

Invasive house mice facing a changing environment on the Sub-Antarctic Guillou Island (Kerguelen Archipelago)

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Abstract

Adaptation to new environments is a key feature in evolution promoting divergence in morphological structures under selection. The house mouse (*Mus musculus domesticus*) introduced on the Sub-Antarctic Guillou Island (Kerguelen Archipelago) had and still has to face environmental conditions that likely shaped the pattern and pace of its insular evolution. Since mouse arrival on the island, probably not more than two centuries ago, ecological conditions dramatically differed from those available to their Western European commensal source populations. In addition, over the last two decades, the plant and animal communities of Guillou Island were considerably modified by the eradication of rabbits, the effects of climate change and the spread of invasive species detrimental to native communities. Under such a changing habitat, the mouse response was investigated using a morphometric quantification of mandible and molar tooth, two morphological structures related to food processing. A marked differentiation of the insular mice compared with their relatives from Western Europe was documented for both mandibles and molar shapes. Moreover, these shapes changed through the 16 years of the record, in agreement with expectations of drift for the molar, but more than expected by chance for the mandible. These results suggest that mice responded to the recent changes in food resources, possibly with a part of plastic variation for the mandible prone to bone remodelling. This pattern exemplifies the intricate interplay of evolution, ecology and plasticity that is a probable key of the success of such an invasive rodent facing pronounced shifts in food resources exploitation under a changing environment.

Introduction

The success of an introduced species to establish itself and spread in a new environment relies on its ability to cope with environmental conditions that may strongly differ from its native area. Therefore, introduced species offer emblematic cases to investigate how adaptation proceeds (Lambrinos, 2004). Facing this challenge can

trigger rapid changes over short time-scales where ecological and evolutionary processes can interact (Lambrinos, 2004; Bradshaw & Holzapfel, 2006; Kinnison & Hairston, 2007). Ultimately, any species on an oceanic island was at one point an introduced species. In that respect, insular evolution may provide valuable insights onto the pace of changes of a species facing a new environment, with longer hindsight than available for modern introduced species. In agreement, insular species have been shown to be often characterized by rapid and pronounced evolution soon after arrival (Sondaar, 2000; Millien, 2006; Nagorsen & Cardini, 2009).

Among invasive species, the house mouse (*Mus musculus* L.) is known to be one of the hundred 'worst

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world invaders' according to the Invasive Species Specialist Group (ISSG – http://www.issg.org/worst100_species.html). Indeed, this species has been able to successfully colonize highly diverse environments, from central heating ducts and refrigerated stores to the most remote and apparently inhospitable environments (Berry, 1981, 1996). The small Guillou Island (145 ha), part of the Sub-Antarctic Kerguelen Archipelago (48°25'–50°S, 68°27'–70°35 E), offers an exemplary case to study how house mice (*Mus musculus domesticus*) have faced a new and changing environment.

The Kerguelen Archipelago is situated about 4 000 km away from the African and Australian coasts and was discovered in 1772. Mice have been reported to be abundant on these remote islands by the end of the 19th century (Kidder, 1876), suggesting them to have been introduced by sealers probably as soon as the beginning of the 1800s (Chapuis *et al.*, 1994). It is not possible to precisely date the colonization of Guillou Island. It can be supposed, however, that starting from the main island of the Archipelago (Grande Terre), mice should have invaded Guillou Island more recently, probably around 100 years ago.

From a genetic point of view, all mice from the Kerguelen Archipelago share the same mitochondrial haplotype except in two remote islands (Hardouin *et al.*, 2010). No other evidence of successful multiple colonizations was found, in agreement with observations on other Sub-Antarctic Islands, suggesting resilience to re-invasion for this rodent species (Hardouin *et al.*, 2010). Based on historical records and genetic data (mitochondrial D-loop haplotype as well as autosomal and Y chromosomal microsatellites), Kerguelen mice are attributed to the *Mus musculus domesticus* sub-species with a Western European origin (Hardouin *et al.*, 2010).

At their arrival on Sub-Antarctic islands such as the Kerguelen Archipelago, mice had to face obvious environmental stresses such as temperature conditions much harsher than those prevailing in their source populations of Western Europe (Berry & Peters, 1975; Berry *et al.*, 1978, 1979; Avenant & Smith, 2003). Regarding ecological conditions, the Kerguelen Archipelago only partly fulfil conditions of predation release usually observed on oceanic islands and supposed to drive insular rodent evolution (Michaux *et al.*, 2002). The introduced feral cat (*Felis silvestris catus*) maintained relatively high predation pressure on the house mouse (Pontier *et al.*, 2002). A potential competitor is also present since the black rat (*Rattus rattus*) occurs on the archipelago (Chapuis *et al.*, 1994).

More subtle changes included differential food availability, leading to a shift in diet towards an increased proportion of invertebrate preys (Copson, 1986; Le Roux *et al.*, 2002). Recently, mice had to face an extensive rearrangement of the ecosystem on Guillou

Island resulting in further changes of their food resources. A restoration programme involving rabbit (*Oryctolagus cuniculus*) eradication by poisoning since 1994, a regression of the native vegetation cover against invasive plants in relation to climate change since the end of the 1990s, and the spread of an invasive Carabid beetle affecting terrestrial macro-invertebrate communities over the last 15 years (Chapuis *et al.*, 2001, 2004; Laparie *et al.*, 2010; Lebouvier *et al.*, 2011) should have contributed to change in both quantity and quality of the foraging resources available to mice.

A two steps process of response can thus be expected on Guillou Island. First, adaptation to the new food resources must have occurred, with a possible component of neutral differentiation accompanying bottleneck and drift in the colonizing population. Second, the recent changes in food resources may have triggered a response over the last 15 years. Deciphering this sequence of response to changes in food resources requires a focus on characters involved in the nutrition process. Two phenotypic characters evidently related to feeding behaviour and characterized by contrasting properties were investigated: mandible and molar tooth (Fig. 1). The mandible, bearing incisor and molar teeth as well as the insertions of masticatory muscles, is under strong selection for an efficient food handling but also prone to plastic bone remodelling in response to the diet along the animal's life (Renaud & Auffray, 2010; Renaud *et al.*, 2010), making it likely to be influenced by local conditions (Renaud, 2005; Renaud & Michaux, 2007). In contrast, molar teeth are only

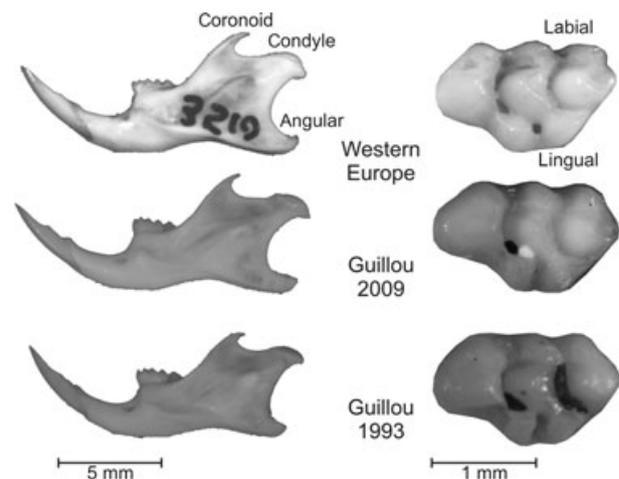


Fig. 1 Examples of mandibles (to the left) and first upper molar (to the right) of the house mouse (*Mus musculus domesticus*) in a continental Western European locality (Gardouch, France, top panel) and in the Sub-Antarctic Guillou Island (Kerguelen Archipelago) in a recent (2009, mid panel) and an older sample (1993, bottom panel).

affected by wear once erupted. Both characters have been shown to exhibit variations in response to biotic and abiotic changes in their environment, along biogeographical variations on the continent (e.g. Cucchi, 2008; Siahsharvie *et al.*, 2012) as well as on numerous islands (Davis, 1983; Michaux *et al.*, 2007b; Renaud & Auffray, 2010). Therefore, mandible and molar tooth appear appropriate characters for tracking the mouse response to new environments such as in the Kerguelen Archipelago. The size and shape of the two characters were quantified using geometric morphometrics along a time series on Guillou Island starting one year before the initiation of the restoration programme (1993) and continuing with subsequent steps in 2001, 2008 and 2009.

