



**HAL**  
open science

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

Sabrina Renaud, Claire Delépine, Ronan Ledevin, Benoît Pisanu, Jean-Pierre Quéré, Emilie A. Hardouin

### ► **To cite this version:**

Sabrina Renaud, Claire Delépine, Ronan Ledevin, Benoît Pisanu, Jean-Pierre Quéré, et al.. A sharp incisor tool for predator house mice back to the wild. *Journal of Zoological Systematics and Evolutionary Research*, 2019, 57, pp.989-999. <10.1111/jzs.12292>. <hal-02336353>

**HAL Id: hal-02336353**

**<https://hal.science/hal-02336353v1>**

Submitted on 21 Nov 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons CC BY-NC-ND 4.0 - Attribution - Non-commercial use - No Derivative Works - International License

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

2 SABRINA RENAUD <sup>1\*</sup>, CLAIRE DELEPINE <sup>1</sup>, RONAN LEDEVIN <sup>2</sup>, BENOIT PISANU <sup>3</sup>, JEAN-PIERRE QUERE <sup>4</sup>,  
3 EMILIE A. HARDOUIN <sup>5</sup>

4

5

6 <sup>1</sup> Laboratoire de Biométrie et Biologie Evolutive, UMR5558 CNRS Université Lyon 1, Campus de la  
7 Doua, 69100 Villeurbanne, France

8 <sup>2</sup> UMR5199 PACEA, Université de Bordeaux, Allée Geoffroy Saint Hilaire (Bat. B8), 33615 PESSAC,  
9 France

10 <sup>3</sup> Centre d'Ecologie et des Sciences de la Conservation, UMR 7204, Sorbonne Universités, Muséum  
11 National d'Histoire Naturelle, CNRS, Université Pierre et Marie Curie, 61 rue Buffon, F-75005 Paris,  
12 France

13 <sup>4</sup> Centre de Biologie et Gestion des Populations (INRA / IRD / Cirad / Montpellier SupAgro), Campus  
14 international de Baillarguet, CS 30016, F-34988, Montferrier-sur-Lez Cedex, France

15 <sup>5</sup> Department of Life and Environmental Sciences, Faculty of Sciences and Technology, Bournemouth  
16 University, Christchurch House, Talbot Campus, Poole, Dorset, BH12 5BB, UK

17

18 Running title: Incisor shape of predator house mice

19

20 \* Corresponding author:

21 Sabrina Renaud

22 E-mail: [Sabrina.Renaud@univ-lyon1.fr](mailto:Sabrina.Renaud@univ-lyon1.fr)

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 km<sup>2</sup>) 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 19<sup>th</sup> 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  $\mu\text{m}$  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  $\pm 0.042$ ).

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  $\text{group}$ :  $\log(\text{BW}) = 46.9\%$  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

#### 437 *Acknowledgements*

438 We thank Renaud Lebrun (ISEM, Montpellier) for his contribution in managing CT scans, produced  
439 within the technical facilities of the MRI platform and of the labEx CeMEB. We also warmly thank the  
440 horse stable Les Peupliers (Balan) for their authorization and support during the trapping, and Jean-  
441 Louis Chapuis for his initiation and long-lasting participation to the IPEV program on Guillou. The  
442 manuscript benefited from stimulating discussions within the GDR 'Phenotypic Plasticity' and from  
443 constructive comments from two anonymous reviewers. This study was supported by the French  
444 Polar Institute (IPEV programme 136) and by the CNRS (Zone Atelier de Recherches sur  
445 l'Environnement Antarctique et Subantarctique).

