ORIGINAL ARTICLE

Mandibles and molars of the wood mouse, *Apodemus sylvaticus* (L.): integrated latitudinal pattern and mosaic insular evolution

Sabrina Renaud[1]* and Johan R. Michaux[2,3]

[1]*Paléoenvironnements et Paléobiosphère, UMR 5125 CNRS - Université Lyon 1, Campus de la Doua, 69622 Villeurbanne, France,* [2]*Unité de Recherches en Zoogéographie, Institut de Zoologie, Quai Van Beneden 22, 4020 Liège, Belgique and* [3]*Centre de Biologie et de Gestion des Populations (CBGP), UMR 1062, Campus International de Baillarguet, CS 30016, 34988, Montferrier/Lez (Montpellier) Cedex, France*

ABSTRACT

Aim The distinct nature of island populations has traditionally been attributed either to adaptation to particular insular conditions or to random genetic effects. In order to assess the relative importance of these two disparate processes, insular effects were addressed in the European wood mouse, *Apodemus sylvaticus* (Linnaeus, 1758).

Location Wood mice from 33 localities on both mainland and various Atlantic and western Mediterranean islands were considered. This sampling covers only part of the latitudinal range of *A. sylvaticus* but included the two main genetic clades identified by previous studies. Islands encompass a range of geographical conditions (e.g. small islands fringing the continent through large and isolated ones).

Methods The insular syndrome primarily invokes variations in body size, but ecological factors such as release from competition, niche widening and food availability should also influence other characters related to diet. In the present study, the morphology of the wood mice was quantified based on two characters involved in feeding: the size and shape of the mandibles and first upper molars. The size of the mandible is also a proxy for the body size of the animal. Patterns of morphological differentiation of both features were estimated using two-dimensional outline analysis based on Fourier methods.

Results Significant differences between mainland and island populations were observed in most cases for both the mandibles and molars. However, molars and mandibles displayed divergent patterns. Mandible shape diverged mostly on islands of intermediate remoteness and competition levels, whereas molars exhibited the greatest shape differentiation on small islands, such as Port-Cros and Porquerolles. A mosaic pattern was also displayed for size. Body and mandible size increased on Ibiza, but molar size remained similar to mainland populations. Mosaic patterns were, however, not apparent in the mainland populations. Congruent latitudinal variations were evident for the size and shape of both mandibles and molars.

Main conclusions Mosaic evolution appears to characterize insular divergence. The molar seems to be more prone to change with reduced population size on small islands, whereas the mandible could be more sensitive to peculiar environmental conditions on large and remote islands.

Keywords
Apodemus sylvaticus, body size, dentition, Fourier analysis, island syndrome, mandible shape, morphometrics, Rodentia.

*Correspondence: Sabrina Renaud, UMR 5125 CNRS – Université Lyon 1, Bât. Géode, 2 rue Dubois, Campus de la Doua, F-69622 Villeurbanne Cedex, France.
E-mail: sabrina.renaud@univ-lyon1.fr

INTRODUCTION

The interaction between contingency and determinism drives morphological evolution, but the relative importance of both processes remains the subject of considerable discussion (e.g. Losos *et al.*, 1998). Insular evolution presents a unique opportunity to address this question as chance effects generating divergence are likely to be enhanced due to the process of colonization and subsequent drift in a reduced population. On the other hand, directional evolutionary trends can be favoured on islands where isolation enhances the ability of a population to track local environmental optima (Whittaker, 1998; Lenormand, 2002). Both contingency and determinism have been invoked to explain the insular evolution of rodent populations (Berry, 1973, 1996). The wood mouse, *Apodemus*, is of interest in this framework because of its notable ability to colonize islands. Insular populations often display a larger body size than their closest mainland relatives, suggesting the occurrence of an 'insular syndrome' (Angerbjörn, 1986; Michaux *et al.*, 1996b, 2002b; Lomolino, 2005). On the other hand, some insular populations show apparently random morphological differentiation (Renaud & Millien, 2001; Renaud & Michaux, 2003). In contrast, deterministic factors seem to play an important role in structuring a latitudinal gradient in response to environmental conditions in mainland populations (Renaud & Millien, 2001; Renaud & Michaux, 2003).

As latitudinal gradients may cause morphological differentiation, with a response amplitude that is itself variable with latitude (Yom-Tov *et al.*, 1999), focusing on a limited latitudinal range may help decipher the key factors shaping insular divergence within a given climatic realm. The European wood mouse, *Apodemus sylvaticus* (Linnaeus, 1758), has been present in the Western Palearctic region for the past 3 Myr (Michaux & Pasquier, 1974). From this wide geographical distribution, we focus on only south-western European populations. Within this geographical range, size and shape should display a simple gradient with latitude (Renaud & Michaux, 2003). The range restriction might impede the detection of gross latitudinal variation but will enhance the detection of insular divergence.

A previous study (Renaud & Michaux, 2003) was based on the analysis of the mandible. This character varies with body size (Renaud, 2005) and is involved in the feeding function. Hence, its variation with several ecological factors invoked in the insular syndrome, such as increased body size (Foster, 1964; Lomolino, 1985; Michaux *et al.*, 2002b) and diversification of the diet (Orsini & Cheylan, 1988), is unsurprising. Other characters, such as the teeth, are less likely to vary with body size at an intraspecific level but are also involved in the feeding function. Such features are important for evolutionary studies of small mammals since analysis of the fossil record of small mammals relies heavily on teeth. Generalization of conclusions drawn from studies based on body or mandible size to fossil data requires direct evidence that teeth co-vary with other characters.

Finally, the genetic structure of the European wood mouse is now well understood based on mitochondrial cytochrome *b* sequences and variations in restriction fragment length polymorphism (Michaux *et al.*, 1996b, 1998a, 2002a, 2003; Libois *et al.*, 2001). Therefore, patterns of morphological divergence can be interpreted within a well-defined phylogenetic framework. Two main clades emerge, corresponding to a western and central European group and to one of Italo-Balkanic affinity. Overall, Mediterranean insular populations of wood mice are closely related to mainland relatives, in agreement with Holocene dates for the colonization by the wood mouse of large Mediterranean islands (Vigne, 1999). Sicilian populations are an exception in that they emerge as a differentiated clade.

Morphological differentiation between populations was estimated based on the size and shape of mandibles and first upper molars using Fourier analysis of the outline. Results were compared between characters and sorted by genetic grouping, insularity and latitudinal location. Pattern coherency and deviation identified key factors determining the morphological differentiation. Size vs. shape and mandible vs. molar emerged as characters varying according to different factors, and prone to either ecological or genetic influences.

MATERIALS AND METHODS

Materials

The study is based on a sample set of 239 wood mice (*A. sylvaticus*): 216 mandibles and 239 first upper molars (M1) were measured (Table 1). Only mature specimens with the third molar erupted were considered. The mice were either trapped by J. R. Michaux and R. Libois, or were obtained from the Museum National d'Histoire Naturelle (Paris, France). All animals from the former collection were diagnosed genetically as *A. sylvaticus*. These specimens were trapped in 33 localities (Table 1) on the mainland and islands (Fig. 1). The mainland localities were selected as reference points from each island. The islands were selected to sample variable geographical conditions and included small (< 20 km^2) to large islands ($> 20{,}000$ km^2) and islands close to (distance < 10 km) or remote from (distance > 200 km) the mainland. Corsica, Sardinia and Ibiza were isolated during the Pleistocene, whereas other islands were connected to the mainland during sea-level low-stands of the last glacial–interglacial cycles (Van Andel, 1989, 1990).

The majority of wood mice belong to the western European genetic clade, with the exception of Corsica, Sardinia and Elba, which were occupied by wood mouse populations of the Italo-Balkanic genetic clade. Sicily is characterized by wood mice belonging to a distinct genetic group related to the Italo-Balkanic clade. Sicily is a large, elevated island, separated from the mainland only by the Strait of Messina. The strong currents in the Strait of Messina at present form an efficient barrier to gene flow from the mainland, but the depth of the strait (*c.* 80 m) allowed connections between Sicily and the mainland during the Late Pleistocene.

Table 1 Localities and groups used in statistical analyses, with latitude and number of items analysed

Group	M/I	GENET	Country	Locality	Coll	Latitude	M1	Md
FR-IEV	M	WEUR	France	St Méen	MHNH	48.2	6	6
FR-CH	M	WEUR	France	Lignières	MHNH	46.8	10	11
FR-LA	M	WEUR	France	Marais Poitevin	JRM	46.8	2	1
				Puceul	MHNH	47.6	21	21
FR-OL	I	WEUR	France	Oléron	JRM	45.9	15	15
FR-RE	I	WEUR	France	Ré	JRM	46.2	7	6
FR-YEU	I	WEUR		Yeu	JRM	46.7	1	1
FR-NM	I	WEUR		Noirmoutier	JRM	47.0	3	3
FR-MTP	M	WEUR	France	Montpellier	JRM	44.0	14	14
FR-S	M	WEUR	France	Cap Lardier	JRM	43.3	6	4
				Esterel	JRM	43.4	2	1
				La Penne	JRM	43.9	4	4
				Cévennes	JRM	44.4	3	2
FR-PORQ	I	WEUR	France	Porquerolles	JRM	43.0	11	8
FR-PTCR		WEUR		Port Cros	JRM	43.0	7	4
CO	I	IBK	France	Bonifacio	JRM	41.4	3	3
				Fango	JRM	42.4	5	5
IT-CAL	M	IBK	Italy	Gambaria	JRM	38.2	2	3
IT-TARQ		IBK		Tarquinia	JRM	42.3	32	25
IT-GROS		IBK		Grosseto	JRM	42.8	6	5
IT-SARD	I	IBK	Italy	Sardinia	JRM	40.9	3	2
ELBA	I	IBK		Elba	JRM	42.8	1	1
SIC-FIC	I	SIC	Italy	Ficuzza	JRM	37.7	7	6
SIC-GRAT	I	SIC		Grateri	JRM	38.0	8	9
SIC-M	I	SIC		Marettimo	JRM	38.0	1	1
PYR	M	WEUR	France	Banyuls	JRM	42.5	10	6
			Spain	Figueras	JRM	42.3	5	5
			Spain	Fabian	JRM	42.6	4	4
SP-MUR	M	WEUR	Spain	Murcia	JRM	38.0	15	15
SP-ALC	M	WEUR	Spain	Alcoy	JRM	38.7	10	10
SP-N		WEUR		Nogais	JRM	42.8	3	3
				Posada	JRM	43.2	3	3
IBIZ	I	WEUR	Spain	Ibiza	JRM	38.9	9	9

M1, first upper molar; Md, mandible; M/I, mainland/island; Coll, collection (MNHN, Museum National d'Histoire Naturelle, Paris, France; JRM, Johan R. Michaux); GENET, genetic clade (WEUR, western European; IBK, Italo-Balkanic; SIC, Sicily).