Materials and methods

Sample collection

A total of 70 skulls were prepared for morphometric analyses from mice trapped on Guillou Island during the middle-end of the breeding period (March–April 1993, April 2001, May 2008 and May 2009). Six Western European populations were used for comparison (France: Gardouch and Montpellier; Italy: Lombardy, Reggiolo, and San Bernardino [ISEM and CBGP collections]; Germany: Cologne-Bonn surroundings [MPI Plön collection]). The European animals were trapped in all seasons and during a more than 30 years period, from the 1970s to the 2000s.

All mice considered were sub-adults and adults, the criteria being the eruption of the third molars that occurs at weaning. Sex was recorded for most animals and allowed for investigating the effect of sexual dimorphism. For each mouse, the mandible and the first upper molar (UM1) were measured. Details of the sampling are provided on Table 1.

Table 1 Localities of trapping and labels used on the Figures with numbers of mandibles (Md) and first upper molars (UM1) measured. Numbers of mandibles and UM1 measured are different because of different preservation.

Area	Locality	Labels	Md	UM1
Kerguelen	Guillou 1993	G1993	18	22
	Guillou 2001	G2001	13	20
	Guillou 2008	G2008	17	20
	Guillou 2009	G2009	22	22
France	Gardouch	FR-GARD	68	68
	Montpellier	FR-MONTP	19	13
Italy	Lombardy	IT-LOMB	16	15
	Reggiolo	IT-REG	7	7
	San Bernardino	IT-SBER	14	18
Germany	Cologne-Bonn	GER-CB	14	14

Outline analysis

The mandible shape was quantified by the 2D outline of the mandibular bone, the hemi-mandible being placed flat on its lingual side. The starting point was defined at the intersection between the incisor and the bone at the upper edge of the mandible. The molar shape was approximated by the 2D outline of the tooth seen from the occlusal surface, the focus being made towards the basis of the crown, a place late affected by wear (Renaud, 2005). The starting point was positioned at the anteriormost part of the tooth using a standardization based on the best-fitting ellipse to the outline (Renaud *et al.* 2006). Such an outline analysis has been shown on both characters to efficiently describe fine-scale geographical variations in mice (Renaud, 2005; Renaud & Michaux, 2007; Renaud & Auffray, 2010).

Each outline was defined by a set of 64 points that was analysed using a Fourier-based method. The variation of the distance of each point to the centre of gravity of the outline as a function of the cumulative distance along the outline was decomposed into a sum of trigonometric functions of decreasing wavelengths (harmonics), each being weighted by two Fourier coefficients (FCs). The zero harmonic, proportional to the outline size, was used as size estimator and to standardize all other FCs so they represent shape variables only. The outline area was further registered as additional size estimator. The higher the rank of the harmonics, the more details they represent on the outline. The shape of a mouse mandible or molar tooth is adequately described by the first seven harmonics, that is, by 14 variables (Renaud & Michaux, 2003; Renaud *et al.*, 2011).

Statistical analyses

Total shape variation was initially described by 14 variables (FCs) for each mandible or molar. This information was first summarized using principal component analyses (PCA) on the matrix of variance-covariance. This provided synthetic shape axes to display the pattern of differentiation. To increase the power of the statistical analyses, subsequent tests were not performed on the set of FCs but on the set of principal axes representing more than 5% of variance, a procedure allowing a dimensionality reduction of the data set (Sheets *et al.*, 2006). Further statistical analyses were performed on these reduced data sets (one for the mandible, one for the molar).

The occurrence of sexual dimorphism was investigated using analyses of variance (ANOVA) on univariate size variables, and multivariate analysis of variance (MANOVA) on the multivariate shape data sets. A model including as factors locality, sex and their interaction was used.

For both the mandible and the molar, the relationship between the two size estimators (area of the outline, and zero harmonic of the Fourier analysis) was evaluated using a linear regression. The zero harmonic was thereafter used as univariate size estimator. Between-group size differences were tested using ANOVA and associated pairwise tests.

Shape differentiation was first investigated using multivariate analyses of covariance (MANCOVA) performed using as factors the locality/year of trapping and as covariate the size variable, to evaluate the importance of both factors in driving the shape differentiation. The interaction term (locality \times size) allowed estimating whether allometric patterns were shared among populations and if so, could explain a part of the inter-population differentiation. Among-group, shape differences were further tested using multivariate analyses of variance (MANOVA) completed by pairwise Hotelling's tests.

These tests are widely used but have the drawback, as any parametric tests, to rely on assumptions regarding the distribution of the variables, their variance and the balanced sampling of the groups. To strengthen our conclusions, this classical approach was completed by a permutation procedure allowing a comparison of the observations to a null hypothesis derived of the same sample. Morphological Euclidean distances in size and shape (based on the total data set, i.e. the FCs, or on the reduced data set, i.e. the PCs $>$ 5%) were computed between successive years in Guillole islands, and between Guillole samples and the pool of Western European specimens. To assess if these distances were higher than expected between Western European populations, 1000 populations of 20 and 15 specimens each (hence close to the sample size of the Guillole and Western European samples) were simulated from the pool of Western European specimens. Distances among these simulated populations were computed. The comparison of the distances involving Guillole specimens to these distributions allowed assessing if they were larger than expected among random continental populations.

Statistics were performed using Systat v. 12 and NTSys 2.2.

Results

Sexual dimorphism

Differences between sexes did not appear significant in any cases, either for size or for shape of the mandible and the molar (Table 2). This is in agreement with previous results repeatedly showing that the effect of sex was negligible in wild populations of mice (Davis, 1983; Valenzuela-Lamas *et al.*, 2011; Ledevin *et al.*, 2012). Age has also been shown to have minor effects (Davis, 1983; Renaud, 2005). All animals were therefore pooled together in subsequent analyses.

Table 2 Test of sexual dimorphism in house mice from Western Europe and Guillole Island. The effects of locality, sex and their potential interaction (Loc. \times Sex) were tested. Probabilities of ANOVA are given for univariate size variables, of MANOVA for multivariate shape data sets. Shape was summarized by the first three axes for the mandible and first five axes for the molar (all explaining more than 5% of the total variance) of a principal component analysis on the variables of the outline analysis (FCs).

	Md Size	UM1 Size	Md Shape	UM1 Shape
Locality	<0.001	<0.001	<0.001	<0.001
Sex	0.133	0.786	0.475	0.099
Loc. \times Sex	0.991	0.999	0.865	0.589

Mandible and molar size

Mandible and molar size were estimated by the area of the outline and the zero harmonic of the Fourier analysis. Both variables were highly significantly correlated (mandible: $R^2 = 0.986$, $P < 0.001$; molar: $R^2 = 0.997$, $P < 0.001$).

