



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

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Two species of field mice, *Apodemus argenteus* and *A. speciosus*, occur in sympatry across the Japanese archipelago. The inter- and intraspecific patterns of morphological differentiation have been evaluated, using a Fourier analysis of the mandible outline. The relative importance of the effect of insular isolation and latitudinal climatic gradient on the size and shape of the two species was assessed by a comparison of the populations from the large island of Honshu and the surrounding small-island populations. The size variation in *A. argenteus* is correlated with the climatic gradient whilst the shape variation corresponds mainly to a random differentiation of the small-island populations from a Honshu-like basic morphological pattern. *A. speciosus* displays increased size on small islands, and its shape variation is related to both the climatic gradient and insularity. Finally, the two species are differentiated by both the size and shape of the mandible across the Japanese archipelago, suggesting that interspecific competition between both species is reduced via niche partitioning. Our results emphasize the importance of insular isolation on shape differentiation, but a part of the morphological differentiation is also related to the latitudinal climatic gradient. Isolation on small islands could have favoured such a response to environmental factors by lowering the gene flow that prevents almost any significant differentiation within Honshu populations.

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INTRODUCTION

Island vertebrate species, when compared to equivalent mainland species, are often characterized by peculiar morphological traits. A classical example is the evolution of body size of island mammals (Case, 1978). Large mammals tend to become smaller on islands, while small mammals tend to display the reverse trend (Foster, 1964; Van Valen, 1973; Lomolino, 1985). Insular gigantism, for example, has been well described in rodents (e.g. Angerbjörn, 1986) and is more generally thought to be part of an ‘insular syndrome’ involving changes in demography, reproduction, and behaviour (Adler & Levins, 1994). The extent of this body size increase seems to be related to the size of the island, and to be more pronounced on small islands

(Case, 1978). However, within this general trend of insular gigantism, a few cases of insular dwarfism in rodents have been described and linked to local environmental factors that have favoured small body size (Ganem *et al.*, 1995).

Although changes in body size induced by insular isolation are relatively well known, similar trends in shape of morphological traits have been less investigated. Again, examples of morphological differentiation have been reported for insular rodent populations, e.g. the mandible of the house mouse, *Mus musculus*, on British islands (Scriven & Bauchau, 1992), skeletal characters of the black rat, *Rattus norvegicus*, on Mediterranean islands (Granjon & Cheylan, 1990), and cranial traits in the Mediterranean field mouse, *Apodemus sylvaticus* (Libois, Fons & Bordenave, 1993; Michaux *et al.*, 1996). Morphological differences observed between mainland and island populations have often been interpreted as being

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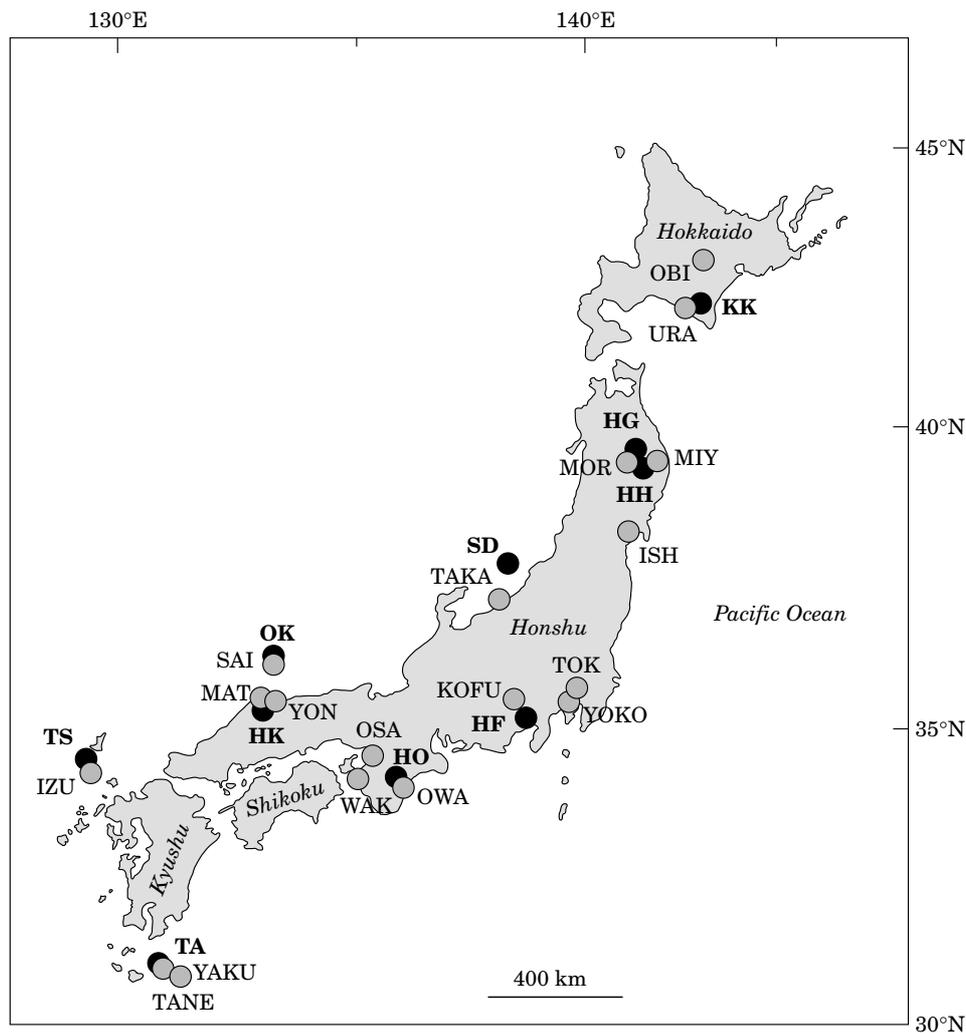


