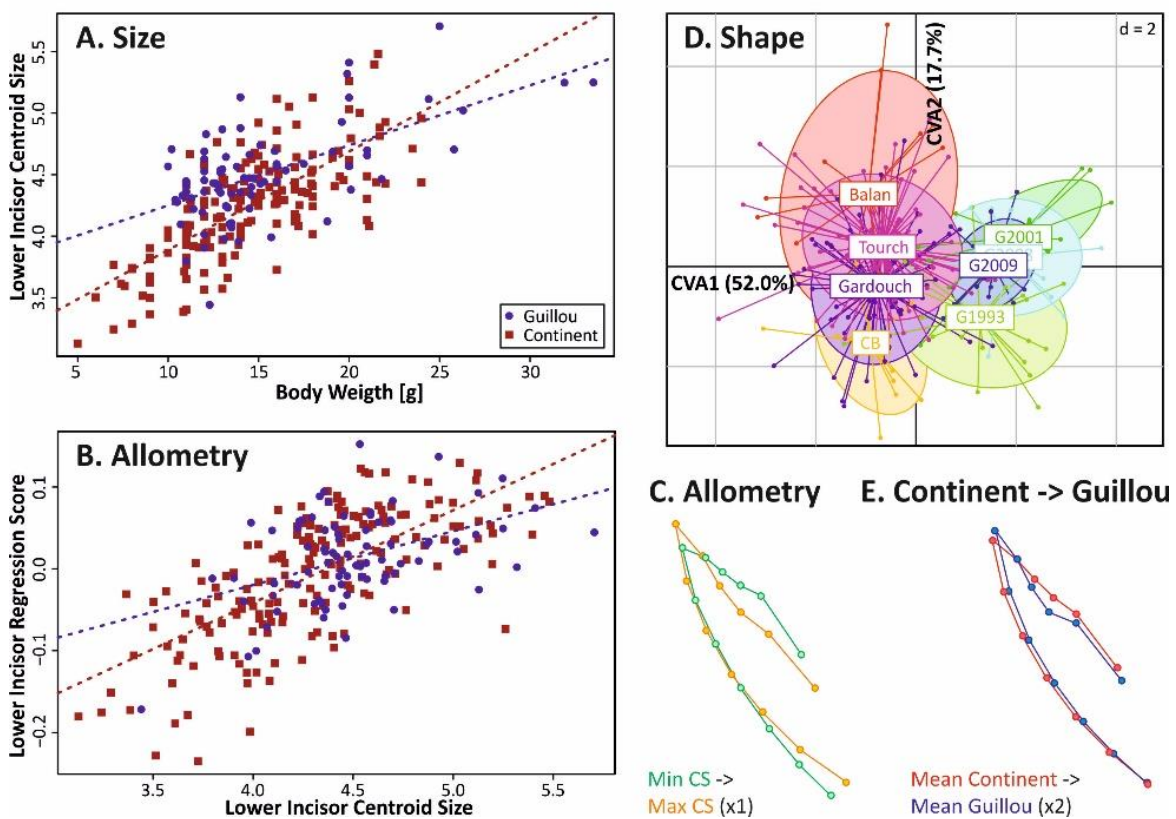


Figure 3. Upper incisor size and shape variation. A. Incisor centroid size vs. body weight. B. Allometric shape difference between the smallest and the largest incisor (no magnification). C. Differentiation in incisor shape between the different mouse populations, along the first two axes of a Canonical Variate Analysis on the aligned coordinates. Guillou populations: light green, G1993; green, G2001; light blue, G2008; dark blue: G2009. Continental populations: red: Balan; pink: Tourch; violet: Gardouch; yellow: CB. D. Mean shape difference between continental and Guillou incisors (magnification: x2).