Outline analysis

The outline describes the overall shape of morphological features. For molars, it describes the relative position and swelling of the cusps. The outline of the bone in mandibles provides a good description of the processes involved in the insertion of the masticatory muscles, as well as of the alveolar region hosting the cheek teeth and the incisors. In both cases, outline analysis is an appropriate method to use to describe subtle geographical changes in characters related to feeding (Renaud & Michaux, 2003; Renaud, 2005).

The first upper molar was described by its two-dimensional projection viewed from the occlusal surface. The starting point was the maximum of curvature at the fore-part of the tooth. The outline of the mandible describes the projection of the hemi-mandible put flat on its side with the lingual side down. As the incisors can be free moving and the molars missing, only the outline of the mandibular bone was considered. The starting point was defined at the meeting point of the incisor and the bone at the upper edge of the mandible. For both the mandible and the first upper molar (M1), we sampled 64 points equally spaced along the outline.

A radial Fourier transform (RFT) was applied to the M1 outlines (Renaud, 1999; Renaud & Michaux, 2003). Using the *x,y*-coordinates of the points, 64 radii (i.e. distance of each point to the centre of gravity of the outline) were calculated. Using the RFT, the initial data set was described by the variations of the radius as a function of the cumulative distance along the outline. This function is approximated by a finite sum of trigonometric functions of decreasing wavelength, the harmonics. Each harmonic was weighted by two Fourier coefficients (FC), A_n and B_n. The zero harmonic A_0 is proportional to the size of each outline and was used to standardize all other the Fourier coefficients in order to eliminate isometric size effects and to concentrate on shape information only.
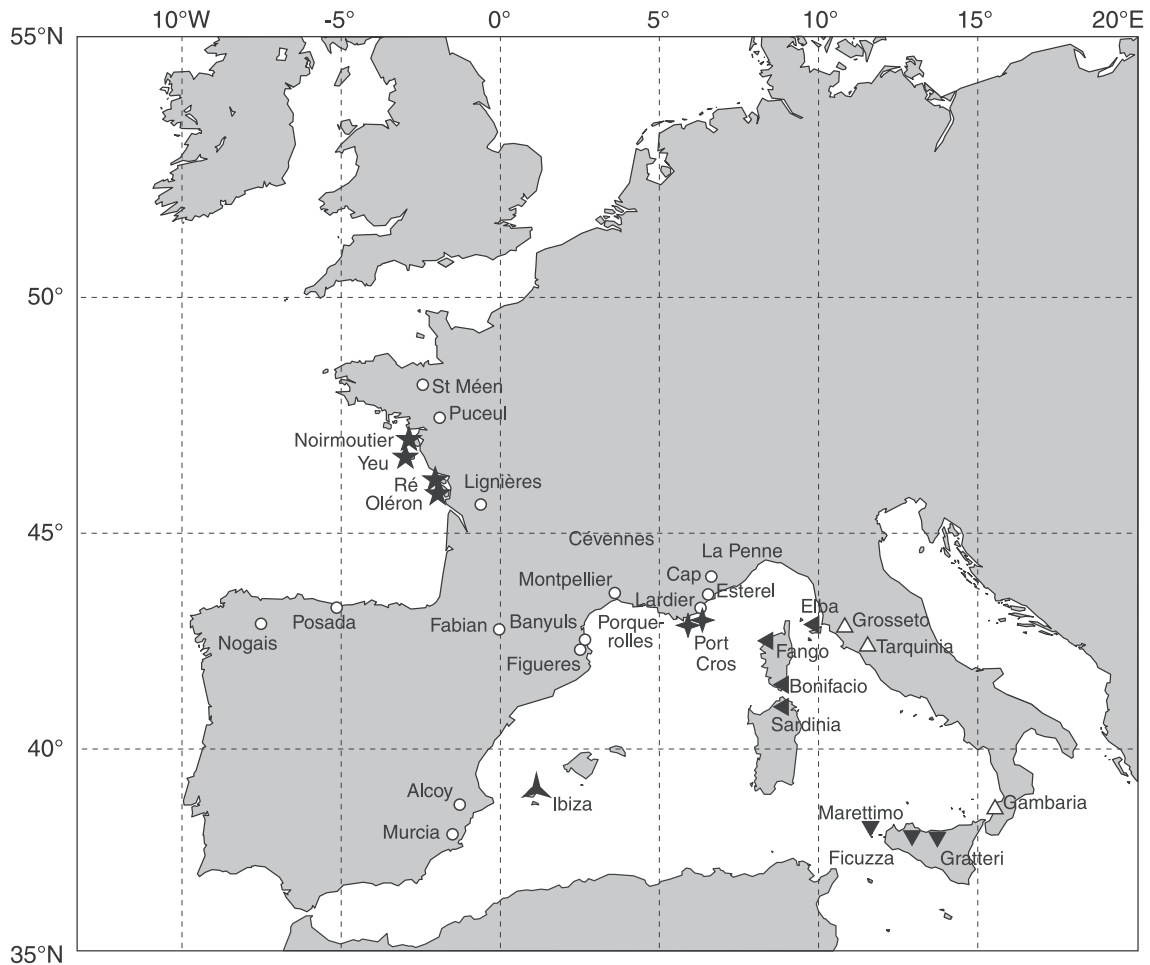
Figure 1 Sampling localities of the animals considered in the present study. Open symbols: mainland (circles, western European clade; triangles, Italo-Balkanic clade), filled symbols: islands.

An alternative approach to the analysis of outline data is the elliptic Fourier transform (EFT). This method is based on separate Fourier decompositions of the incremental changes along x and y as a function of the cumulative length along the outline (Kuhl & Giardina, 1982; Ferson *et al.*, 1985). Any harmonic corresponds to four coefficients: A_n and B_n for x, and C_n and D_n for y, defining an ellipse in the xy-plane. The coefficients of the first harmonic, describing the ellipse best fitting the original outline, were used to standardize the size and orientation. The Fourier coefficients (FC) of one harmonic cannot be considered as independent because the variations along x and y are related when considering a closed outline. Additionally, performing a Fourier analysis on both, the variations of x and y lead to twice the number of FC as RFT for the same number of harmonics. Although the high number of variables can be viewed as a shortcoming, EFT provides very accurate reconstructions, even for complex outlines, and makes visual inspection of the results easier, especially when dealing with shape variations of a complex structure like the mandible. As both methods provide similar results when compared

(Renaud & Michaux, 2003), RFT was used for the analysis of the simple molar outline whereas EFT was chosen to describe the morphological variation of the mandible.

Previous studies on related rodents showed that using the first nine harmonics for the M1 (Renaud, 1999) and the first seven harmonics for the mandible (Renaud & Michaux, 2003) offer a good compromise between measurement error, information content and number of variables to be considered.

Statistical analyses

Estimation of size

The size of the mandible or of the M1 was estimated by a univariate parameter, derived from the outline analysis. For the M1, the RFT provides the zero-harmonic amplitude A_0 as a size estimator. The size of each mandible was estimated by the area of the ellipse corresponding to the first harmonic (area H1). The relationship of these two parameters with latitude was investigated using simple linear regression.

Reduction of multivariate shape variation on synthetic axes

The shape of each outline was described by a set of Fourier coefficients: 24 for the mandible (EFT: four FCs per six harmonics, the FCs of the first harmonic being excluded from the analysis) and 18 for the M1 (RFT: two FCs per nine harmonics). These sets of coefficients have been analysed using multivariate statistics. The differences between localities were tested using a multivariate analysis of variance (MANOVA, test considered: Wilks' lambda). Canonical axes were estimated using the MANOVA analyses, and these allowed us to display the morphological variability on a few synthetic shape axes.

The biological interpretation of these axes relies on visualizing the shape changes they describe. Reconstructions can be obtained from a set of FCs corresponding to scores on the synthetic axes, thereafter using an inverse Fourier transform. Such a set of coefficients was obtained by calculating the multivariate regression parameters of FCs (dependent variables) upon the relevant synthetic axis (independent variable). In this case, the multivariate regression corresponds to the set of all single regressions of FCs upon the synthetic axis (Monti *et al.*, 2001).

The relationship of the synthetic shape axes with (geographical) latitude was investigated using simple linear regression on the first two canonical axes from the MANOVA.

Multivariate analysis of the morphological variability

Although clearly useful for visualizing the pattern of morphological shape variability, expressing this variation on a few synthetic axes leads to a loss of information. Therefore, the relationships of shape with clade, size, latitude and insularity were also investigated using a range of complementary approaches.

The relationship of shape and univariate parameters such as size and latitude was investigated using multivariate regression, with the Fourier coefficients as the dependent variables. As a significant relationship between size and shape may cause a latitudinal shape variation through allometry, the residuals of a multivariate regression of the FCs on size were also regressed onto latitude. A similar approach was used on mainland samples, in order to identify a possible differentiation among the two clades. The residuals of a regression of the FCs onto latitude were tested for clade differences.

Euclidean distances were calculated among group means for shape and size. The relationships between various data sets were investigated using a comparison of the distance matrices (Mantel *t*-test).

The amount of morphological divergence on each island was compared with different physical and ecological characteristics of the islands. In order to take into account the effect of latitude and of the various mainland source populations (Michaux *et al.*, 2002a; Goüy de Bellocq *et al.*, 2003), the amount of divergence was estimated as the difference between the mean value of the island and the closest mainland relative. Simple differences were calculated between size estimators, retaining a sign indicator to show a size increase or decrease. To estimate the amount of morphological difference in a fully multivariate way, the shape difference was estimated as Euclidean distance on the FCs. Data from Marettimo islet were excluded, firstly since the population was sampled by a single animal and second because it is an intricate case of isolation from the insular population of Sicily.

Finally, the physical and ecological parameters were categorized and their influence on the morphological divergence investigated using a one-way analysis of variance. As sample size was small ($N = 12$) we used the Kruskall–Wallis test, which is a nonparametric analogue.

All statistical analyses were performed using Systat 10 and NTsys-pc 2.1 (Rohlf, 2000).