Figure 1. Location of the *Apodemus* samples across the Japanese archipelago, and of the climatological stations used for the comparisons. Labels as in Tables 1 and 2.

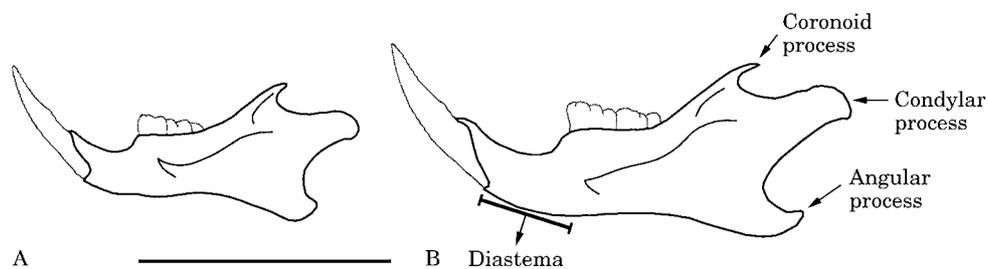
due to stochastic differences following the founder effect and subsequent divergence due to genetic drift. Alternatively, changes in shape, much like changes in body size, may be associated with environmental gradients, e.g. rodent molar shape by a gradient of temperature and humidity (Renaud, 1999). Currently, the relative importance of environmentally induced adaptive responses and stochastic processes due to isolation itself for observed changes in morphology of isolated populations, is as yet unknown.

This study aimed to identify the patterns of morphological differentiation associated with both insular conditions and climatic gradients on rodents. We focused on two species of field mice, *Apodemus argenteus* and *A. speciosus*, widely distributed across the Japanese archipelago. These two species resemble each other morphologically, although *A. argenteus* is one third of the body weight of *A. speciosus* (Oka, 1992).

They also display differences in ecology. *A. speciosus* occupies open fields and secondary lowland forests, whilst *A. argenteus* tends to occupy natural mountain forests, although they frequently co-occur in many forests (Kaneko, 1982; Oka, 1992). In areas of sympatry, a vertical habitat segregation occurs. *A. argenteus* is a skilful tree-climber, whilst *A. speciosus* occupies only terrestrial habitats (Abe, 1986; Sekijima & Soné, 1994). The sampling localities used in the present study are located both on the main Japanese island, Honshu, and on various smaller islands (Fig. 1). An evaluation of the importance of insular isolation for these rodents was thus conducted by employing a comparison between the populations from the large island of Honshu and the surrounding small islands. In addition, these samples cover the entire range of climatic variation encountered in the Japanese archipelago, ranging from cool-temperate conditions in the

Table 1. The sample size of the different groups considered in this study and their corresponding latitude (°E) and longitude (°N)

	Locality	Code	Lat.	Long.	<i>A. argenteus</i>	<i>A. speciosus</i>
Honshu	Mt. Fuji	HF	35.2	128.9	11	11
	Mt. Goyo	HG	39.8	141.6	5	—
	Mt. Hayachine	HH	39.4	141.7	4	7
	Kisuki-Cho	HK	35.3	133.1	—	6
	Mt. Odaigahara	HO	34.2	136.0	10	—
Islands	Mt. Poroshiri	KK	42.2	142.9	10	5
	Oki isl.	OK	36.2	133.3	—	10
	Sado isl.	SD	37.8	138.6	9	15
	Tane isl.	TA	31.0	130.7	2	16
	Tsushima isl.	TS	34.5	128.9	10	19

**Figure 2.** Mandible of *Apodemus argenteus* (A) and *A. speciosus* (B) in lateral view. Scale bar is 1 cm.

north, to warm-tropical in the south. Comparing the intraspecific patterns of morphological differentiation of the two field mouse species provides an opportunity for the assessment of the response of each to a similar environmental pressure. Furthermore, an interspecific comparison permits the identification of the unique morphological response of each species to the same environment.