446

447 **References**

- 448 Adams, C. D., & Otarola-Castillo, E. (2013). Geomorph: An r package for the collection and analysis of  
 449 geometric morphometric shape data. *Methods in Ecology and Evolution*, 4, 393-399.
- 450 Burn-Murdoch, R. A. (1993). The effect of the consistency of the diet on eruption rates and lengths of  
 451 incisor teeth in rats. *Archives of Oral Biology*, 38, 699-706.
- 452 Chapuis, J.-L., Frenot, Y., & Lebouvier, M. (2004). Recovery of native plant communities after  
 453 eradication of rabbits from the subantarctic kerguelen islands, and influence of climate  
 454 change. *Biological Conservation*, 117, 167-179.
- 455 Chapuis, J.-L., Le Roux, V., Asseline, J., & Kerleau, F. (2001). Eradication of rabbits (*oryctolagus*  
 456 *cuniculus*) by poisoning on three islands of the subantarctic kerguelen archipelago. *Wildlife*  
 457 *Research*, 28, 323-331.
- 458 Copson, G. R. (1986). The diet of the introduced rodents *mus musculus* l. And *rattus rattus* l. On sub-  
 459 antarctic macquarie island. *Australia Wildlife Research*, 13, 441-445.
- 460 Cox, P. G., & Jeffery, N. (2011). Reviewing the morphology of the jaw-closing musculature in squirrels,  
 461 rats, and guinea pigs with contrast-enhanced microct. *The Anatomical Record*, 294, 915-928.
- 462 Evans, A. R., Wilson, G. P., Fortelius, M., & Jernvall, J. (2007). High-level similarity of dentitions in  
 463 carnivorans and rodents. *Nature*, 445, 78-81.
- 464 Fabre, P.-H., Hautier, L., Dimitrov, D., & Douzery, E. J. P. (2012). A glimpse on the pattern of rodent  
 465 diversification: A phylogenetic approach. *BMC Evolutionary Biology*, 12, 88.
- 466 Fabre, P.-H., Herrel, A., Fitriana, Y., Meslin, L., & Hautier, L. (2017). Masticatory muscle architecture in  
 467 a water-rat from australasian (murinae, *hydromys*) and its implication for the evolution of  
 468 carnivory in rodents. *Journal of Anatomy*, 231, 380-397.
- 469 Ghalambor, C. K., McKay, J. M., Carroll, S. P., & Reznick, D. N. (2007). Adaptive versus non-adaptive  
 470 phenotypic plasticity and the potential for contemporary adaptation in new environments.  
 471 *Functional Ecology*, 21, 394-407.
- 472 Hammer, Ø., Harper, D. A. T., & Ryan, P. D. (2001). Past: Paleontological statistics software package  
 473 for education and data analysis. *Palaeontological Electronica*, 4, 1-9.
- 474 Harari, D., Hermolin, G., & Harari, O. (2005). The effect of age on morphology and eruption of the  
 475 lower incisors in mature rats. *Archives of Oral Biology*, 50, 953-958.
- 476 Hardouin, E., Chapuis, J.-L., Stevens, M. I., van Vuuren, J. B., Quillfeldt, P., Scavetta, R. J., et al. (2010).  
 477 House mouse colonization patterns on the sub-antarctic kerguelen archipelago suggest  
 478 singular primary invasions and resilience against re-invasion. *BMC Evolutionary Biology*, 10,  
 479 325.
- 480 Jernvall, J., Hunter, J. P., & Fortelius, M. (1996). Molar tooth diversity, disparity, and ecology in  
 481 cenozoic ungulate radiations. *Science*, 274, 1489-1492.
- 482 Kidder, J. H. (1876). The natural history of the kerguelen island. *The American Naturalist*, 10, 481-484.
- 483 Klevezal, G. A., & Shchepotkin, D. V. (2012). Incisor growth rate in rodents and the record of the  
 484 entire annual cycle in the incisors of *marmota baibacina centralis*. *Biology Bulletin*, 39, 684-  
 485 691.
- 486 Landry, S. O. (1970). The rodents as omnivores. *The Quarterly Review of Biology*, 45, 351-372.
- 487 Laparie, M., Lebouvier, M., Lalouette, L., & Renault, D. (2010). Variations of morphometric traits in  
 488 populations of an invasive carabid predator (*merizodus soledadinus*) within a sub-antarctic  
 489 island. *Biological Invasions*, 12, 3405-3417.
- 490 Le Roux, V., Chapuis, J.-L., Frenot, Y., & Vernon, P. (2002). Diet of the house mouse (*mus musculus*)  
 491 on guillou island, kerguelen archipelago, subantarctic. *Polar Biology*, 25, 49-57.
- 492 Lebouvier, M., Laparie, M., Hullé, M., Marais, A., Cozic, Y., Lalouette, L., et al. (2011). The significance  
 493 of the sub-antarctic kerguelen islands for the assessment of the vulnerability of native  
 494 communities to climate change, alien insect invasions and plant viruses. *Biological Invasions*,  
 495 13, 1195-1208.
- 496 Lowe, S., Browne, M., Boudjelas, S., & De Poorter, M. 2000. 100 of the world's worst invasive alien  
 497 species. A selection from the global invasive species database. pp. 1-12): The Invasive Species