RESULTS

Patterns of shape differentiation of the mandible and molars

Significant morphological differentiation was found for both molars and mandibles (MANOVA on Fourier coefficients: $P < 0.001$). The percentage of among-group variance displayed by the first two axes was limited (35.3% for the mandibles and 44.1% for the molars), but most of the divergence expressed on subsequent axes corresponds to some local differentiation. Only the results based on the first two axes are shown and discussed in detail (Fig. 2).

The pattern of mandible differentiation is shown in Fig. 2(a). The mandibles of the two mainland clades appear to be very similar, although some difference appears on axis CA2. The Italian mandibles are grouped on the range of the variation exhibited by the western European group, possibly because of a more restricted latitudinal range.

Insular mandible morphologies scatter around the mainland populations. Except for Port-Cros, Porquerolles and the Sicilian islet Marettimo, islands tend to display negative values along axes CA1 and CA2 (Fig. 2a). The most divergent insular populations on the first canonical plane are Porquerolles (along CA1), Sardinia (along CA2), Corsica and the single specimen from Elba (along CA1). Reconstructed outlines allow a visual interpretation of the canonical plot. The mandible traits characterizing the insular populations are subtle. Positive differences along CA1 include mandibles with a smooth alveolar region, a reduced coronoid process, and a wide and rounded angular process. Negative deviations are displayed by mandibles with a pronounced alveolar region, prominent coronoid process and a slender angular process. The second axis has massive mandibles with a straight lower border displaying positive divergence, whereas negative divergence was seen in slender mandibles with an undulating outline and prominent processes.

The differentiation between the two mainland clades is better expressed by the shape of the first upper molar (Fig. 2b). Sample means from mainland Italy and western Europe are
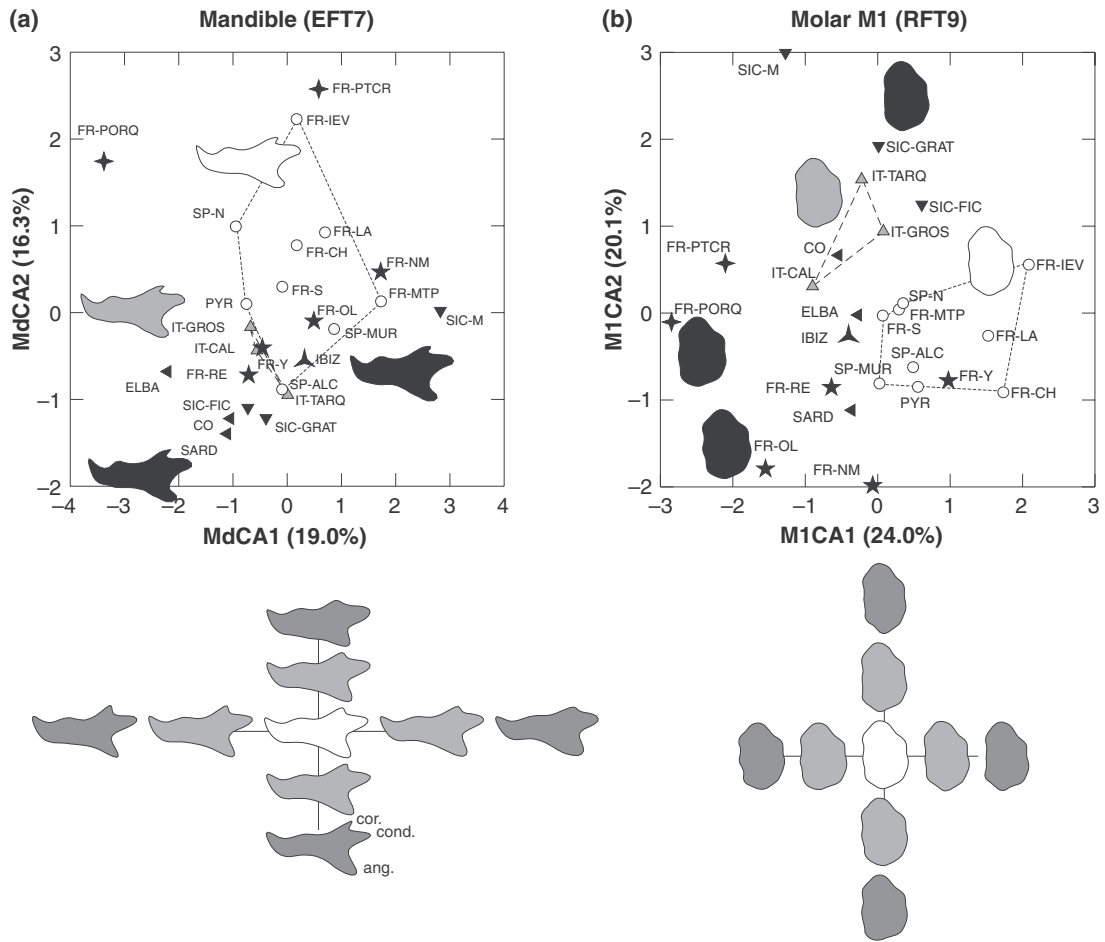
Figure 2 Shape variability of the mandible (a) and first upper molar (b), estimated by the first two axes of a canonical analysis on the EFT Fourier coefficients of the mandible and the RFT coefficients of the M1 outline. Symbols correspond to the average value per geographical group. Dotted lines enclose the range of variation of the mainland western European and Italo-Balkanic clades. Reconstructed outlines on the plot visualize the average shape of some geographical groups (EFT mandible, seven harmonics, RFT M1, nine harmonics). Below, shape changes corresponding to the canonical axes are depicted. The open outline corresponds to the average shape; light grey outlines to the maximum values of the axes on the plot; dark grey outlines to exaggerated variations on the axes (mandible: scores = 10/–10 and M1: scores = 5/–5). On the lowermost upper mandible outline, abbreviations indicate the name of the processes: cor., coronoid; cond., condylar; ang., angular.

segregated without overlap on axes CA1 and CA2. Except for Corsica and the single M1 from Yeu, insular morphologies scatter outside the mainland range. Sicilian populations (that constitute a peculiar genetic clade related to the Italian one) are grouped towards positive CA2 values but still remain close to the Italian group. These two genetically related clusters are further differentiated along axis CA3 (11.9%, data not shown). Other insular populations are displaced towards negative CA1 values. The most divergent M1 samples on the first two canonical axes are Porquerolles and Port-Cros (along CA1), the single tooth from Marettimo (towards positive CA2), Noirmoutier and Oléron (towards negative CA2).

Reconstructed outlines indicate that molars with a straight labial side (on the left of the outline) and a pointed fore and back part are displaced on the positive side of the first axis, whereas molars with an undulating labial side and a prominent

posterior labial cusp are shifted to the negative side. The second axis opposes slender (positive CA2) to massive molars (negative CA2).

Interplay of the latitudinal gradient with insular divergence

A primary way of investigating the influence of geography on morphological differentiation is to plot the results of the canonical analyses as a function of latitude (Fig. 3a,c). Since insular effects might interfere with the latitudinal gradient, the existence of a linear relationship between the canonical axes and latitude was tested on mainland samples only. The gradient appeared to be expressed on axis CA2 for mandibles (linear correlation of group means with latitude, $N = 12$, CA1: $R = 0.190$, $P = 0.553$; CA2: $R = 0.788$, $P = 0.002$) and on
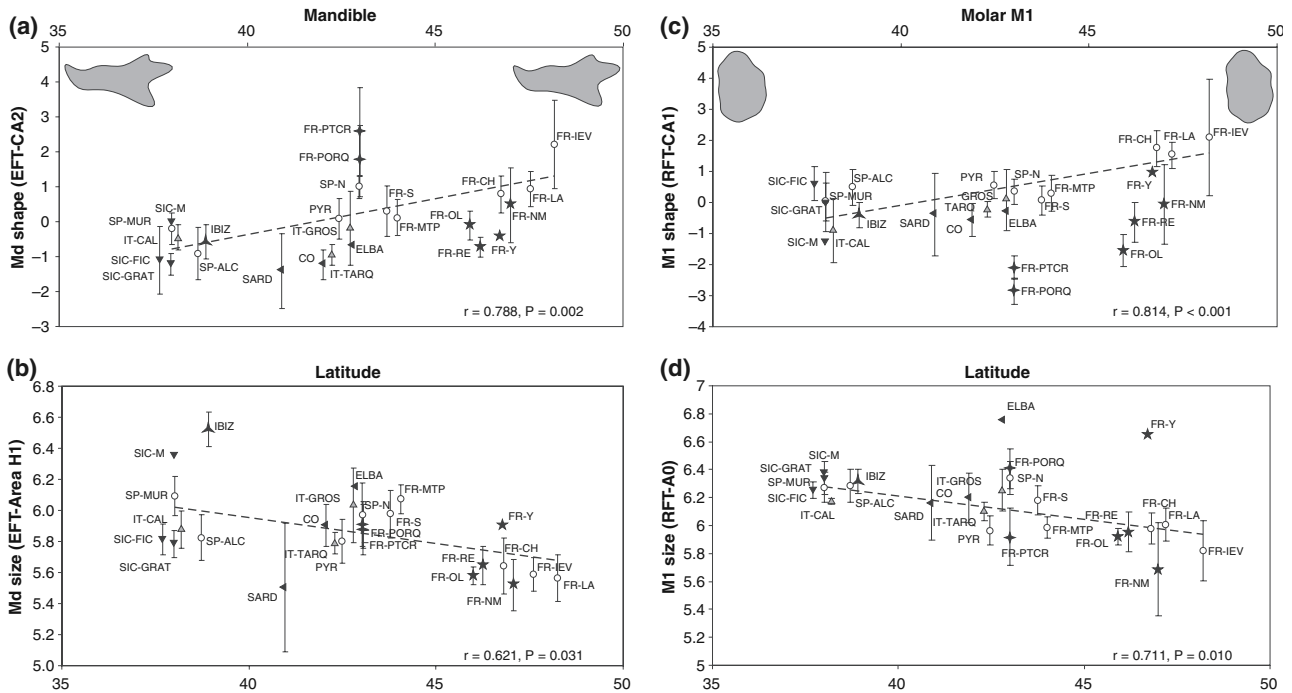
Figure 3 Shape and size variations of the mandible (a, b) and the M1 (c, d) with latitude. Shape is estimated by the second (mandible) and first (M1) canonical axes, size is estimated by the area of the first ellipse (EFT) for the mandible and the zero harmonic (RFT) for the M1. Symbols correspond to average values per geographical groups ± standard error (95%). Dotted line: regression of the mainland samples vs. latitude. R and P values are given on the graphs (regression of the group means on latitude, N = 12). The outlines visualize the corresponding shape change.

axis CA1 for molars (linear correlation of group means with latitude, N = 12, CA1: R = 0.449, P < 0.001; CA2: R = 0.020, P = 0.801).