Mandible size varied significantly among groups (ANOVA: $P < 0.001$). Differences were seldom significant among samples from Western Europe, and no significant differences emerged among the different years sampled in Guillole Island (Table 3). In contrast, significant differences emerged between Guillole and Western European samples (Table 3). Guillole mandibles tended to be smaller than the continental ones (Fig. 2). Differences were marked for the oldest year sampled on Guillole (1993) with 5 of 6 comparisons with Western European samples being highly significant ($P < 0.001$; Table 3). The exception is constituted by the Italian group from Reggio (IT-REG) probably due to its small sample size. Differences tended to fade out through time. The most recent Guillole sample (2009) was only significantly different ($P < 0.01$) from 2 of 6 Western European samples. This corresponded to a progressive increase of mandible size on Guillole Island from 1993 to 2009 (Fig. 2).

In contrast, despite overall significant differences (ANOVA: $P < 0.001$), molar size was not different between Guillole and Western European samples. Few differences occurred within continental groups, and none among the successive years in Guillole Island (Table 4, Fig. 2).

Mandible shape differentiation

The total variance in mandible shape was efficiently summarized on the first three principal axes of a PCA on the FCs, each representing more than 5% of variance and totalling more than 85% of the total variance (PC1 = 54.2%, PC2 = 24.4%, PC3 = 8.0%, subsequent axes $<$ 5%). The pattern of differentiation is mostly driven by an opposition along the first axis between

Table 3 Probabilities of mandible size differences among groups from Guillo Island and Western Europe. Size was estimated by the zero harmonic of the 2D outline analysis. Probabilities of pairwise Tukey's tests (with Bonferroni correction) are provided. In bold significant probabilities ($P < 0.05$).

	G1993	G2001	G2008	G2009	FR-GARD	FR-MONTP	GER-CB	IT-LOMB	IT-REG	IT-SBER
G1993	–									
G2001	1	–								
G2008	1	1	–							
G2009	0.124	1	1	–						
FR-GARD	<0.001	0.041	0.821	1	–					
FR-MONTP	<0.001	<0.001	<0.001	0.006	0.010	–				
GER-CB	<0.001	<0.001	<0.001	0.009	0.002	1	–			
IT-LOMB	<0.001	<0.001	0.009	0.271	0.752	1	1	–		
IT-REG	0.159	0.764	1	1	1	1	0.701	1	–	
IT-SBER	<0.001	<0.001	0.003	0.095	0.247	1	1	1	1	–

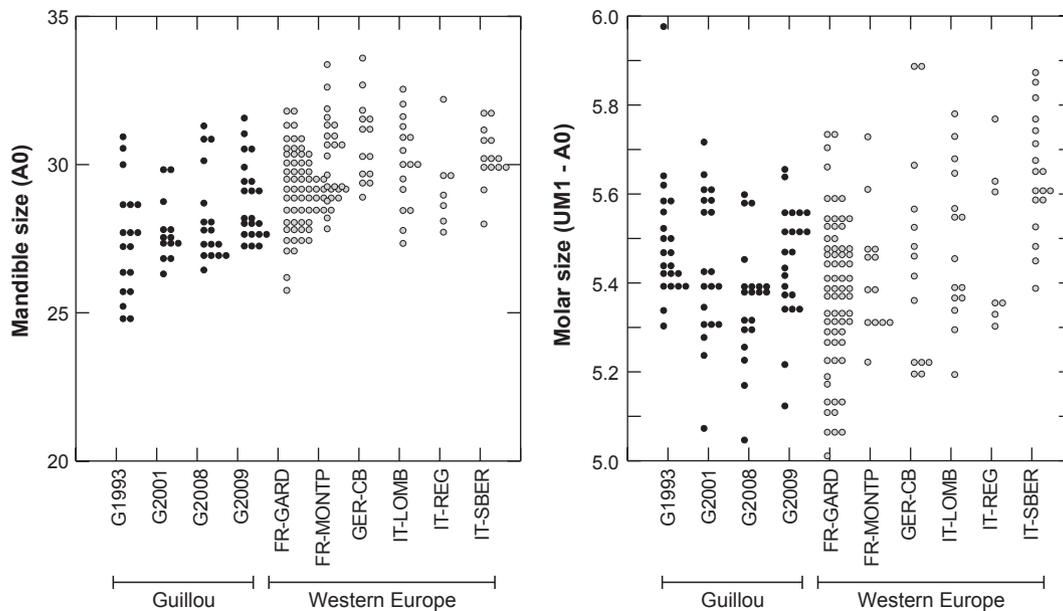


Fig. 2 Mandible and molar size in Guillo Island and Western European samples. Left, Mandible size distribution; right, molar size distribution in the various samples. Size was estimated by the zero harmonic of the outline analyses. Each dot corresponds to a specimen.

Guillo Island and Western European samples (Fig. 3). Despite some scattering along the second and especially the third axes within these continental samples, none of the pairwise differences emerged as significant (Table 5), evidencing stability through space and also through time since continental animals were trapped over more than 30 years. In contrast, significant differences existed between years in Guillo Island, being particularly marked among distant years. The oldest sample (1993) was highly significantly different ($P < 0.0001$) from the most recent ones (2008, 2009), the intermediate sample (2001) still differed from the most recent sample (2009) and no difference emerged between 2001–2008 and 2008–2009 (Table 5). This change through time corresponded to a progressive

extension of the coronoid and angular processes (Fig. 4), and to a progressive convergence of Guillo Island mandibles towards Western European morphologies (Figs 1 and 4). Indeed, while the oldest Guillo Island sample (1993) strongly differed from all continental groups (6 of 6 comparisons with $P < 0.0001$), the most recent sample (2009) only displayed one highly significant difference ($P < 0.0001$) when compared to the six continental groups (Table 5).

Molar shape differentiation

Molar shape total variance was more scattered on successive principal axes, with the first five axes representing more than 5% of variance and totalling more than

Table 4 Probabilities of first upper molar (UM1) size differences among groups from Guillou Island and Western Europe. Size was estimated by the zero harmonic of the 2D outline analysis. Probabilities of pairwise Tukey's tests (with Bonferroni correction) are provided. In bold significant probabilities ($P < 0.05$).

	G1993	G2001	G2008	G2009	FR-GARD	FR-MONTP	GER-CB	IT-LOMB	IT-REG	IT-SBER
G1993	-									
G2001	1	-								
G2008	0.447	1	-							
G2009	1	1	1	-						
FR-GARD	0.230	1	1	1	-					
FR-MONTP	1	1	1	1	1	-				
GER-CB	1	1	1	1	1	1	-			
IT-LOMB	1	1	1	1	0.903	1	1	-		
IT-REG	1	1	1	1	1	1	1	1	-	
IT-SBER	0.143	0.006	<0.001	0.009	<0.001	0.010	0.041	0.261	1	-

85% of the total variance (PC1 = 36.3%, PC2 = 23.6%, PC3 = 12.8%, PC4 = 8.1%, PC5 = 5.3%, subsequent axes < 5%). The main signal of differentiation corresponded to an opposition along the first principal axis of the Guillou and Western European samples (Fig. 3). Samples from Western Europe were scattered along the second and third axes, as were the different years sampled in Guillou. Noteworthy, a temporal trend occurred along the third axis, showing a sequence from 1993 to 2009, the two samples of consecutive years (2008 and 2009) being indeed very close in the morphological space.