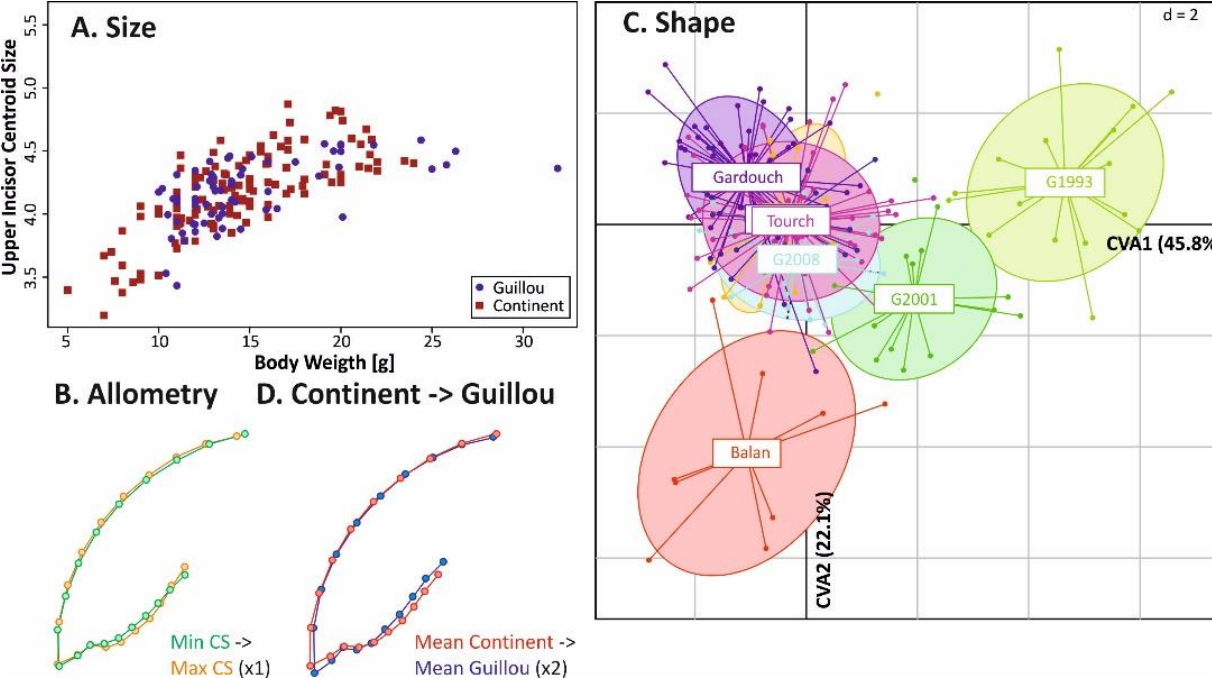
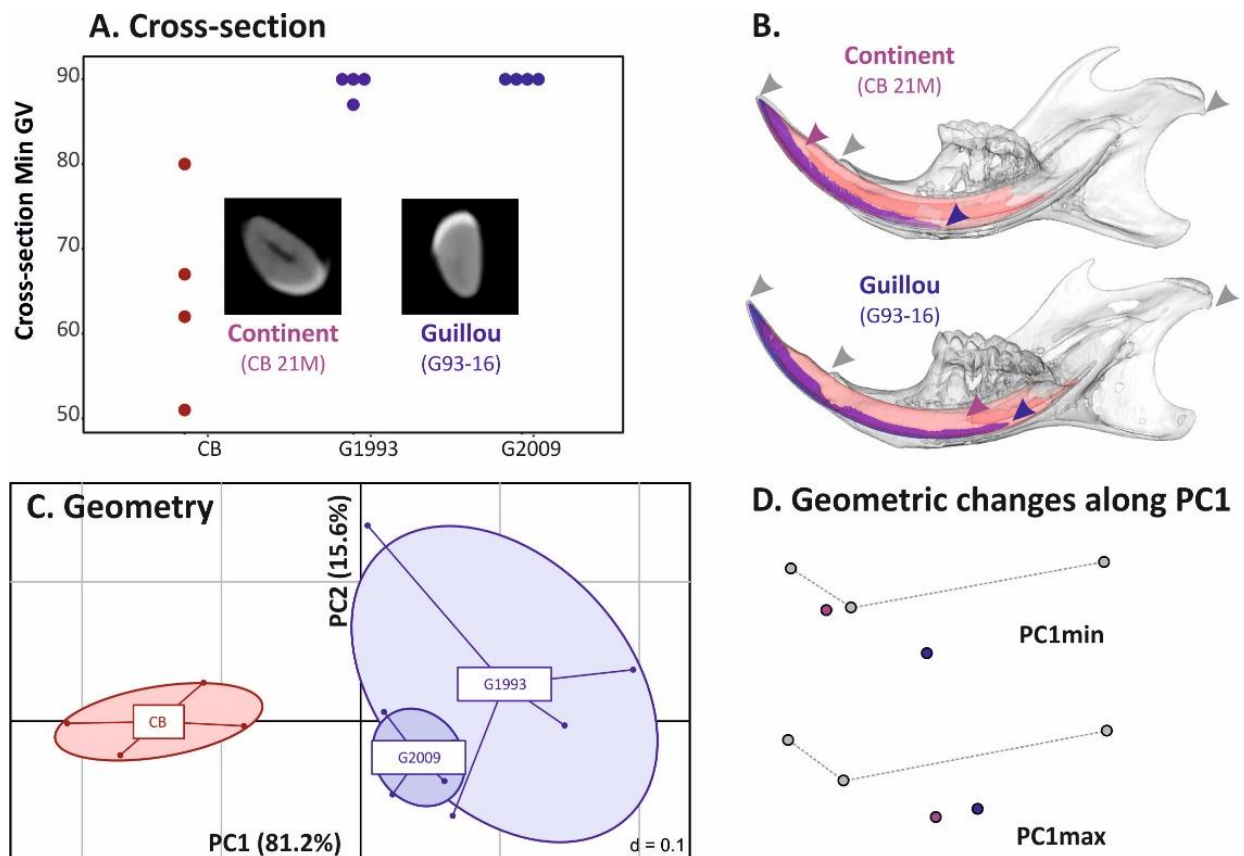
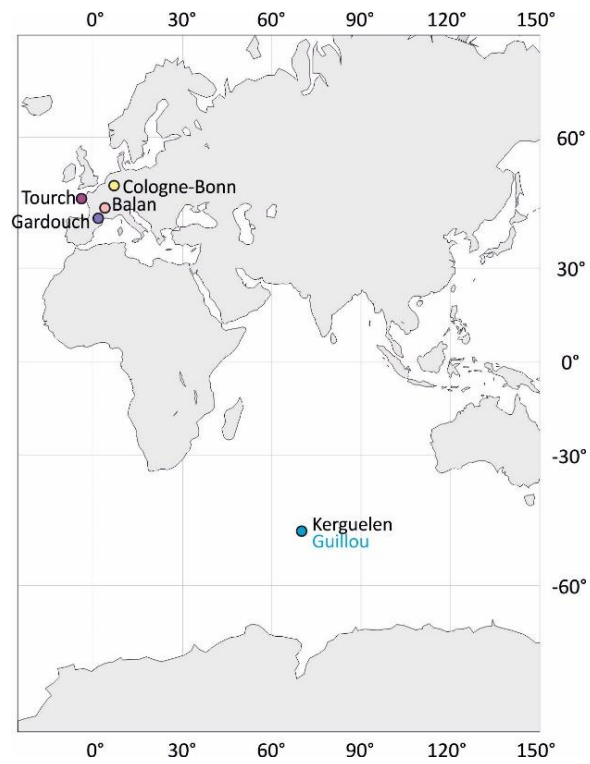