Most insular populations match the latitudinal gradient for the mandible (Fig. 3a) and for the molar shape (Fig. 3c). However, in both cases, the few populations that depart from the overall gradient originate from islands. Sardinia, Porquerolles and Port Cros are the most obvious cases of departure of the mandible from the general gradient along axis CA2.

The latitudinal gradient is also expressed on molar shape (Fig. 3c). As with mandibles, some insular populations depart from the general trend. The most obvious cases are the Mediterranean French islands Porquerolles and Port-Cros, followed by the Atlantic French islands Oléron, Ré and Noirmoutier.

The size of the characters also varies with latitude and insularity (Fig. 3b,d). Mandible size on the mainland decreases towards higher latitudes (N = 12, R = 0.621, P = 0.031). Most of the island populations follow this trend as well. The population from Ibiza provides the only obvious case of insular gigantism based on mandible size.

The size of the molars for mainland populations is correlated in a similar way with latitude (N = 12, R = 0.711, P = 0.010). Teeth from Yeu and Elba, unfortunately both sampled by a single specimen, constitute departures from the overall trend.

Multivariate comparisons between shape, size, genetics and latitude

The canonical analyses of the Fourier coefficients showed that the pattern of morphological differentiation was the result of a complex interplay between the influences of latitude, insularity and genetic differences among clades. Furthermore, as size shows a latitudinal gradient, the shape gradient might be linked to an allometric effect, which would imply different causal effects. To disentangle these various factors, multivariate multiple regressions were undertaken using the Fourier coefficients as dependent variables.

Firstly, using mainland only and mainland vs. island populations, the existence of the latitudinal shape gradient was confirmed using multivariate multiple regressions of the Fourier coefficients on latitude (Table 2). In order to separate a possible allometric effect causing this shape gradient, FCs were regressed on size and the residuals were compared with latitude (Table 2). A significant latitudinal effect persisted after removing the size effect for both mandibles and molars.

Secondly, multivariate analyses of variance were applied to test for a systematic effect of insularity and genetic clades on mandible and molar shape (Table 3). The clade effect proved important on raw FCs, but weakened significantly after removing the latitudinal effect for the mandible. However, the clade effect remained highly significant for molars (Table 3). This result corroborates the pattern emerging from

Table 2 Multivariate regressions of the shape parameters with latitude and size

Character	Dependent variables	Independent variable	Samples	N	P
Mandible shape	EFT7–FC	Latitude	Mainland + islands	216	< 0.0001
Molar shape	RFT9–FC	Latitude	Mainland + islands	239	< 0.0001
Mandible shape	EFT7–FC	Latitude	Mainland	143	0.0001
Molar shape	RFT9–FC	Latitude	Mainland	158	< 0.0001
Mandible shape	EFT7–FC	Mandible size (area H1)	Mainland + islands	216	< 0.0001
	Residuals EFT7–FC/size	Latitude	Mainland + islands	216	0.0003
Molar shape	RFT9–FC	Molar size (A_0)	Mainland + islands	239	< 0.0001
	Residuals RFT9–FC/size	Latitude	Mainland + islands	239	0.0020

EFT, elliptic Fourier transform; RFT, radial Fourier transform; FC, Fourier coefficients; N, number of specimens considered; P, probability.

Table 3 MANOVAS on shape coefficients

Character	Dependent variables	Grouping variable	Samples	N	P
Mandible shape	EFT7–FC	Clade	Mainland	143	0.0001
	EFT7–FC/lat.	Residuals Clade	Mainland	143	0.0016
Molar shape	RFT9–FC	Clade	Mainland	158	< 0.0001
	Residuals RFT9–FC/lat.	Clade	Mainland	158	< 0.0001
Mandible shape	Residuals EFT7–FC/lat.	Insularity	Mainland + islands	216	0.1136
Molar shape	Residuals EFT7–FC/lat.	Insularity	Mainland + islands	239	< 0.0001

EFT, elliptic Fourier transform; RFT, radial Fourier transform; FC, Fourier coefficients; lat., latitude; N, number of specimens considered; P, probability.

the canonical analysis, suggesting a stronger genetic effect on molar shape rather than on mandible shape.

Finally, the existence of a typical insular signature was investigated, once the latitude effect was removed, by considering residuals of a multiple regression of the FCs on latitude (Table 3). No systematic morphological shift corresponding to an insular trend was detected for mandibles. This may be due to a random scatter of the insular morphologies around the mainland ones as shown on the canonical plane (Fig. 2a). However, such an 'insular trend' existed for the molars. Most island samples are clustered in the canonical plane (Fig. 2b) with the exception of Sicilian molars.

Comparing patterns of differentiation between mandibles and molars

Table 4 shows the correlation of average scores for the different size and shape parameters of the mandible and molars with both islands and the mainland. The correlation is weaker when considering insular samples alone (Fig. 4), especially with respect to shape differentiation. Overall, differentiation in size of mandibles and molars seems to be positively correlated (Fig. 4a; linear regression, $N = 12$, $R = 0.579$, $P = 0.048$). Wood mice from Ibiza constitute a departure from the common trend. However, shape differentiation of mandibles and molars seems to be uncoupled since the amount of insular divergence of both characters is not correlated ($N = 12$, $R = 0.184$, $P = 0.567$), even tending towards a negative regression if the outlying sample from Yeu is excluded ($N = 11$, $R = -0.357$, $P = 0.282$).

Identification of the causal factors of these patterns of differentiation is somewhat intractable as they are likely to mix latitude and various possible factors associated with insular conditions (Table 5). Physical parameters such as area, elevation and distance from the mainland can be considered as proxies for overall environmental conditions.

Table 4 Comparison between mandible and molar size and shape. Euclidean distances were computed for each variable (univariate estimates of size) or set of variables (FCs). Matrices of distances were then compared using a Mantel test. The coefficient of correlation R is given, as well as P = 1 (the probability than a random R is larger than the observed R). Group means are considered

Variable 1	Variable 2	Samples	N	R	P
M1 shape RFT9–FCs	Md shape EFT7–FCs	Mainland + islands	25	0.32735	0.0112
M1 size (A_0)	M1 shape RFT9–FCs	Mainland + islands	25	0.29266	0.0129
Md size (area H1)	Md shape EFT7–FCs	Mainland + islands	25	0.28993	0.0097
M1 size (A_0) (area H1)	Md size	Mainland + islands	25	0.22066	0.0268

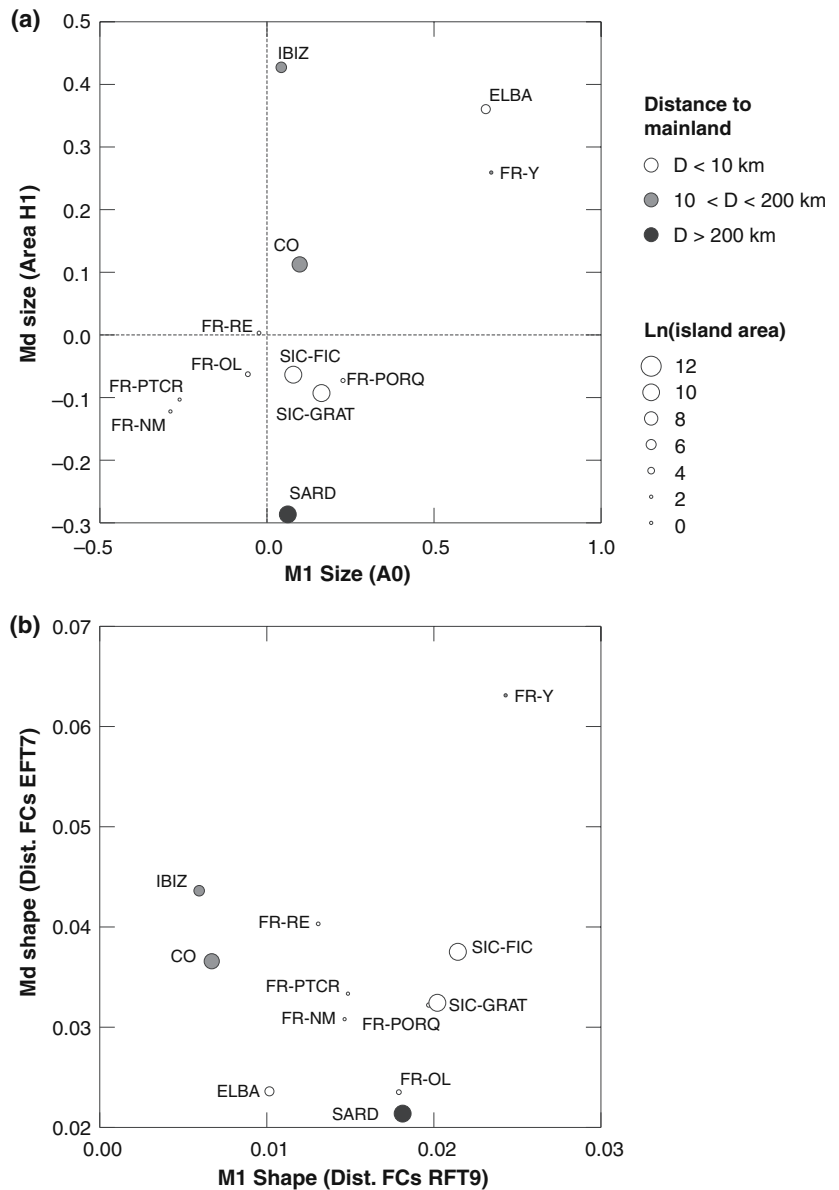N, initial number of samples involved in the calculation of the distances.

Figure 4 Insularity effect on size (a) and shape (b) of molar vs. mandible of the wood mouse. (a) The size divergence is estimated as the difference between the mean value of the size estimator (A_0 for molars and area H1 for the mandible) on one island and its closest mainland relative. (b) The divergence in shape is estimated as the Euclidean distance between the mean values of the FCs (RFT9 for the molar and EFT7 for the mandible) of one island and its closest mainland relative. The size of each symbol is proportional to the logarithm of the island area.