- 498 Specialist Group (ISSG) a specialist group of the Species Survival Commission (SSC) of the  
 499 World Conservation Union (IUCN).
- 500 Martin, S. A., Alhajeri, B. H., & Steppan, S. J. (2016). Dietary adaptations in the teeth of murine  
 501 rodents (muridae): A test of biomechanical predictions. *Biological Journal of the Linnean*  
 502 *Society*, 119, 766-784.
- 503 Meredith, A. L., Prebble, J. L., & Shaw, D. J. (2015). Impact of diet on incisor growth and attrition and  
 504 the development of dental disease in pet rabbits. *Journal of Small Animal Practice*, 56, 377-  
 505 382.
- 506 Millien-Parra, V. (2000). Species differentiation among muroid rodents on the basis of their lower  
 507 incisor size and shape: Ecological and taxonomical implications. *Mammalia*, 64, 221-239.
- 508 Müller, J., Clauss, M., Codron, D., Schulz, E., Hummel, J., Fortelius, M., et al. (2014). Growth and wear  
 509 of incisor and cheek teeth in domestic rabbits (*oryctogalus cuniculus*) fed diets of different  
 510 abrasiveness. *Journal of Experimental Zoology, Part A*, 321A, 283-298.
- 511 Ness, A. R. (1956). The response of the rabbit mandibular incisor to experimental shortening and to  
 512 the prevention of its eruption. *Proceedings of the Royal Society of London, Biological Sciences*  
 513 *(serie B)*, 146, 129-154.
- 514 Pampush, J. D., Spradley, J. P., Morse, P. E., Harrington, A. R., Allen, K. L., Boyer, D. M., et al. (2016).  
 515 Wear and its effects on dental topography measures in howling monkeys (*alouatta palliata*).
- 516 Pang, Y. W. Y., Feng, J., Daltoe, F., Fatscher, R., Gentleman, E., Gentleman, M. M., et al. (2016).  
 517 Perivascular stem cells at the tip of mouse incisors regulate tissue regeneration. *Journal of*  
 518 *Bone and Mineral Research*, 31, 514-523.
- 519 Popowics, T. E., & Fortelius, M. (1997). On the cutting edge: Tooth blade sharpness in herbivorous  
 520 and faunivorous mammals. *Annales Zoologici Fennici*, 34, 73-88.
- 521 Renaud, S., Dufour, A.-B., Hardouin, E. A., Ledevin, R., & Auffray, J.-C. (2015). Once upon multivariate  
 522 analyses: When they tell several stories about biological evolution. *PLoS ONE*, 10, e0132801.
- 523 Renaud, S., Gomes Rodrigues, H., Ledevin, R., Pisanu, B., Chapuis, J.-L., & Hardouin, E. A. (2015). Fast  
 524 morphological response of house mice to anthropogenic disturbances on a sub-antarctic  
 525 island. *Biological Journal of the Linnean Society*, 114, 513-526.
- 526 Renaud, S., Hardouin, E. A., Pisanu, B., & Chapuis, J.-L. (2013). Invasive house mice facing a changing  
 527 environment on the sub-antarctic guillou island (kerguelen archipelago). *Journal of*  
 528 *Evolutionary Biology*, 26, 612-624.
- 529 Renaud, S., Hardouin, E. A., Quéré, J.-P., & Chevret, P. (2017). Morphometric variations at an  
 530 ecological scale: Seasonal and local variations in feral and commensal house mice.  
 531 *Mammalian Biology*, 87, 1-12.
- 532 Renaud, S., Ledevin, R., Pisanu, B., Chapuis, J.-L., Quillfeldt, P., & Hardouin, E. A. (2018). Divergent in  
 533 shape and convergent in function: Adaptive evolution of the mandible in sub-antarctic mice.  
 534 *Evolution*, 72, 878-892.
- 535 Rohlf, F. J., & Slice, D. (1990). Extensions of the procrustes method for the optimal superimposition of  
 536 landmarks. *Systematic Zoology*, 39, 40-59.
- 537 Rowe, K. C., Achmadi, A. S., & Esselstyn, J. A. (2016). Repeated evolution of carnivory among indo-  
 538 australian rodents. *Evolution*, 70, 653-665.
- 539 Samuels, J. X., & Van Valkenburgh, B. (2009). Craniodental adaptations for digging in extinct  
 540 burrowing beavers. *Journal of Vertebrate Paleontology*, 29, 254-268.
- 541 Schlager, S. 2017. Morpho and rvcg - shape analysis in {r}. In G. Zheng, S. Li & G. Székely (Eds)  
 542 *Statistical shape and deformation analysis*. pp. 217-256): Academic Press.
- 543 Sharpe, P. T. (2016). Dental mesenchymal stem cells. *Development*, 143, 2273-2280.
- 544 Smith, V. R., Avenant, N. L., & Chown, S. L. (2002). The diet and impact of house mice on a sub-  
 545 antarctic island. *Polar Biology*, 25, 703-715.
- 546 Steppan, S. J., & Schenk, J. J. (2017). Muroid rodent phylogenetics: 900-species tree reveals  
 547 increasing diversification rates. *PLoS ONE*, 12, e0183070.
- 548 Taylor, A. C., & Butcher, E. O. (1951). The regulation of eruption rate in the incisor teeth of the white  
 549 rat. *Journal of Experimental Zoology*, 117, 165-188.