This pattern is supported by pairwise tests (Table 6). Few differences emerged among continental samples. A temporal trend is displayed on Guillou, with the oldest sample (1993) being significantly different ($P < 0.05$) from the recent years (2008 and 2009), but no differences emerging between samples from 2001, 2008 and 2009.

Despite this trend throughout the Guillou record, the differentiation from the continent was held constant, with Guillou in all years being significantly ($P < 0.05$) different from all 6 Western European localities.

The differentiation among Guillou samples corresponded to subtle differences in molar shape (Fig. 4), with an anterior lingual cusp slightly more prominent in 1993. Compared with continental mice, Guillou molars have a more developed anterior part, whereas the lingual cusps are shifted anteriorly in Gardouch mice, reducing the anteriormost part of the tooth.

Morphological distances through time and space

Significant differences were displayed even among continental populations. To assess if the difference among Guillou samples and between Guillou and the continental pool was indeed larger than what could be anyway expected among continental populations, distances

were computed among pairs of random groups sampled from the continental pool (1000 repetitions). Morphological distances among successive years in Guillou Island, and distances between Guillou and the continental pool were computed and compared with the expected distribution for random continental groups (Fig. 5).

Mandible and molar shape distances between years in Guillou tended to be larger between distant years (1993–2001) than between consecutive years (2008–2009). These distances were, however, in the range of expected distances between random continental groups for molar shape, as they were for molar and mandible size. They were larger than the expected distances between random continental groups for mandible shape only (Fig. 5; Table 7).

Regarding differences between Guillou and the continent, distances in molar shape were larger than random at any time (Table 7). This was also the case regarding mandible size and shape, but for the oldest years sampled in Guillou only (1993 and 2001). The morphological distances diminished through time towards the range encountered between random continental samples.

Discussion

This study evidenced several major points. First, mice from the Sub-Antarctic Guillou Island were indeed morphologically differentiated from the Western European populations, considered the most probable source area of the initial colonizers of the Kerguelen Archipelago based on historical and genetic data (Hardouin *et al.*, 2010). Second, the mandible difference between Guillou mice and their continental relatives, while being important in 1993, decreased in recent years. Finally, the mandible and the molar were the two morphological characters considered and they evidenced a complex mixing of congruent and discrepant patterns.

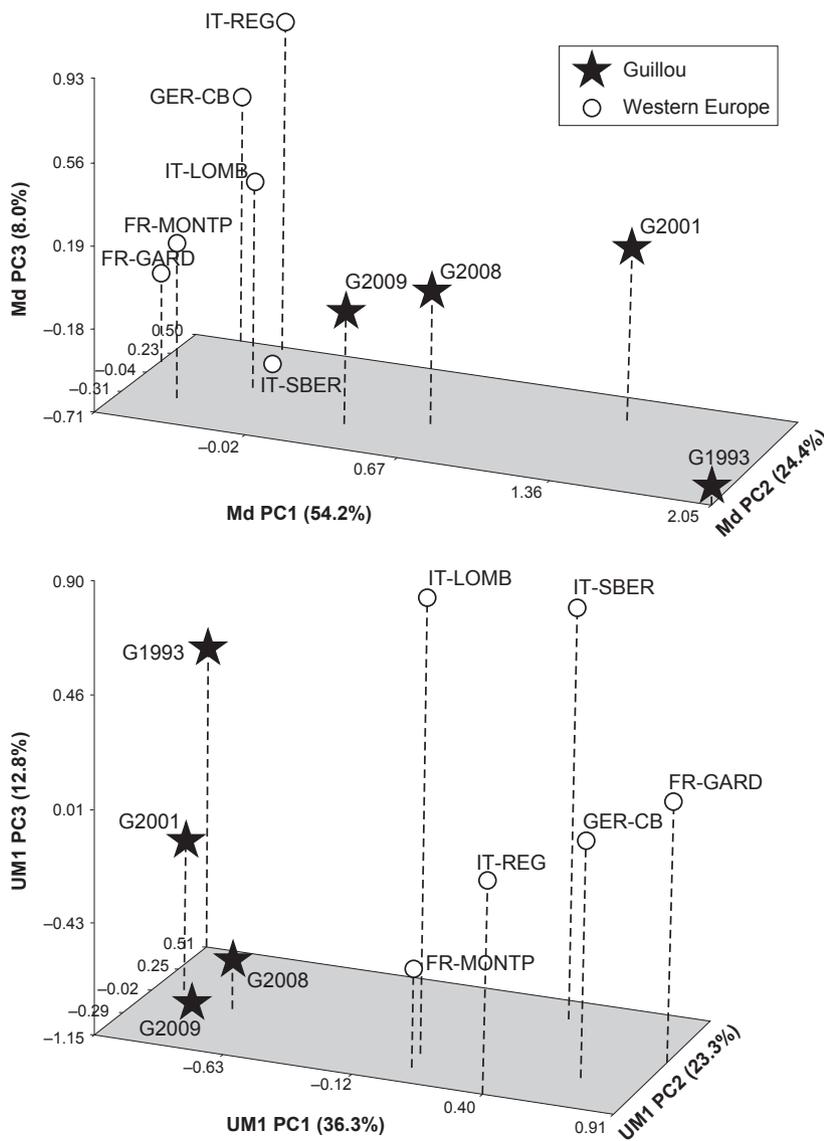


Fig. 3 Mandible and molar shape differences between Guillou Island and Western European samples. Upper panel, mandible shape; lower panel, molar shape. The average value of each locality is represented in a morphospace defined by the first three axes of a PCA on the Fourier coefficients of the outline analysis.

Insular divergence: ecological conditions and isolation promoting evolution

A former genetic study, including mitochondrial DNA and microsatellite data on autosomal and Y chromosomes, evidenced a strong genetic differentiation of the Kerguelen house populations, likely due to founder effect and subsequent drift (Hardouin *et al.*, 2010). From the mainland of Kerguelen Archipelago, Guillou Island represented a further event of colonization involving another founder effect, since Guillou mice are well differentiated within the Kerguelen pool (Hardouin *et al.*, 2010). Genetic data further evidenced resilience against late invasion, as shown by the homogeneity of the population (Hardouin *et al.*, 2010). Compared with the source area where house mice mostly experienced commensal

conditions, the climatic and ecological conditions on Sub-Antarctic islands like Guillou also differed in many ways, challenging the invasive population to adapt to new environmental conditions. Altogether, the combination of random and adaptive processes should favour a rapid initial evolution following colonization, with a subsequent return to background evolutionary rate similar to continental conditions (Millien, 2006).

In agreement with this expectation and with numerous examples of insular evolution in mice (e.g. Davis, 1983; Michaux *et al.*, 2007b; Renaud & Auffray, 2010), a pronounced morphological differentiation of the house mice was evidenced on Guillou Island, based on mandible and molar shape. Indeed, the morphological distances involved were in most cases higher than between simulated continental groups (Fig. 5). The

Table 5 Probabilities of mandible shape differences among groups from Guillou Island and Western Europe. Shape was summarized by the first three axes (all explaining more than 5% of the total variance) of a principal component analysis on the variables of the outline analysis (FCs). Probabilities of pairwise Hotelling's tests are provided (with Bonferroni correction). In bold significant probabilities ($P < 0.05$).