Ecological parameters such as competition and predation, however, are known to play an important role in island evolution. These parameters are difficult to assess reliably for all the islands, because predators and competitors may vary greatly from one island to another, and density is also a crucial factors that cannot be easily quantified. Quantitative comparisons were performed between morphological estimators and these various parameters of the islands, but comparisons with ecological factors should be considered with caution.

The size of M1 did not correlate with any investigated parameter (Table 6). The size of the mandible was related to island area, although in a nonlinear fashion. Mandibular size increased on islands of intermediate size (Ibiza, Corsica, Elba) and/or at intermediate distances to the mainland (Yeu, Corsica, Ibiza). In the case of Sardinia, the very large size of

the island may counterbalance its remoteness, possibly leading to a decrease in mandible size.

The amount of shape divergence of M1 was related to island area but was minimal on islands of intermediate size (Elba, Corsica, Ibiza). The shape of the mandible varied with the remoteness of the island. As in the case of mandible size, intermediate levels of remoteness (Yeu, Ibiza, Corsica) promoted divergence of mandible shape. Finally, the number of competitors on the island was also related to divergence of mandible shape, with maximal divergence for three competitors (Yeu, Ibiza, Ré).

DISCUSSION

The most striking result from this study is the discrepancy between a latitudinal mainland gradient in size and shape of

Table 5 Characteristics of the islands, physical (area, maximum altitude, distance to continent) and ecological (number of competitors of the wood mice and qualitative estimation of the competition pressure, including density effects). The amount of morphological differentiation is estimated as the difference in size (estimated by A_0 for the M1 and area H1 for the mandible) or as Euclidean distances on FCs for estimating shape divergence, between the island value and the one of the closest mainland population of the same genetic clade. Data from Michaux (1996), Michaux *et al.* (1996b, 2002a,b) and Vigne (1999)

Island	Island area (km²)	No. of competitors	Competition (low/high)	Dist. to mainland (km)	Altitude (m)	Age of colonization (years)	Differential island/mainland	M1 size (A_0)	Md size (area H1)	M1 shape [d(RFT9)]	Md shape [d(EFT7)]
Oléron	17.5	4	0	Bridge	Low	> 5000	OL-FRCH	−0.057	−0.063	0.018	0.024
Ré	9.3	3	0	4	Low	> 5000	RE-FRCH	−0.024	0.003	0.013	0.040
Yeu	2.2	3	0	20	Low	> 5000	YEU-FRCH	0.671	0.259	0.024	0.063
Noirmoutiers	4.9	4	0	Pathway	Low	> 5000	NM-FRCH	−0.289	−0.122	0.015	0.031
Porquerolles	12.5	2	1	3	142	> 5000	PORQ-FRS	0.228	−0.073	0.020	0.032
Port Cros	6.4	2	1	9.2	196	> 5000	PTCR-FRS	−0.261	−0.103	0.015	0.033
Corsica	8722	4	0	82	2710	5000	CO-ITTARQ	0.098	0.113	0.007	0.037
Sardinia	23,833	4	0	220	1834	5000	SARD-ITTARQ	0.062	−0.286	0.018	0.021
Elba	224	4	1	8	1019	> 5000	E-ITTARQ	0.655	0.361	0.010	0.024
Sicily	25,460	4	0	2	1950	> 5000	SICFIC-ITCAL	0.079	−0.063	0.021	0.038
							SICGRAT-ITCAL	0.163	−0.093	0.020	0.032
Ibiza	541	3	0	180	475	5000	IB-SPMUR	0.043	0.427	0.006	0.044

Table 6 Relationship between size and shape divergence and insular characteristics (cf. Table 5). The dependent variable was size difference or shape distance for the M1 and the mandible; grouping variable was derived from the insular characteristics in Table 5. The differences between the categories were tested using a Kruskall–Wallis test, a nonparametric analogue of a one-way analysis of variance. Significant effects (5%) are in bold. Some insular characteristics were discontinuous (number of competitors, competition level, age of colonization). Others were continuous and had to be categorised: (1) island area, three categories (< 20 km², 20 < area < 20,000 km², > 20,000 km²), (2) distance to mainland, three categories (< 10 km, 10 km < D < 200 km, > 200 km), (3) altitude, three categories (< 200 m, 200 < A < 1500 m, > 1500 m)

		Size		Shape	
Type of parameter	Parameter	M1 size (A_0)	Md size (area H1)	M1 shape [d(RFT9)]	Md shape [d(EFT7)]
Physical	Island area	0.520	**0.048**	**0.027**	0.784
	Distance to continent	0.596	**0.048**	0.677	**0.048**
	Altitude	0.527	0.093	0.149	0.938
Historical	Age of colonization	0.683	0.683	0.102	1.000
Ecological	Number of competitors	0.952	0.127	0.853	**0.040**
	Competition level	0.518	0.926	0.782	0.405

both mandibles and molars as opposed to various individual patterns of differentiation on islands.

The possible causes of the clinal gradients are discussed first, as they provide a background to consider the factors influencing insular differentiation.

North–south size gradient of mandibles and molars on mainland

Geographical variations in body size of the wood mouse, *Apodemus sylvaticus*, have been reported for over 50 years (Ursin, 1956; Saint Girons, 1966; Niethammer, 1969; Kahmann & Niethammer, 1971; Alcàntara, 1991). Our results using mandible and molar size as proxies for body size support the increase in body size from the north to the south contrary

to Bergmann's rule (Bergmann, 1847) within the considered latitudinal range.

The northward decrease in body size has been interpreted in many ways. Firstly, *A. sylvaticus* displays a distribution area shifted towards warmer environments compared with the related *Apodemus flavicollis*. Accordingly, its relative fitness is thought to decrease towards colder environments, leading to a progressive decrease in size (Niethammer, 1969). Another, not mutually exclusive, interpretation relates to the size variations of *A. sylvaticus* resulting from competition pressure exerted by *A. flavicollis* (Alcàntara, 1991). The negative effect of *A. flavicollis* on *A. sylvaticus* has been documented in Sweden (Hoffmeyer & Hansson, 1974). In such environments, resource partitioning occurs that could favour a small size for *A. sylvaticus* (Angelstam *et al.*, 1987). As the competition

pressure of the colder-adapted *A. flavicollis* is likely to decrease towards warmer environments (Alcàntara, 1991), such inter-actions might contribute to the latitudinal size pattern observed. Character displacement in *A. sylvaticus* caused by the elimination of *A. flavicollis* supports this argument, and has been reported in the fossil record (Tchernov, 1979). The same study, however, provided evidence of a size increase of the wood mouse during cold intervals, in agreement with Bergmann's rule. The validity of these observations may be questioned as the discrimination of teeth from *A. sylvaticus* and *A. flavicollis* is extremely difficult. Tchernov's (1979) conflicting results can be reconciled by the results of a study of the latitudinal variation of the wood mouse across Europe (Renaud & Michaux, 2003), which included more data than the previous study of Alcàntara (1991). Size variations over Europe are nonlinear, showing an increase in size towards both the northern and southern range of the distribution area, with minimal size at around 45–50° N (Renaud & Michaux, 2003). Such a pattern suggests the balancing effects of two factors: Bergmann's effect to the north, and increased relative fitness of *A. sylvaticus* towards the South. The degree of seasonality, correlated in the Western Palearctic with longitude, may further interfere with latitudinal size variations (Meiri *et al.*, 2005). However, our sampling was restricted to south-western Europe and should limit the impact of such an effect.

The results of Renaud & Michaux (2003) are based on mandible size. The extent to which results based on the size of a character can be extrapolated to the size of other morpho-logical traits, and be considered as estimators of body size, has yet to be investigated more fully. Within a population of wood mice, mandible size appears to be correlated with body length whereas molar size is not (Renaud, 2005). The present study, however, shows that on the mainland the mandibles and molars provide very congruent patterns of size variations. These results support the idea that above the intrapopulation level, molar size provides a reliable estimator of the general size of the animals and this is corroborated by data obtained at a larger taxonomic scale (Legendre, 1989).

Congruent shape variation of mandibles and molars: clinal adaptive response on mainland

Shape variation with geography has received less attention than variation of size with geography. Nevertheless, a growing body of evidence suggests that clinal changes also affect cranial (Fadda & Corti, 2001), mandibular (Duarte *et al.*, 2000; Renaud & Millien, 2001) and dental (Renaud, 1999) traits in rodents. Such clinal variation in shape may be related to an allometric variation with size. In this study, however, this hypothesis may be discarded. Even after removing a possible allometric effect, a correlation between shape and latitude remains (Table 2). Specific causal factors for shape variation should thus exist apart from factors related to size differences.

For mandibles and teeth, clinal variations can be interpreted as relating to changes in the feeding behaviour, as these two characters are heavily involved in the mastication process. The

latitudinal widening of the mandible, associated with a more pronounced development of the coronoid and angular processes, previously observed on *A. sylvaticus* and interpreted as a possible response to a clinal change in average diet and behaviour (Renaud & Michaux, 2003), is confirmed by this work (Fig. 3). The consistent latitudinal variation displayed by the first molar (increased width towards the south) supports the adaptive interpretation. At a larger taxonomic scale, a broadening of the first upper molar has been related to a more herbivorous diet in murine rodents (Renaud & Michaux, 2004; Renaud *et al.*, 2005). The broadening of the molar increases the surface of occlusion between upper and lower cheek teeth, favouring the consumption of more abrasive food, such as grass. The pattern of morphological differentiation observed among distantly related genera might thus be displayed at the intraspecific level as well, albeit in a reduced way.

Differential response of mandibles and molars to genetic divergence

As the western European wood mouse populations are the results of post-glacial recolonization from the Iberic refugial zone (Michaux *et al.*, 2003), our work suggests that the formation of the size and shape gradients has occurred in less than *c.* 16,000 years. Building the genetic divergence of *c.* 5% between the western European and Italo-Balkanic clades was a considerably longer process (Michaux *et al.*, 1996a, 1998b, 2003). This divergence is interpreted as the consequence of repeated isolation on both sides of the alpine barrier during Quaternary glacial cycles. The *c.* 1 Myr vicariant process did not cause any important difference in mandible shape (Renaud & Michaux, 2003; this study), although the European environmental gradient caused a morphological differentiation of the mandible on a much shorter time span. The mainland samples from both clades, however, can be separated based on molar shape. The molar seems thus to be more sensitive to genetic isolation than the mandible. These results are in agreement with observations on northern German wood mouse populations (Renaud, 2005) suggesting that tooth shape would vary according to the amount of gene flow whereas mandible shape is related to local selective pressures. Similar results have also been found in other organisms such as fishes (Linde *et al.*, 2004). The sensitivity of molar shape to genetic isolation is further supported by the differentiation of the molars from Sicily. Sicilian wood mice belong to a distinct subclade related to the Italian stock, which diverged at least 800,000 years ago (Michaux *et al.*, 1998b; Libois *et al.*, 2001).