550 van Aarde, R. J., & Jackson, T. P. (2007). Food, reproduction and survival in mice on sub-antarctic  
551 marion island. *Polar Biology*, 30, 503-511.

552

553

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  $\mu$ 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  $\mu$ 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	<b>0.0002</b>	0.0804	<b>0.0002</b>	<b>0.0001</b>	-			
	G2001	Guillou	19	<b>0.0007</b>	<b>0.0001</b>	<b>0.0002</b>	<b>0.0004</b>	<b>0.0001</b>	-		
	G2008	Guillou	20	<b>0.0005</b>	<b>0.0001</b>	<b>0.0001</b>	<b>0.0009</b>	<b>0.0001</b>	0.7118	-	
	G2009	Guillou	22	<i>0.0065</i>	<b>0.0007</b>	<i>0.0026</i>	<b>0.0006</b>	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	<b>0.0008</b>	0.3080	-					
	Tourch	Continent	59	0.0847	0.0645	<b>0.0001</b>	-				
	G1993	Guillou	16	<i>0.0026</i>	<b>0.0010</b>	<b>0.0001</b>	<b>0.0001</b>	-			
	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	<b>0.0001</b>	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

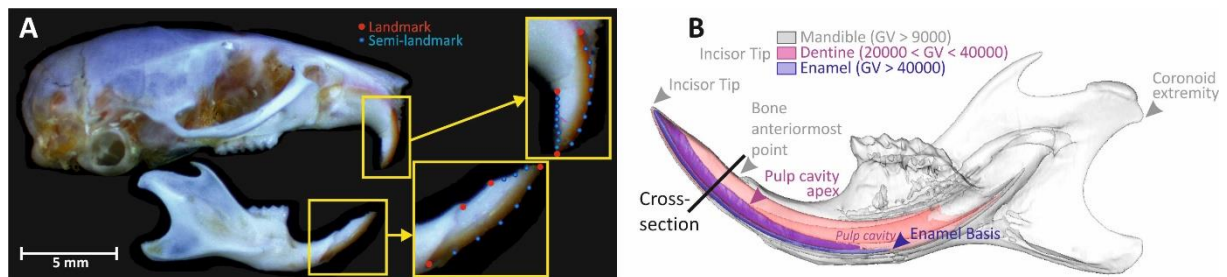
618 Upper panel: lower incisor; shape variables: first three PC axes. Lower panel: upper incisor; shape  
619 variables: first four PC axes. N: sample size. Probabilities of pairwise permanova are provided, based  
620 on 9999 permutations. In italics:  $P < 0.01$ ; in bold  $P < 0.001$ .