	G1993	G2001	G2008	G2009	FR_GARD	FR-MONTP	IT-LOMB	IT-REG	IT-SBER	GER-CB
G1993	–									
G2001	0.00714	–								
G2008	<0.00001	0.3953	–							
G2009	<0.00001	0.00181	1	–						
FR_GARD	<0.00001	<0.00001	<0.00001	<0.00001	–					
FR-MONTP	<0.00001	<0.00001	0.00022	0.00829	1	–				
IT-LOMB	<0.00001	0.00001	0.01026	0.21975	0.08517	1	–			
IT-REG	<0.00001	0.00086	0.01896	0.05786	0.78155	1	1	–		
IT-SBER	<0.00001	0.00002	0.00684	0.11202	0.50095	1	1	1	–	
GER-CB	<0.00001	<0.00001	0.00006	0.00030	1	1	1	1	0.74022	–

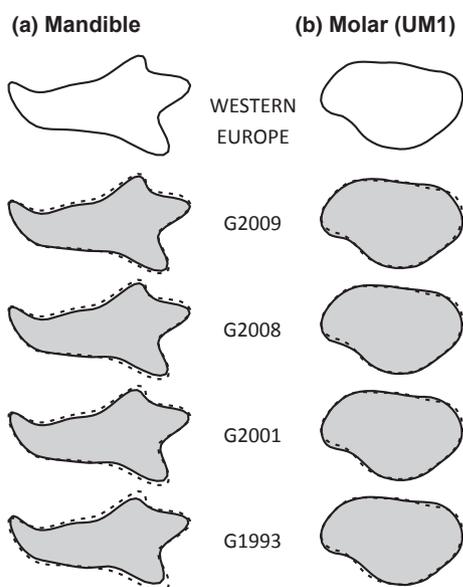


Fig. 4 Reconstructed outlines visualizing mean shape differences between the Western European sample of Gardouch (white) and successive year in Guillou (grey). (a) Mandible. (b) Molar (UM1). To facilitate the visualization of the shape changes, the mean outline of the Western European group (dashed line) was superimposed to the outlines of Guillou.

respective roles of random and adaptive processes on this differentiation are difficult to disentangle, but several points can be mentioned to consider this case of insular evolution into a broader context.

House mice in Guillou Island have been shown to display a generalist and opportunistic feeding behaviour, with both plant and invertebrates being consumed (Le Roux *et al.*, 2002). In contrast, although diet is likely prone to considerable variations depending on local food availability, house mice tend to display a mainly granivorous diet in continental environment

(Whitaker, 1966; Newsome, 1969). The important component of invertebrate preys for Guillou mice may have selected for different shape of characters involved in food handling such as tooth and mandible. Invertebrate preys are softer than vegetal matter. Their handling should require less strength in the mastication muscles inserted on the angular process of the mandible. In contrast, the incisor part of the mandible works as a blade to catch prey (Michaux *et al.*, 2007a). Guillou mandibles are characterized by reduced processes, a relatively well developed incisor part, and overall a mandible smaller than in continental mice. These changes matched the pattern of differentiation expected based on the functional requirements of a diet based on soft prey. Variation in tooth shape might also be involved in subtle resource partitioning (Dayan & Simberloff, 1994; Ledevin *et al.*, 2012), although the functional relationship between tooth shape change and mechanical properties involved in grinding and mastication is less straightforward than for the mandible. The fact that insular conditions may exert selective pressure on teeth radically different from what is experienced on mainland is further exemplified by the amazing survival to adulthood of a molarless rat feeding on soft food of animal origin on another insular environment (Bover *et al.*, 2010).

A sequence of morphological change in Guillou mice: drift and/or response to changing environment?

Beyond the differentiation characterizing Guillou mice, a further intriguing result of this study was that this differentiation was not stable through time. Both regarding mandible and molar shape, a temporal sequence of evolution was displayed, with the oldest sample (1993) differing from the most recent ones (2008 and 2009), whereas these consecutive years displayed almost no differentiation.

Table 6 Probabilities of molar shape differences among groups from Guillo Island and Western Europe. Shape was summarized by the first five axes (all explaining more than 5% of the total variance) of a principal component analysis on the variables of the outline analysis (FCs). Probabilities of pairwise Hotelling's tests are provided (with Bonferroni correction). In bold significant probabilities ($P < 0.05$).

	G1993	G2001	G2008	G2009	FR_GARD	FR-MONTP	IT-LOMB	IT-REG	IT-SBER	GER-CB
G1993	–									
G2001	0.10292	–								
G2008	0.03069	1	–							
G2009	0.00223	1	1	–						
FR_GARD	<0.00001	<0.00001	<0.00001	<0.00001	–					
FR-MONTP	0.00002	<0.00001	0.00001	<0.00001	<0.00001	–				
IT-LOMB	0.00004	<0.00001	<0.00001	<0.00001	0.00003	0.03010	–			
IT-REG	0.00337	0.00898	0.02938	0.00163	0.06850	0.32125	1	–		
IT-SBER	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	0.00058	1	1	–	
GER-CB	<0.00001	<0.00001	<0.00001	<0.00001	0.92771	0.08633	1	1	1	–

A comparison with simulated groups derived from the Western European continental pool allowed a comparison of the morphological distances involved with a model of evolution among continental populations. Differentiation can also occur among commensal mice even at a quite small spatial scale (e.g. Auffray *et al.*, 1996), because of local environmental conditions and genetic differentiation among populations acting like demes in a relative isolation from each other (Pocock *et al.*, 2004). Distance among random continental populations can thus be considered an approximation of the background evolution occurring on mainland conditions, and corresponding to a random walk due to a combination of fluctuating selection, random processes and possibly stabilizing selection.

The temporal evolution of Guillo mice regarding molar morphology did not depart from this model of background evolution. This suggests that the temporal differences observed could be attributed to nonoriented processes, most probably drift. Even though the time elapsed between the two most distant years appears short from an evolutionary point of view (16 years), mice in a Sub-Antarctic environment experience massive demographic crashes every winter (e.g. van Aarde & Jackson, 2007) that could promote drift from one year to the other.

The pattern was different regarding mandible shape. The morphological distance between the two most distant years 1993–2001 appeared as higher than expected by chance. Together with the fact that mandible differentiation of Guillo mice from Western European relatives, very pronounced in 1993, tended to fade away through time, this suggested that an oriented, nonrandom process of morphological evolution occurred in Guillo Island since 1993.

Indeed, a range of environmental changes occurred in this time interval in Guillo Island that could have modified the selective pressures on the mandible morphology in relation to ecological conditions. The major events were the rabbit eradication in 1994 and the

changes in the climatic conditions since the late 1990s. The former was accompanied by a momentary decrease of feral cat abundance that might have changed the predation pressure on mice. Both the rabbit eradication and the changes in the climatic conditions impacted on the dynamics of plant communities (Chapuis *et al.*, 2001, 2004; Robin *et al.*, 2011), with the consequence for the mice of changed access to some food items. Whereas the endemic flora was adapted to cold and humid conditions, repeated summer droughts since the 1990s favoured the spread of invasive species (e.g. *Taraxacum* gr. *officinale*) to the detriment of native plants (e.g. *Acaena magellanica*) except some Poacea (*Festuca erecta*) (Chapuis *et al.*, 2004; Lebouvier *et al.*, 2011). Moreover, invertebrates, which are a key component of the mouse diet (Le Roux *et al.*, 2002), were also affected by climate changes. The recent spread of the invasive predator beetle *Merizodus soledadinus* during the 1990s (Laparie *et al.*, 2010) further impacted the invertebrate community and food resources for mice, since it is a predator for most invertebrate species and it is also a new prey for mice. Overall, this situation might have contributed to a diversification of the mouse diet from an insect- and worm-based diet towards one with dominance of other items, including vegetal parts, seeds and flowers of various plants, especially grasses (Poacea) that constitute a major component of their diet elsewhere in the world (Whitaker, 1966; Newsome, 1969). This change in the average quality of the food items might have changed the selective pressures and/or muscular loadings on the mandible, favouring stronger and more robust mandibles and thus leading to morphotypes similar to those of the mainland relatives, despite persistent differences in the nature of the food items consumed. This suggests that environmental changes could have driven the observed dedifferentiation of the mandible observed, but further studies of the diet of Guillo mice are required to validate this hypothesis.