Based on the few genetic studies available, mandibles and molars do not differ greatly in the number of genes involved. Quantitative trait locus (QTL) mapping on inbred strains of mice led to the identification of 25 QTL for the mandible shape (Klingenberg *et al.*, 2001) and 18 QTL for the molar shape (Workman *et al.*, 2002). Twelve of these QTL may be common between molar and mandible shape (Workman *et al.*, 2002). The position of the cusps is determined early during development by a patterning cascade (Jernvall &

Thesleff, 2000) and significant changes in the final shape of the tooth can be caused by slight changes early in development (Jernvall, 2000; Salazar-Ciudad & Jernvall, 2002; Kangas *et al.*, 2004). This mode of development makes a single developmental module with interdependence of cusps. Furthermore, once the molars are erupted they should only vary with wear (Renaud, 2005). The mandible, however, seems to consist of at least two modules corresponding to the alveolar region and the ascending ramus (Atchley *et al.*, 1992; Mezey *et al.*, 2000) and the shape of the mandible is influenced by bone remodelling occurring due to an interaction between muscular functioning and mandible growth (Lightfoot & German, 1998; Bresin *et al.*, 1999). These factors may make mandible shape more prone to change in response to environmental factors than molar shape.

These results suggest that molars, which are significantly more abundant in the fossil record than fragile mandibles, can provide valuable information on the phylogenetic background of populations even at an intraspecific scale. However, the assumption of tracking the phylogenetic background of populations using morphometric analyses of teeth may be challenged. Firstly, given recent developmental data, slight changes early in development can induce major change in the final shape of the tooth. Second, different genes or changes in gene expression can produce similar morphologies (Cheverud *et al.*, 1997; Klingenberg *et al.*, 2001; Klingenberg & Leamy, 2001) that increase the likelihood of convergent evolution. Third, dental characters may experience rapid changes during periods of environmental changes (Kangas *et al.*, 2004; Renaud *et al.*, 2005). However, our results, together with recent data on the intraspecific variation of the mouse tooth (Cucchi, 2005), support the molar as a marker of genetic divergence, at least for relatively recent events.

Mosaic evolution of mandible and molar shape on islands

The pattern observed on the mainland suggests that molars should differentiate according to patterns of gene flow. The response time for molars should be slower than for mandibles, which could vary rapidly due to various environmental factors. Insular populations are prone both to breakdown of gene flow and to important changes in ecological factors compared with the mainland populations. Island populations, therefore, should display more intricate patterns than populations on the mainland, which have an integrated mandible–molar latitudinal signal.

Mandibles tend to diverge more on islands of intermediate remoteness (Yeu, Corsica, Ibiza) and intermediate competition pressure (Yeu, Ré, Ibiza). Divergence on Ibiza may be influenced by allometric divergence due to the very large size of the animals. The island's remoteness and middle level of competition pressure may cause environmental conditions distinct from the mainland, such as a widening of the wood mouse niche permitting a more varied diet (Orsini & Cheylan, 1988), therefore causing mandible shape to change.

Conversely, molar differentiation is minimal on islands of intermediate size. This effect is due to the differentiation of the Sicilian molars. Despite the large size of this island, it is occupied by a specific genetic clade (Michaux *et al.*, 1998b), and the molar difference is linked to a genetic divergence. Additionally, molar differentiation is important on small islands such as Porquerolles (12.5 km^2), Port-Cros (6.4 km^2), Oléron (17.5 km^2), Noirmoutier (4.9 km^2) and Ré (9.3 km^2), where a reduced population size seems to trigger the process. Notably, the existence of an artificial bridge linking an island to the mainland does not seem to increase gene flow enough to homogenize island and mainland populations, as shown by the results from the islands of Oléron and Noirmoutier. This can be explained partly by research showing that resident populations of rodents on small islands limit introgression from mainland genes by an increased aggressiveness towards immigrants (Granjon & Cheylan, 1989, 1990). Accordingly, genetic data suggest that the proximity to the mainland does not impede significant genetic divergence on smaller islands (Michaux *et al.*, 2002b). An adaptive component for such molar divergence on small islands is suggested by a common trend of insular molars to be wider, interpreted on the mainland as allowing the consumption of more resistant food. This response might be enhanced by a long history of isolation of a reduced population favouring genetic divergence.

A differential response of mandibles and molars to (1) ecological factors and (2) genetic differentiation is supported by the pattern of mosaic evolution observed on the islands we studied. These data corroborate the results based on mainland patterns of differentiation.

Insular effect on size: mosaic variation, gigantism and macrodonty

Fewer genes are apparently required to control the size of mandibles and molars than the shape (Workman *et al.*, 2002). Size, however, is known to vary with numerous factors (Nevo, 1989; Ganem *et al.*, 1995; Dayan & Simberloff, 1998; Kingsolver & Pfennig, 2004) and the large influence of the endocrine system on size may explain the lability of this character (Bünger & Hill, 1999; Dupont & Holzenberger, 2003). The discrepancies in the response of molar and mandible shape to various factors may therefore not be valid for size. Yet, our results point to distinct factors influencing molar and mandible size (Table 6).

Size is, however, a commonly studied feature, and understanding the factors underlying its differentiation is of general interest. A well-documented effect of insularity on small mammals is a trend towards larger size (Foster, 1964; Lomolino, 1985, 2005). This effect has been described for body size, and it is usually interpreted as due to a weakening of interspecific competition (Angerbjörn, 1986; Dayan & Simberloff, 1998) or predation (Michaux *et al.*, 2002b), allowing the animals to converge towards an optimal energetic size (Damuth, 1993). The consequent change in the niche

width of insular rodents leads to the modification of many life-history traits compared with mainland relatives (Libois *et al.*, 1993; Adler & Levins, 1994). Some authors, however, noted that size varied differently depending on the character considered, and that the feeding apparatus (namely the length of the molar row) tended to be more heavily modified than other body or cranial measurements (Orsini & Cheylan, 1988; Vigne *et al.*, 1993). This discrepancy points to the importance of mosaic evolution for size as well as shape, which should lead to a complex expression of the insular syndrome: larger body size (gigantism) and/or larger teeth (macrodonty).

In this study, only the wood mouse from Ibiza constitutes a clear case of insular gigantism (Michaux *et al.*, 2002b), associated with extremely large mandibles. The corresponding molars, however, display a size coherent with the mainland latitudinal gradient. In general, increase in mandible size seemed to be prevalent on islands of intermediate size and remoteness.

Ibiza has been colonized recently (*c.* 5000 years) by the wood mouse, probably as an anthropogenic introduction (Vigne & Alcover, 1985; Vigne, 1999). The short time span has, nonetheless been sufficient for the mandible and body size to achieve extreme values. However, colonization occurred at approximately at the same time on Corsica and Sardinia (Vigne & Alcover, 1985; Vigne, 1999), and the wood mouse does not display any significant size increase on these islands. This discrepancy may be related to three factors. Firstly, Ibiza is much more isolated from the mainland than the other islands we analysed. Second, Corsica and Sardinia are much larger than Ibiza and the large population size could damper divergence. Third, although numbers of competitor species are not very different (Table 2), Corsica and Sardinia display a very high population density of the two major competitor species of the wood mouse, the black rat, *Rattus rattus* (Linnaeus 1758), and the domestic mouse, *Mus musculus* (Linnaeus 1758). Such an important competition pressure restricts the wood mouse to elevated areas in Corsica, whereas the domestic mouse and the black rat dominate in the plains (Granjon & Cheylan, 1988). Ecological release would thus be important on Ibiza, favouring larger body size, and therefore larger mandible size, but not on Corsica and Sardinia.

The reverse case (i.e. a molar larger than expected), is displayed on Elba, Yeu and to a lesser extent on Porquerolles. Published data indicate a larger body size for the wood mice on these islands (Elba: Kahmann & Niethammer, 1971; Porquerolles: Libois & Fons, 1990), although a shorter mandible is evident on Porquerolles (Libois & Fons, 1990). In the three cases, our study provided scant evidence of a mandible larger than would be expected according to the latitudinal gradient (Fig. 3). We suggest that these populations exhibit a macrodont rather than a gigantism trend. These islands were land-bridged during the low sea-level of the last glacial cycle and this might have led to strong similarities of the initial gene pool on the islands and continent, with divergence occurring thereafter by vicariance. Such a process has been invoked to explain

larger body size on islands that were refugial during cold periods (Millien & Damuth, 2004). In our study, however, such an effect is unlikely as it should have led to a smaller body size of the island population, especially given the direction of the latitudinal gradient. Notwithstanding, time since vicariance was sufficient for molar divergence to accumulate. Drift may have enhanced the speed of divergence, owing to the reduced population size on smaller islands. On these islands, however, the wood mouse currently experiences severe competition due to high population densities of rats and domestic mice. This ecological pressure is recent compared to the putative old isolation, and it is related to the historical intensification of human activities (Vigne & Valladas, 1996).

Provided that the mandible can respond more rapidly than the molar to environmental changes, the surprising pattern of large teeth associated with mandibles close to the expected size might be the result of an ongoing process of adjustment to new ecological conditions, where: (1) after the last glacial maximum wood mice evolved under released competition pressure leading to larger body size, larger mandibles and eventually larger molars; (2) the introduction of successful competitors like the rat and the domestic mouse triggered a decrease in size of the competitively inferior wood mouse (Yom-Tov *et al.*, 1999); and/or (3) body and mandible size would already be back close to the mainland size, although molar size, being slower to evolve, lagged and displayed larger sizes than expected. This interpretation remains hypothetical and needs further investigation. However, our results point consistently to mosaic evolution as a result of the different response time of distinct characters. In this instance, historical processes are key to interpreting the modern situation. The mosaic pattern of size differentiation can in any event explain discrepancies between measures of size on the same rodent populations based on different characters such as mandibles and incisors (Renaud & Millien, 2001; Millien, 2004), without pointing to a given character as systematically the best proxy for body size.

Adaptation or random effects: what drives the insular evolution?