Figure 4. Internal structure of the lower incisor in a subset of continental (brown dots) and Guillou mice (green and blue dots), based on μ CT-scan data. A. Minimal grey value of a cross-section of the lower incisor between the bevel and the insertion in the mandibular bone. B. Example of a continental and a Guillou mandible, with mandible, dentine and enamel segmented with constant grey value thresholds. Arrowheads point to the five landmarks describing the internal structure of the incisor relative to the mandible. Grey arrowheads: tip of the incisor, anteriormost point of the bone along the incisor and coronoid posterior extremity. Purple arrowhead: apex of the pulp cavity; blue arrowhead: enamel basis. The pulp cavity extends much more anteriorly in continental mice. C. Plot of the PCA based on the aligned coordinates of the five landmarks. Brown: Cologne-Bonn (CB), in blue: Guillou 1993 and in darkblue: Guillou 2009. D. Visualization of the deformation along PC1. Right: configuration at the minimum value along PC1; left, configuration at the maximum value along PC1.

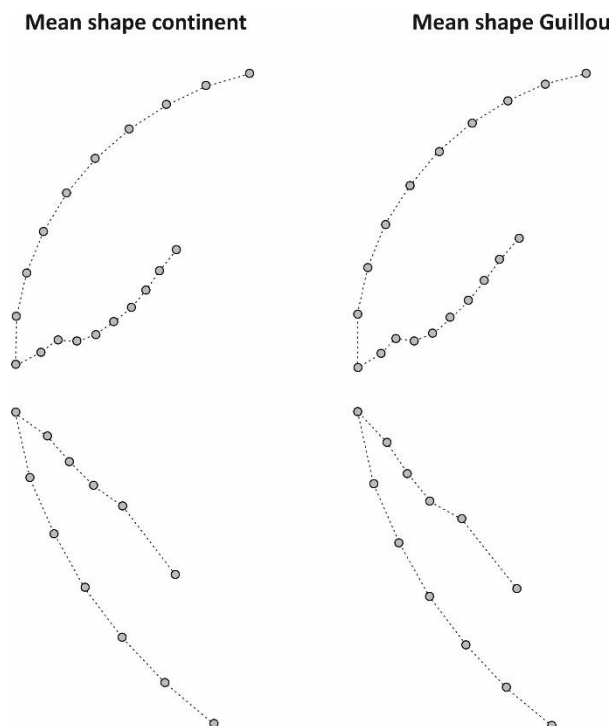


Supplementary Figures

Supplementary Figure 1. Map of the localities considered in this study.



Supplementary Figure 2. Consensus shape of the upper and lower incisors for continental and Guillou mice.



Supplementary Figure 3. Cross-section of lower incisor between the basis of the bevel and the insertion of the bone, for Cologne-Bonn (brown, upper row), Guillou 1993 (green, mid row) and Guillou 2009 (blue, lower row) mice. Right, location of the cross-section on a mandible (bone in violet, dentine in pink, enamel in orange).