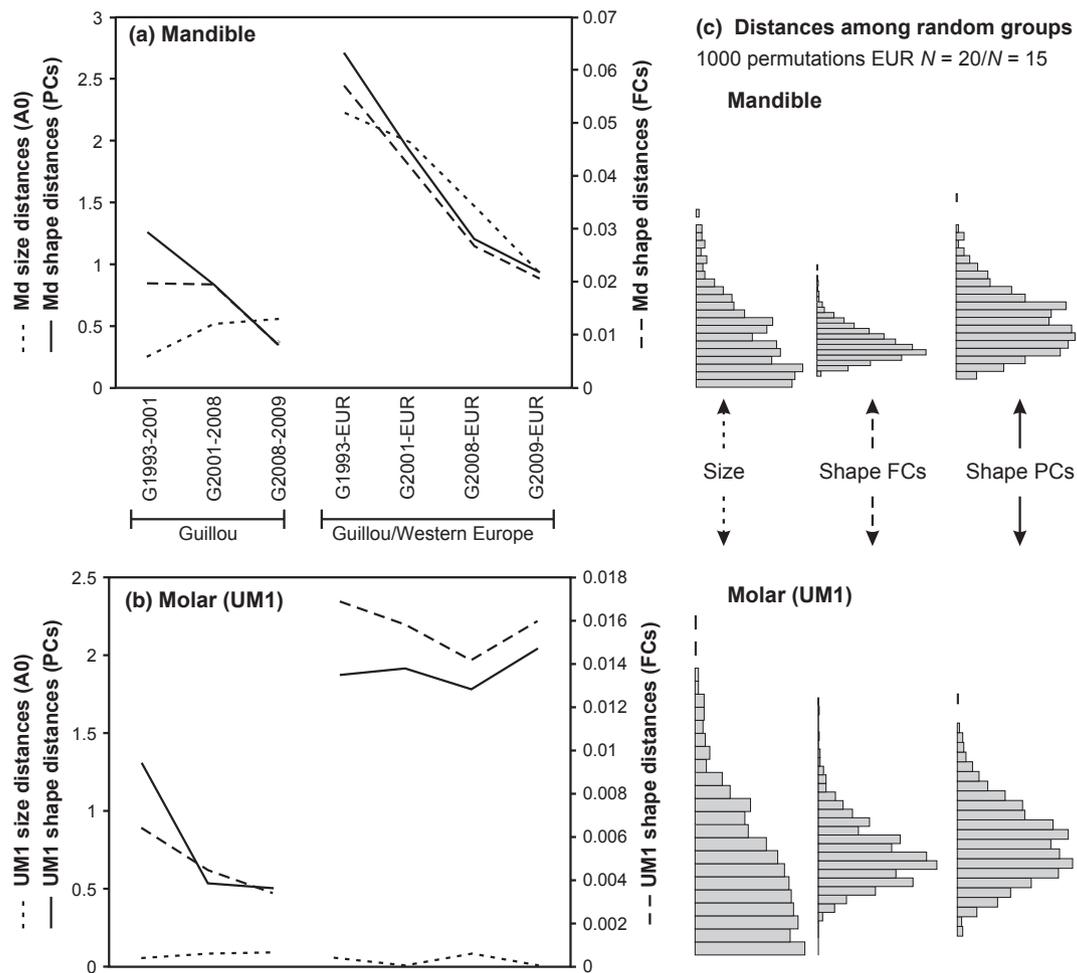


Fig. 5 Morphological distances between successive samples in Guillou Island, and between successive Guillou samples and the average Western Europe sample. (a) Mandible distances. (b) Molar distances. Size distances (dotted line) were estimated based on the zero harmonic of the outline analysis. Shape distances were estimated using two multivariate approaches: (1) based on all Fourier coefficients (FCs) up to the 14th harmonic (dashed line) and (2) on the set of principal axes summarizing more than 5% of the total variance each (three for the mandible, five for the molar) (full line). (c) Distribution of morphological distances among simulated groups derived from the Western European pool. Two groups of 20 and 15 specimens were randomly extracted from the Western European pool of specimens (138 for the mandible and 135 for the molar). The procedure was repeated 1 000 times. The distribution of the morphological distances between these simulated European groups provided a comparison basis for the observed distances involving Guillou samples.

Different characters, different developmental properties, different response to changing conditions?

The contrast between a trend in mandible shape vs. nonoriented limited changes in molar shape provides evidences that each character can tell its own story, as much as a gene only provides clues about the selective regime it was submitted to (e.g. Rodríguez *et al.*, 2010; Turmelle *et al.*, 2011). It suggests that mandible shape was prone to track rapid changes in the local environment of the mice, whereas molar shape did not display oriented trend through time. This supports other convergent results suggesting that at the intra-specific

level, mandible shape varies much more according to local environmental factors than molar shape does (Renaud, 2005; Renaud & Michaux, 2007; Ledevin *et al.*, 2012). It has been shown that the shape of the mandible changes in a plastic way when muscular functioning is modified during the post-weaning life of the animal, either in the pathological case of muscular dystrophy (Ravosa *et al.*, 2007; Renaud *et al.*, 2010) or in the more realistic case of changes in food consistency (Mavropoulos *et al.*, 2004; Renaud *et al.*, 2010). Hence, the phenotypic response of the mandible may include a part of plastic component allowed by a permanent bone remodelling. Note that an adaptive role of plastic morphological changes has been proposed, by helping

Table 7 Comparison between observed morphological distances and distances between random groups from Western Europe. Two random groups were computed ($N = 20$ and $N = 15$) from of the initial values the Western European pool (1 000 repetitions). The observed distances between successive years on Guillou Island, and between Guillou Island and the Western European pool, were compared to the distances expected among the simulated groups. Number of random observations higher than observed is indicated (1 000 indicates that all random distances were smaller than observed). In bold a value corresponding to a chance higher than 0.95% that the observed value is larger than random.

	Mandible			UM1		
	Size	Shape (FCs)	Shape (3 PCs)	Size	Shape (FCs)	Shape (5 PCs)
G1993-G2001	762	996	997	542	881	980
G2001-G2008	699	996	910	759	487	220
G2008-G2009	395	550	302	811	160	183
G1993-EUR	1000	1000	1000	503	1000	1000
G2001-EUR	1000	1000	1000	46	1000	1000
G2008-EUR	999	1000	990	781	1000	1000
G2009-EUR	946	999	950	76	1000	1000

the animal to face changing resources and hence, by allowing the first step of successful survival in a new environment (Losos *et al.*, 2001; Aubret *et al.*, 2004). The plasticity of a phenotypic character like the mandible is likely related to a behavioural plasticity in diet. The plasticity in such ecological traits and their morphological consequence may be crucial to successfully invade an insular environment strongly departing from the source area, and to face environmental perturbations occurring at an accelerated pace. This does not preclude the evolution of heritable differences that may involve the same mandible parts as plastic variations do, namely zones of insertion of the masticatory muscles (Young & Badyaev, 2010).