The pattern of insular variation of size and shape on various characters appears to be the consequence of a complex interplay of factors including ecology, physical environment and genetic determinism. Morphological differentiation on islands might thus appear as a collection of distinctive cases, and trying to decipher general rules in this complex pattern might lead only to 'just-so' stories, as highlighted by Berry (1996). The number of replicates included in this study allows us to reach a number of firm conclusions:

Firstly, the integrated latitudinal gradient observed on the mainland contradicts insular mosaic evolution. It illustrates that discrepant evolution between characters is a characteristic of insular evolution. On the mainland, gene flow can counteract adaptation to local conditions because it limits the changes of gene frequency favoured by selection

(Lenormand, 2002). However, genetic mixing over Europe is probably not important enough to prevent any adaptive latitudinal differences in gene frequencies (Davis & Shaw, 2001) and mitochondrial DNA markers fail to reveal any significant geographical structure within the western European clade (Michaux *et al.*, 2003). Gene flow might nevertheless buffer small-scale environmental variations and favour a clinal pattern on the mainland.

Second, the breakdown of gene flow on islands should leave the populations free to track local environmental conditions. Alternatively, the importance of contingent effects is enhanced on islands, due to the founder effect and subsequent drift in populations of reduced effective size. Founder effects should be more important on remote islands that are difficult to colonize, whereas drift should be favoured on small islands. Despite these random effects, most of the post-colonization changes on islands are attributed to adaptation (Berry, 1996; Losos *et al.*, 1998; Pergams & Ashley, 2001). Our results support both the importance of contingency and adaptation in the patterns of morphological differentiation. The latter corresponds to common trends shared by independent insular populations whereas the former will be expressed as shape features unique to one island. Such patterns are exhibited in multivariate space on axes subsequent to the first ones, displaying the major trends of latitude and/or adaptive insular effect (see also Renaud & Millien, 2001; Renaud & Michaux, 2003). They are thus not mutually exclusive of adaptive trends and their importance in the pattern of the morphological differentiation will depend on the relative impact of the other factors.

Lastly, the breakdown of gene flow on islands allows adaptive changes to occur very rapidly compared to the accumulation of genetic divergence (Vitt *et al.*, 1997; Brehm *et al.*, 2001). The importance of adaptation is supported in this study by common trends emerging from the different insular populations that share similar molar morphological features. Nevertheless, the complexity of the factors involved constitutes a limit to any predictive scenario. The pattern of morphological divergence will rely on the character and the specific environment (e.g. the island), with additional influence from competition, predation, degree of isolation, population size and vegetation. We suggest that a combined morphological, developmental and genetic approach can shed light on the constraints on responses of organisms to natural selection.

ACKNOWLEDGEMENTS

REFERENCES

Adler, G.H. & Levins, R. (1994) The island syndrome in rodent populations. *The Quarterly Review of Biology*, **69**, 473–490.

Alcàntara, M. (1991) Geographical variation in body size of the Wood Mouse *Apodemus sylvaticus* L. *Mammal Review*, **21**, 143–150.

Angelstam, P., Hansson, L. & Phersson, S. (1987) Distribution borders of field mice *Apodemus*: the importance of seed abundance and landscape composition. *Oikos*, **50**, 123–130.

Angerbjörn, A. (1986) Gigantism in island populations of wood mice (*Apodemus*) in Europe. *Oikos*, **47**, 47–56.

Atchley, W.R., Cowley, D.E., Vogl, C. & McLellan, T. (1992) Evolutionary divergence, shape change, and genetic correlation structure in the rodent mandible. *Systematic Biology*, **41**, 196–221.

Bergmann, C. (1847) Über die Verhältnisse der Wärmeökonomie der Thiere zu ihrer Grösse. *Göttinger Studien*, **3**, 595–708.

Berry, R.J. (1973) Chance and change in British long-tailed field mice (*Apodemus sylvaticus*). *Journal of Zoology, London*, **170**, 351–366.

Berry, R.J. (1996) Small mammal differentiation on islands. *Philosophical Transactions of the Royal Society, London B*, **351**, 753–764.

Brehm, A., Khadem, M., Jesus, J., Andrade, P. & Vicente, L. (2001) Lack of congruence between morphometric evolution and genetic differentiation suggests a recent dispersal and local habitat adaptation of the Madeiran lizard *Lacerta dugesii*. *Genetics Selection Evolution*, **33**, 671–685.

Bresin, A., Kiliardis, S. & Strid, K.-G. (1999) Effect of masticatory function on the internal bone structure in the mandible of the growing rat. *European Journal of Oral Sciences*, **107**, 35–44.

Bünger, L. & Hill, W. (1999) Role of growth hormone in the genetic change of mice divergently selected for body weight and fatness. *Genetical Research, Cambridge*, **74**, 351–360.

Cheverud, J.M., Routman, E.J. & Irschick, D.J. (1997) Pleiotropic effects of individual gene loci on mandibular morphology. *Evolution*, **51**, 2006–2016.

Cucchi, T. (2005) Le commensalisme de la souris et les premières sociétés néoliyhiques méditerranéennes. Thèse, Muséum National d'Histoire Naturelle, Paris.

Damuth, J. (1993) Cope's rule, the island rule and the scaling of mammalian population density. *Nature*, **365**, 748–750.

Davis, M.B. & Shaw, R.G. (2001) Range shift and adaptive responses to Quaternary climate change. *Science*, **292**, 673–679.

Dayan, T. & Simberloff, D. (1998) Size patterns among competitors: ecological character displacement and character release in mammals, with special reference to island populations. *Mammal Review*, **28**, 99–124.

Duarte, L.C., Monteiro, L.R., Von Zuben, F.J. & Dos Reis, S.F. (2000) Variation in mandible shape in *Thrichomys apereoides* (Mammalia: Rodentia): geometric analysis of a

complex morphological structure. *Systematic Biology*, **49**, 563–578.

Dupont, J. & Holzenberger, M. (2003) Biology of insulin-like growth factors in development. *Birth Defects Research (Part C)*, **69**, 257–271.

Fadda, C. & Corti, M. (2001) Three-dimensional geometric morphometrics of *Arvicanthis*: implications for systematics and taxonomy. *Journal of Zoological Systematics and Evolutionary Research*, **39**, 235–245.

Ferson, S., Rohlf, F.J. & Koehn, R.K. (1985) Measuring shape variation of two-dimensional outlines. *Systematic Zoology*, **34**, 59–68.

Foster, J.B. (1964) The evolution of mammals on islands. *Nature*, **202**, 234–235.

Ganem, G., Granjon, L., Ba, K. & Duplantier, J.-M. (1995) Body size variability and water balance: a comparison between mainland and island populations of *Mastomys huberti* (Rodentia: Muridae) in Senegal. *Experientia*, **51**, 402–410.

Goüy de Bellocq, J., Sara, M., Casanova, J.C., Feliu, C. & Morand, S. (2003) A comparison of the structure of helminth communities in the woodmouse, *Apodemus sylvaticus*, on islands of the western Mediterranean and continental Europe. *Parasitology Research*, **90**, 64–70.

Granjon, L. & Cheylan, G. (1988) Mécanismes de coexistence dans une guilde de muridés insulaire (*Rattus rattus* L., *Apodemus sylvaticus* L. et *Mus musculus domesticus* Rutty) en Corse: conséquences évolutives. *Zeitschrift für Säugetierkunde*, **53**, 301–316.

Granjon, L. & Cheylan, G. (1989) Le sort des rats noirs (*Rattus rattus*) introduits sur une île, révélé par radio-tracking. *Comptes Rendus de l'Académie des Sciences de Paris, série III*, **309**, 571–575.

Granjon, L. & Cheylan, G. (1990) Adaptations comportementales des rats noirs *Rattus rattus* des îles ouest-méditerranénnes. *Vie Milieu*, **40**, 189–195.

Hoffmeyer, I. & Hansson, L. (1974) Variability in number and distribution of *Apodemus flavicollis* (Melch.) and *A. sylvaticus* (L.) in South Sweden. *Zeitschrift für Säugetierkunde*, **39**, 15–23.

Jernvall, J. (2000) Linking development with generation of novelty in mammalian teeth. *Proceedings of the National Academy of Sciences of the United States of America*, **97**, 2641–2645.

Jernvall, J. & Thesleff, I. (2000) Reiterative signaling and patterning during mammalian tooth morphogenesis. *Mechanisms of Development*, **92**, 19–29.

Kahmann, H. & Niethammer, J. (1971) Die Waldmaus (*Apodemus*) von der Insel Elba. *Senckenbergiana Biologica*, **52**, 381–392.

Kangas, A.T., Evans, A.R., Thesleff, I. & Jernvall, J. (2004) Nonindependence of mammalian dental characters. *Nature*, **432**, 211–214.

Kingsolver, J.G. & Pfennig, D.W. (2004) Individual-level selection as a cause of Cope's rule of phyletic size increase. *Evolution*, **58**, 1608–1612.

Klingenberg, C.P. & Leamy, L.J. (2001) Quantitative genetics of geometric shape in the mouse mandible. *Evolution*, **55**, 2342–2352.

Klingenberg, C.P., Leamy, L.J., Routman, E.J. & Cheverud, J.M. (2001) Genetic architecture of mandible shape in mice: effects of quantitative trait loci analyzed by geometric morphometrics. *Genetics*, **157**, 785–802.

Kuhl, F.P. & Giardina, C.R. (1982) Elliptic Fourier features of a closed contour. *Computer Graphics and Image Processing*, **18**, 259–278.

Legendre, S. (1989) Les communautés de mammifères du Paléocène (Eocène supérieur et Oligocène) d'Europe occidentale: structures, milieu et évolution. *Münchner Geowissenschaftliche Abhandlungen, Reihe A, Geologie und Paläontologie*, **16**, 1–110.

Lenormand, T. (2002) Gene flow and the limits to natural selection. *Trends in Ecology and Evolution*, **17**, 183–189.

Libois, R. & Fons, R. (1990) Le mulot des Iles d'Hyères: un cas de gigantisme insulaire. *Vie et Milieu*, **40**, 217–222.

Libois, R., Fons, R. & Bordenave, D. (1993) Mediterranean small mammals and insular syndrome: biometrical study of the long-tailed field mouse (*Apodemus sylvaticus*) (Rodentia-Muridae) of Corsica. *Bonner Zoologische Beiträge*, **44**, 147–163.

Libois, R., Michaux, J.R., Ramalhinho, M.G., Maurois, C. & Sarà, M. (2001) On the origin and systematics of the northern African wood mouse (*Apodemus sylvaticus*) populations: a comparative study of mtDNA restriction patterns. *Canadian Journal of Zoology*, **79**, 1503–1511.