621

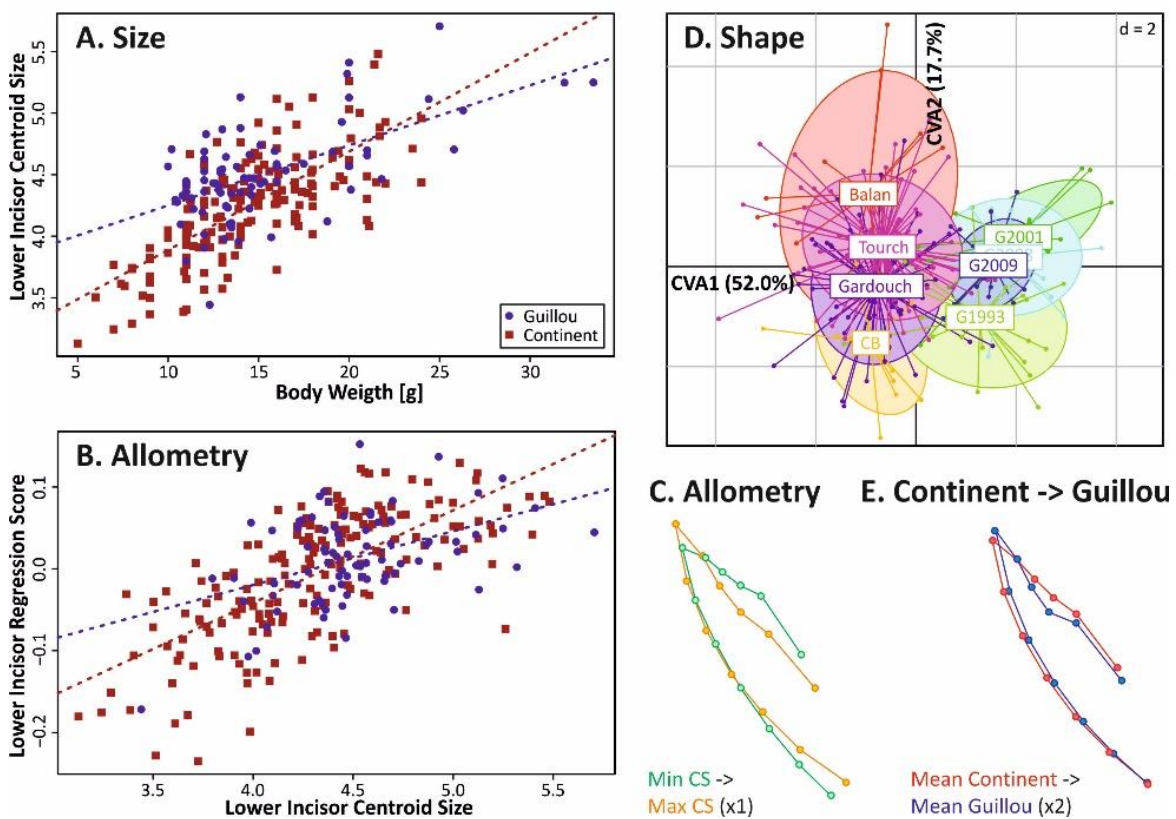
622

623

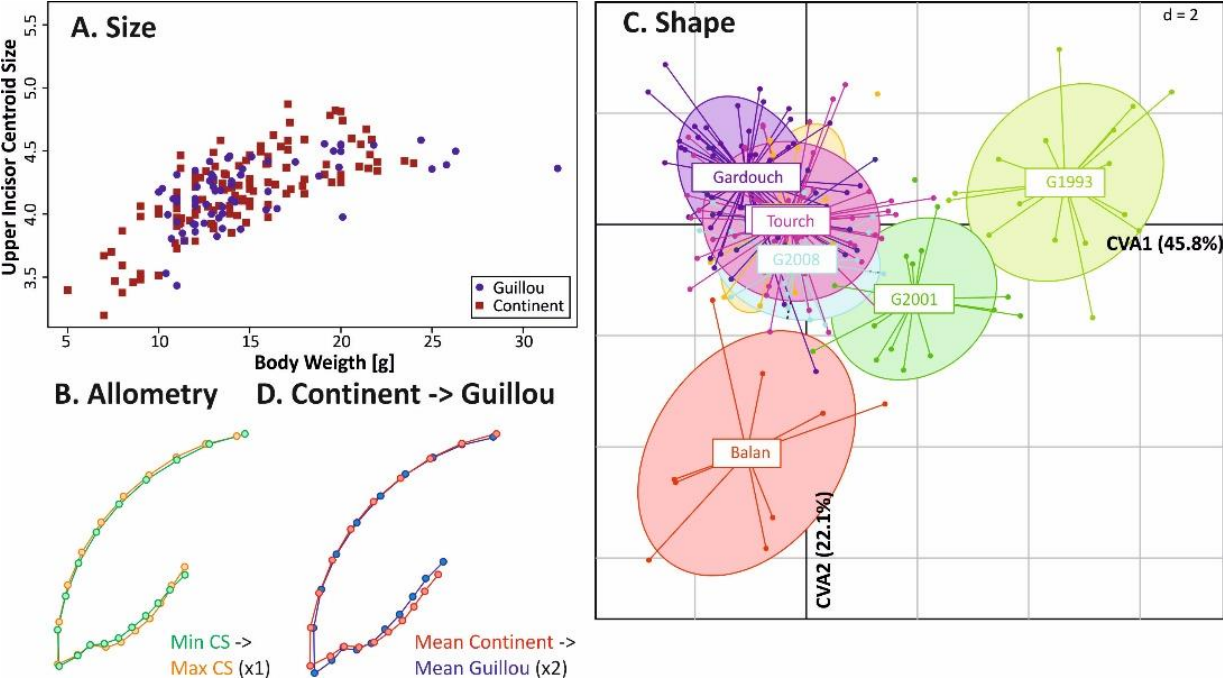
**Figure 1.** Data set for the quantification of the incisors shape and internal structure. (A) Example of right upper and lower incisor on the mouse head, with the set of 2D landmarks and semi-landmarks used for the 2D shape analysis in the yellow inserts. (B) Lingual profile of a right hemi-mandible, based on  $\mu$ 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).