In contrast, the shape of a tooth like the first upper molar is determined early during prenatal ontogeny (e.g. Prochazka *et al.*, 2010) and it is mineralized and erupted early during post-natal life, making a plastic component in the molar shape changes unlikely. Developmental properties can nevertheless play an important role in determining the direction of evolution (e.g. Renaud *et al.* 2006). The anterior extension of the molar observed in Guillou mice is similar to a pattern observed on other islands and it seems to correspond to a direction of change favoured by developmental constraints (Renaud *et al.*, 2011). If such internal constraints tend to make some molar shape easier to evolve, a pronounced evolution might easily occur due to drift on a small and isolated population in conditions of relaxed selection.

Conclusion

A condition of invasive species worldwide success is their adaptability towards new environments. This adaptability has been considered under many facets, including genetic changes (e.g. Hoekstra *et al.*, 2005), physiological (e.g. Lynch, 1992), ecological (Granjon & Cheylan, 1988; Le Roux *et al.*, 2002; Pocock *et al.*, 2004) or demographic (Smith *et al.*, 2002; Ferreira *et al.*, 2006) traits. The

morphometric study of Guillou mice documents a further and often neglected aspect of adaptability, namely a rapid and at least partly plastic morphological response to environmental changes related to the exploitation of food resources by a generalist invader.

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References

- van Aarde, R.J. & Jackson, T.P. 2007. Food, reproduction and survival in mice on sub-Antarctic Marion Island. *Polar Biol.* **30**: 503–511.
- Aubret, F., Shine, R. & Bonnet, X. 2004. Adaptive developmental plasticity in snakes. *Nature* **431**: 261–262.
- Auffray, J.-C., Alibert, P., Latieule, C. & Dod, B. 1996. Relative warp analysis of skull shape across the hybrid zone of the house mouse (*Mus musculus*) in Denmark. *J. Zool (Lond.)* **240**: 441–455.
- Avenant, N.L. & Smith, V.R. 2003. The microenvironment of house mice on Marion Island (sub-Antarctic). *Polar Biol.* **26**: 129–141.
- Berry, R.J. 1981. Town Mouse, Country Mouse: adaptation and adaptability in *Mus domesticus* (*M. musculus domesticus*). *Mammal Rev.* **11**: 91–136.

- Berry, R.J. 1996. Small mammal differentiation on islands. *Philos. T. Roy. Soc. B* **351**: 753–764.
- Berry, R.J. & Peters, J. 1975. Macquarie Island House mice: A genetical isolate on a sub-Antarctic island. *J. Zool. (Lond.)* **176**: 375–389.
- Berry, R.J., Peters, J. & Van Aarde, R.J. 1978. Sub-antarctic House mice: colonization, survival and selection. *J. Zool. (Lond.)* **184**: 127–141.
- Berry, R.J., Bonner, W.N. & Peters, J. 1979. Natural selection in House mice (*Mus musculus*) from South Georgia (South Atlantic Ocean). *J. Zool. (Lond.)* **189**: 385–398.
- Bover, P., Alcover, J.A., Michaux, J. & Renaud, S. 2010. The case of an insular molarless black rat: Effects on lifestyle and mandible morphology. *Arch. Oral Biol.* **55**: 576–582.
- Bradshaw, W.E. & Holzapfel, C.M. 2006. Evolutionary response to rapid climate change. *Science* **312**: 1477–1478.
- Chapuis, J.-L., Boussès, P. & Barnaud, G. 1994. Alien mammals, impact and management in the French subantarctic islands. *Biol. Conserv.* **67**: 97–104.
- Chapuis, J.-L., Le Roux, V., Asseline, J. & Kerleau, F. 2001. Eradication of rabbits (*Oryctolagus cuniculus*) by poisoning on three islands of the subantarctic Kerguelen Archipelago. *Wildl. Res.* **28**: 323–331.
- Chapuis, J.-L., Frenot, Y. & Lebouvier, M. 2004. Recovery of native plant communities after eradication of rabbits from the subantarctic Kerguelen Islands, and influence of climate change. *Biol. Conserv.* **117**: 167–179.
- Copson, G.R. 1986. The diet of introduced rodents *Mus musculus* L. and *Rattus rattus* L. on Subantarctic Macquarie Island. *Aust. Wildl. Res.* **13**: 441–445.
- Cucchi, T. 2008. Uluburun shipwreck stowaway house mouse: molar shape analysis and indirect clues about the vessel's last journey. *J. Archaeol. Sci.* **35**: 2953–2959.
- Davis, S.J.M. 1983. Morphometric variation of populations of House mice *Mus domesticus* in Britain and Faroe. *J. Zool. (Lond.)* **199**: 521–534.
- Dayan, T. & Simberloff, D. 1994. Morphological relationships among coexisting heteromyids: an incisive dental character. *Am. Nat.* **143**: 462–477.
- Ferreira, S.M., Van Aarde, R.J. & Wassenaar, T.D. 2006. Demographic responses of house mice to density and temperature on sub-Antarctic Marion Island. *Polar Biol.* **30**: 83–94.
- Granjon, L. & Cheylan, G. 1988. Mécanismes de coexistence dans une guildes de muridés insulaire (*Rattus rattus* L., *Apodemus sylvaticus* L. et *Mus musculus domesticus* Ruttley) en Corse: conséquences évolutives. *Z. Säugetierkd.* **53**: 301–316.
- Hardouin, E., Chapuis, J.-L., Stevens, M.I., van Vuuren, J.B., Quillfeldt, P., Scavetta, R.J. *et al.* 2010. House mouse colonization patterns on the sub-Antarctic Kerguelen Archipelago suggest singular primary invasions and resilience against re-invasion. *BMC Evol. Biol.* **10**: 325.
- Hoekstra, H.E., Krenz, J.G. & Nachman, M.W. 2005. Local adaptation in the rock pocket mouse (*Chaetodipus intermedium*): natural selection and phylogenetic history of populations. *Heredity* **94**: 217–228.
- Kidder, J.H. 1876. The natural history of the Kerguelen Island. *Am. Nat.* **10**: 481–484.
- Kinnison, M.T. & Hairston, G.J. 2007. Eco-evolutionary conservation biology: contemporary evolution and the dynamics of persistence. *Funct. Ecol.* **21**: 444–454.
- Lambrinos, J.G. 2004. How interactions between ecology and evolution influence contemporary invasion dynamics. *Ecol.ogy* **85**: 2061–2070.
- Laparie, M., Lebouvier, M., Lalouette, L. & Renault, D. 2010. Variations of morphometric traits in populations of an invasive carabid predator (*Merizodus soledadinus*) within a sub-Antarctic island. *Biol. Invasions* **12**: 3405–3417.
- Le Roux, V., Chapuis, J.-L., Frenot, Y. & Vernon, P. 2002. Diet of the house mouse (*Mus musculus*) on Guillou Island, Kerguelen archipelago, Subantarctic. *Polar Biol.* **25**: 49–57.
- Lebouvier, M., Laparie, M., Hull, M.É., Marais, A., Cozic, Y., Lalouette, L. *et al.* 2011. The significance of the sub-Antarctic Kerguelen Islands for the assessment of the vulnerability of native communities to climate change, alien insect invasions and plant viruses. *Biol. Invasions* **13**: 1195–1208.
- Ledevin, R., Quéré, J.-P., Michaux, J.R. & Renaud, S. 2012. Can tooth differentiation help to understand species coexistence? The case of wood mice in China. *J. Zool. Syst. Evol. Res.* **50**: 315–327.
- Losos, J.B., Schoener, T.W., Warheit, K.I. & Creer, D. 2001. Experimental studies of adaptive differentiation in Bahamian *Anolis* lizards. *Genetica* **112–113** **39**: 9–415.
- Lynch, C.B. 1992. Clinal variation in cold adaptation in *Mus domesticus*: verification of predictions from laboratory populations. *Am. Nat.* **139**: 1219–1236.
- Mavropoulos, A., Kiliardis, S., Bresin, A. & Ammann, P. 2004. Effect of different masticatory functional and mechanical demands on the structural adaptation of the mandibular alveolar bone in young growing rats. *Bone* **35**: 191–197.
- Michaux, J.R., Goüy de Bellocq, J., Sara, M. & Morand, S. 2002. Body size increase in rodent populations: a role for predators? *Global Ecol. Biogeogr.* **11**: 427–436.
- Michaux, J., Chevret, P. & Renaud, S. 2007a. Morphological diversity of Old World rats and mice (Rodentia, Muridae) mandible in relation with phylogeny and adaptation. *J. Zool. Syst. Evol. Res.* **45**: 263–279.
- Michaux, J., Cucchi, T., Renaud, S., Garcia-Talavera, F. & Hutterer, R. 2007b. Evolution of an invasive rodent on an archipelago as revealed by molar shape analysis: the house mouse in the Canary islands. *Biogeogr.* **34**: 1412–1425.
- Millien, V. 2006. Morphological evolution is accelerated among island mammals. *PLoS Biol.* **4**: e321.
- Nagorsen, D.W. & Cardini, A. 2009. Tempo and mode of evolutionary divergence in modern and Holocene Vancouver Island marmots (*Marmota vancouverensis*) (Mammalia, Rodentia). *J. Zool. Syst. Evol. Res.* **47**: 258–267.
- Newsome, A.E. 1969. A population study of house mice temporarily inhabiting a South Australian wheat field. *J. Anim. Ecol.* **38**: 341–359.
- Pocock, M.J.O., Searle, J.B. & White, P.C.L. 2004. Adaptations of animals to commensal habitats: population dynamics of house mice *Mus musculus domesticus* on farms. *J. Anim. Ecol.* **73**: 878–888.
- Pontier, D., Say, L., Debias, F., Bried, J., Thioulouse, J., Micol, T. *et al.* 2002. The diet of feral cats (*Felis catus* L.) at five sites on the Grande Terre, Kerguelen archipelago. *Polar Biol.* **25**(11): 833–837.
- Prochazka, J., Pantalacci, S., Churava, S., Rothova, M., Lambert, A., Lesot, H. *et al.* 2010. Patterning by heritage in mouse molar row development. *Proc. Natl Acad. Sci. USA* **107**: 15497–15502.