Lightfoot, P.S. & German, R.Z. (1998) The effects of muscular distrophy on craniofacial growth in mice: a study of heterochrony and ontogenetic allometry. *Journal of Morphology*, **235**, 1–6.

Linde, M., Palmer, M. & Gómez-Zurita, J. (2004) Differential correlates of diet and phylogeny on the shape of the premaxilla and anterior tooth in sparid fishes (Perciformes: Sparidae). *Journal of Evolutionary Biology*, **17**, 941–952.

Lomolino, M.V. (1985) Body size of mammals on islands: the island rule reexamined. *The American Naturalist*, **125**, 310–316.

Lomolino, M.V. (2005) Body size evolution in insular vertebrates: generality of the island rule. *Journal of Biogeography*, **32**, 1683–1699.

Losos, J.B., Jackman, T.R., Larson, A., de Queiroz, K. & Rodríguez-Schettino, L. (1998) Contingency and determinism in replicated adaptive radiations of island lizards. *Science*, **279**, 2115–2118.

Meiri, S., Dayan, T. & Simberloff, D. (2005) Biogeographical patterns in the Western Palearctic: the fasting-endurance hypothesis and the status of Murphy's rule. *Journal of Biogeography*, **32**, 369–375.

Mezey, J.G., Cheverud, J.M. & Wagner, G.P. (2000) Is the genotype–phenotype map modular? A statistical approach using mouse quantitative trait loci data. *Genetics*, **156**, 305–311.

Michaux, J.R. (1996) *Biogéographie du mulot sylvestre (Apodemus sylvaticus) dans le bassin méditerranéen occidental: étude génétique de l'origine des peuplements et microévolution en milieu insulaire.* Université de Liège, Liège.

Michaux, J. & Pasquier, L. (1974) Dynamique des populations de mulots (Rodentia, *Apodemus*) en Europe durant le Quaternaire. Premières données. *Bulletin de la Société Géologique de France*, **7**, 431–439.

Michaux, J.R., Filipucci, M.-G., Libois, R., Fons, R. & Matagnes, R.F. (1996a) Biogeography and taxonomy of *Apodemus sylvaticus* (the woodmouse) in the Tyrrhenian region: enzymatic variations and mithochondrial DNA restriction pattern analysis. *Heredity*, **76**, 267–277.

Michaux, J.R., Libois, R. & Fons, R. (1996b) Différenciation génétique et morphologique du mulot, *Apodemus sylvaticus*, dans le bassin Méditerranéen occidental. *Milieu*, **46**, 193–203.

Michaux, J.R., Libois, R., Ramalhinho, M.G. & Maurois, C. (1998a) On the mtDNA restriction patterns variation of the Iberian wood mouse (*Apodemus sylvaticus*). Comparison with other west Mediterranean populations. *Hereditas*, **129**, 187–194.

Michaux, J.R., Sara, M., Libois, R. & Matagne, R. (1998b) Is the woodmouse (*Apodemus sylvaticus*) of Sicily a distinct species? *Belgian Journal of Zoology*, **128**, 211–214.

Michaux, J.R., Chevret, P., Filipucci, M.-G. & Macholan, M. (2002a) Phylogeny of the genus *Apodemus* with a special emphasis on the subgenus *Sylvaemus* using the nuclear IRBP gene and two mitochondrial markers: cytochrome *b* and 12S rRNA. *Molecular Phylogenetics and Evolution*, **23**, 123–136.

Michaux, J.R., Goüy de Bellocq, J., Sara, M. & Morand, S. (2002b) Body size increase in rodent populations: a role for predators? *Global Ecology and Biogeography*, **11**, 427–436.

Michaux, J.R., Magnanou, E., Paradis, E., Nieberding, C. & Libois, R. (2003) Mitochondrial phylogeography of the woodmouse (*Apodemus sylvaticus*) in the Western Palearctic region. *Molecular Ecology*, **12**, 685–697.

Millien, V. (2004) Relative effects of climate change, isolation and competition on body-size evolution in the Japanese field mouse, *Apodemus argenteus*. *Journal of Biogeography*, **31**, 1267–1276.

Millien, V. & Damuth, J. (2004) Climate change and size evolution in an island rodent species: new perspectives on the island rule. *Evolution*, **58**, 1353–1360.

Monti, L., Baylac, M. & Lalanne-Cassou, B. (2001) Elliptic Fourier analysis of the form of genitalia in two *Spodoptera* species and their hybrids (Lepidoptera: Noctuidae). *Biological Journal of the Linnean Society*, **72**, 391–400.

Nevo, E. (1989) Natural selection of body size differentiation in Spiny mice, *Acomys*. *Zeitschrift für Säugetierkunde*, **54**, 81–99.

Niethammer, J. (1969) Zur Frage der Introgression bei den Waldmäusen *Apodemus sylvaticus* and *A. flavicollis* (Mammalia, Rodentia). *Zeitschrift für zoologische Systematik und Evolutionsforschung*, **7**, 77–156.

Orsini, P. & Cheylan, G. (1988) Les rongeurs de Corse: modifications de taille en relation avec l'isolement en milieu insulaire. *Bulletin d'Ecologie*, **19**, 411–416.

Pergams, O.R.W. & Ashley, M.V. (2001) Microevolution in island rodents. *Genetica*, **112–113**, 245–256.

Renaud, S. (1999) Size and shape variability in relation to species differences and climatic gradients in the African rodent *Oenomys*. *Journal of Biogeography*, **26**, 857–865.

Renaud, S. (2005) First upper molar and mandible shape of wood mice (*Apodemus sylvaticus*) from northern Germany: ageing, habitat and insularity. *Mammalian Biology*, **3**, 157–170.

Renaud, S. & Michaux, J.R. (2003) Adaptive latitudinal trends in the mandible shape of *Apodemus* wood mice. *Journal of Biogeography*, **30**, 1617–1628.

Renaud, S. & Michaux, J. (2004) Parallel evolution in molar outline of murine rodents: the case of the extinct *Malpaisomys insularis* (Eastern Canary Islands). *Zoological Journal of the Linnean Society*, **142**, 555–572.

Renaud, S. & Millien, V. (2001) Intra- and interspecific morphological variation in the field mouse species *Apodemus argenteus* and *A. speciosus* in the Japanese archipelago: the role of insular isolation and biogeographic gradients. *Biological Journal of the Linnean Society*, **74**, 557–569.

Renaud, S., Michaux, J., Schmidt, D.N., Aguilar, J.-P., Mein, P. & Auffray, J.-C. (2005) Morphological evolution, ecological diversification and climate change in rodents. *Proceedings of the Royal Society of London Series B, Biological Sciences*, **272**, 609–617.

Rohlf, F.J. (2000) *NTSYSpc. Numerical taxonomy and multivariate analysis system.* Exeter Software, New York.

Saint Girons, M.-C. (1966) Etude du genre *Apodemus* Kaup, 1829 en France. *Mammalia*, **30**, 547–600.

Salazar-Ciudad, I. & Jernvall, J. (2002) A gene network model accounting for development and evolution of mammalian teeth. *Proceedings of the National Academy of Science*, **99**, 8116–8120.

Tchernov, E. (1979) Polymorphism, size trends and Pleistocene paleoclimatic response of the subgenus *Sylvaemus* (Mammalia: Rodentia) in Israel. *Israel Journal of Zoology*, **28**, 131–159.

Ursin, E. (1956) Geographical variation in *Apodemus sylvaticus* and *Apodemus flavicollis* (Rodentia, *Muridae*) in Europe. *Biologiske Skrifter Kongelige Danske Videnskabernes Selskab*, **8**, 1–46.

Van Andel, T.H. (1989) Late Quaternary sea-level changes and archaeology. *Antiquity*, **63**, 733–745.

Van Andel, T.H. (1990) Addendum to 'Late Quaternary sea-level changes and archaeology'. *Antiquity*, **64**, 151–152.

Vigne, J.-D. (1999) The large 'true' Mediterranean islands as a model of the Holocene human impact on the European vertebrate fauna? Recent data and new reflections. *The Holocene history of the European vertebrate fauna. Modern aspects of research (Workshop, 6th–9th April 1998, Berlin)* (ed. by N. Benecke), pp. 295–322. Deutches Archaeologisches Institut, Eurasian-Abteilung, Berlin.

Vigne, J.-D. & Alcover, J.A. (1985) Incidences des relations historiques entre l'homme et l'animal dans la composition actuelle du peuplement amphibien, reptilien et mammalien des îles de Méditerranée occidentale. Actes 110e Congrès Nat. *Sociétés Savantes (Montpellier, 1985), Section Sciences*, **2,** 79–81.

Vigne, J.-D. & Valladas, H. (1996) Small mammal fossil assemblages as indicators of environmental changes in Northern Corsica during the last 250 years. *Journal of Archaeological Science*, **23,** 199–215.

Vigne, J.-D., Cheylan, G., Granjon, L. & Auffray, J.-C. (1993) Evolution ostéométrique de *Rattus* et de *Mus musculus domesticus* sur de petites îles: comparaison de populations médiévales et actuelles des îles Lavezzi (Corse) et de Corse. *Mammalia*, **57,** 85–98.

Vitt, L.J., Caldwell, J.P., Zani, P.A. & Titus, T.A. (1997) The role of habitat shift in the evolution of lizard morphology: evidence from tropical *Tropidurus*. *Proceedings of the National Academy of Sciences of the United States of America*, **94,** 3828–3832.

Whittaker, R.J. (1998) *Island biogeography: ecology, evolution, and conservation*. Oxford University Press, Oxford.

Workman, M.S., Leamy, L.J., Routman, E.J. & Cheverud, J.M. (2002) Analysis of quantitative trait locus effects on the size and shape of mandibular molars in mice. *Genetics*, **160,** 1573–1586.

Yom-Tov, Y., Yom-Tov, S. & Moller, H. (1999) Competition, coexistence, and adaptation amongst rodent invaders to Pacific and New Zealand islands. *Journal of Biogeography*, **26,** 947–958.

BIOSKETCHES

Sabrina Renaud works on the quantification of morphological evolution using morphometric methods, particularly outline analysis. She combines temporal, palaeontological perspective and spatial, biogeographical studies to investigate patterns, processes and key factors involved in the morphological response to environmental conditions.

Johan R. Michaux studies phylogeographical patterns of various common and threatened mammal species. The study of insular syndrome in wood mice has been one of his long-standing interests.

Editor: Jon Sadler