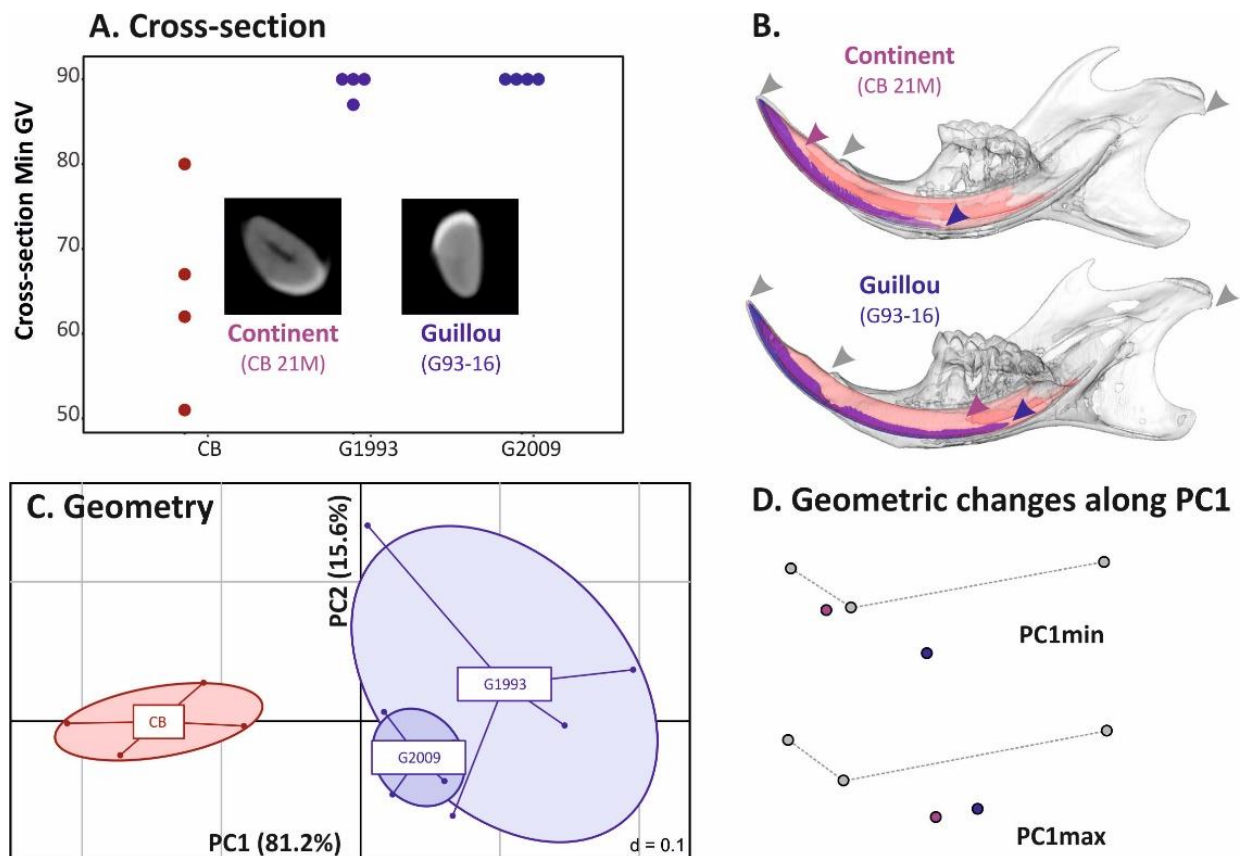
**Figure 2.** Lower incisor size and shape variation. A. Incisor centroid size vs. body weight. B. Allometric incisor shape variation, depicted as the variation of the allometric regression score vs. incisor centroid size. Dotted lines correspond to the linear trends in the two groups (continent and 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  $\mu$ 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.

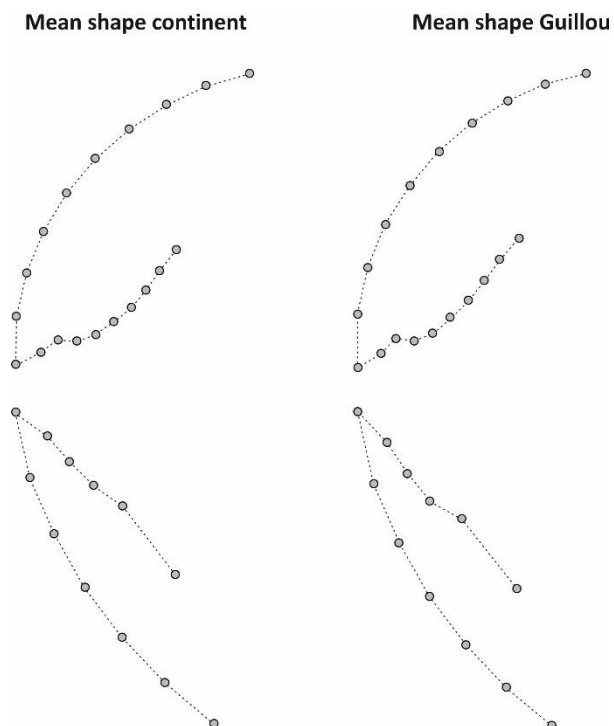


## Supplementary Figures

**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).