- Ravosa, M.J., Klopp, E.B., Pinchoff, J., Stock, S.R. & Hamrick, M. W. 2007. Plasticity of mandibular biomineralization in myostatin-deficient mice. *J. Morphol.* **268**: 275–282.
- Renaud, S. 2005. First upper molar and mandible shape of wood mice (*Apodemus sylvaticus*) from northern Germany: ageing, habitat and insularity. *Mamm. Biol.* **70**: 157–170.
- Renaud, S. & Auffray, J.-C. 2010. Adaptation and plasticity in insular evolution of the house mouse mandible. *J. Zool. Syst. Evol. Res.* **48**: 138–150.
- Renaud, S. & Michaux, J.R. 2003. Adaptive latitudinal trends in the mandible shape of *Apodemus* wood mice. *J. Biogeogr.* **30**: 1617–1628.
- Renaud, S. & Michaux, J.R. 2007. Mandibles and molars of the wood mouse, *Apodemus sylvaticus* (L.): integrated latitudinal signal and mosaic insular evolution. *J. Biogeogr.* **34**: 339–355.
- Renaud, S., Auffray, J.-C. & de La Porte, S. 2010. Epigenetic effects on the mouse mandible: common features and discrepancies in remodeling due to muscular dystrophy and response to food consistency. *BMC Evol. Biol.* **10**: 28.
- Renaud, S., Auffray, J. C. & Michaux, J. 2006. Conserved phenotypic variation patterns, evolution along lines of least resistance, and departure due to selection in fossil rodents. *Evolution* **60**: 1701–1717.
- Renaud, S., Pantalacci, S. & Auffray, J.-C. 2011. Differential evolvability along lines of least resistance of upper and lower molars in island mouse mice. *PLoS ONE* **6**: e18951.
- Robin, M., Chapuis, J.-L. & Lebouvier, M. 2011. Remote sensing of vegetation cover change in islands of the Kerguelen archipelago. *Polar Biol.* **34**: 1689–1700.
- Rodríguez, F., Pérez, T., Hammer, S.E., Albornoz, J. & Domínguez, A. 2010. Integrating phylogeographic patterns of microsatellite and mtDNA divergence to infer the evolutionary history of chamois (genus *Rupicapra*). *BMC Evol. Biol.* **10**: 222.
- Sheets, H.D., Covino, K.M., Panasiwicz, J.M. & Morris, S.R. 2006. Comparison of geometric morphometric outline methods in discrimination of age-related differences in feather shape. *Front. Zool.* **3**: 15.
- Siahsarvie, R., Auffray, J.-C., Darvish, J., Rajabi-Maham, H., Yu, H.-T., Agret, S. et al. 2012. Patterns of morphological evolution in the mandible of the house mouse *Mus musculus* (Rodentia: Muridae). *Biol. J. Linn. Soc.* **105**: 635–647.
- Smith, V.R., Avenant, N.L. & Chown, S.L. 2002. The diet and impact of house mice on a sub-Antarctic island. *Polar Biol.* **25**: 703–715.
- Sondaar, P.Y. 2000. Early human exploration and exploitation of islands. *Tropics* **10**: 203–230.
- Turmelle, A.S., Kunz, T.H. & Sorenson, M.D. 2011. A tale of two genomes: contrasting patterns of phylogeographic structure in a widely distributed bat. *Mol. Ecol.* **20**: 357–375.
- Valenzuela-Lamas, S., Baylac, M., Cucchi, T. & Vigne, J.-D. 2011. House mouse dispersal in Iron Age Spain: a geometric morphometrics appraisal. *Biol. J. Linn. Soc.* **102**: 483–497.
- Whitaker, J.O. 1966. Food of *Mus musculus*, *Peromyscus maniculatus* and *Peromyscus leucopus* in Vigo County, Indiana. *J. Mamm.* **47**: 473–486.
- Young, R.L. & Badyaev, A.V. 2010. Developmental plasticity links local adaptation and evolutionary diversification in foraging morphology. *J. Exp. Zool. (Mol. Dev. Evol.)* **314B**: 434–444.

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