MATERIAL AND METHODS

MATERIAL

Mandibles of 61 specimens of *A. argenteus* and of 89 specimens of *A. speciosus* from 10 localities were measured (Fig. 1; Table 1). These sites are located on Honshu, and on various smaller islands around it. In most localities (nine out of the ten sampled), both species are found in sympatry. However, both species were available for measurements in only six localities. All the material is stored at the National Science Museum, Tokyo. Only mature specimens with the complete eruption of the third molar were considered. Right and left mandibles were mixed, and mirror images of the left mandibles have been considered, in order to compare their shape with right mandibles. Because of

the morphometric method used only complete unaltered lower jaws could be considered, which consequently substantially reduced our sample sizes.

FOURIER ANALYSIS OF THE OUTLINE

Morphometric analyses of mandibles have been proven to efficiently describe taxonomic and geographic variation in rodents (Corti *et al.*, 1996; Demeter, Rácz & Csorba, 1996). In particular, among these methods, outline analysis permits the description of the general shape of the mandible in a very simple way and with a few variables using a Fourier analysis. This method also limits the influence of measurement error by filtering the noise occurring on details of the outline.

The outline corresponds to the two-dimensional projection of the mandible, put on its side with the lingual side down (Fig. 2). Since the incisor was often free moving, and the molars sometimes missing, these features were not included in the study and only the outline of mandibular bone was considered. The starting point of the outline was defined at the meeting point of the incisor and the bone on the upper edge of the mandible. For each mandible, the *x*- and *y*-coordinates of 64 points equally spaced along the outline were extracted semi-automatically using an optical

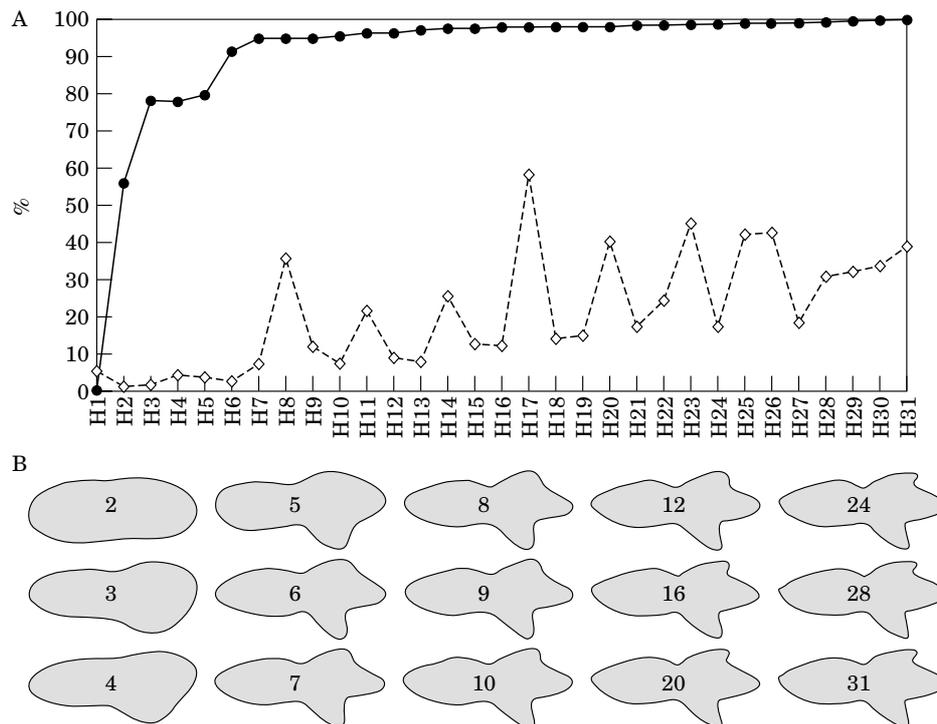


Figure 3. (A) Measurement error (\diamond) and cumulative power (\bullet) as a function of the harmonic order. The measurement error is expressed as the coefficient of variation of the harmonic amplitude of one specimen measured ten times. Cumulative power corresponds to the maximum number of reconstructed outlines based on an increasing number of harmonics from the reconstruction based on the maximum number of harmonics (=31). (B) Some examples of the reconstructed outlines for an increasing number of harmonics.

image analyser. From these coordinates, 64 radii, corresponding to the distance of each point to the centre of gravity of the outline, were calculated. A Fourier transform was then applied to this set of 64 radii. The outline is thus expressed as a finite sum of trigonometric functions of decreasing wavelength (harmonics) according to the formula:

$$r(s) = a_0 + \sum_{n=1}^K [a_n \cos(s/L2\pi n) + b_n \sin(s/L2\pi n)]$$

where r is the radius at the abscissa s along the outline, L the perimeter, K the number of points along the outline, and n the rank of the harmonic (Renaud, 1999). The outline is therefore described by the set of Fourier coefficients a_n and b_n .

To obtain coefficients dependent only on shape, the size is standardized by dividing all the Fourier coefficients by the zeroth harmonic, a_0 , which is proportional to the diameter of the best-fit circle to the digitized outline and can thus be considered as a reliable size estimator (Ehrlich & Weinberg, 1970). A correspondence with a more usual size-estimator can be established by comparing the values of a_0 with the perimeter P_m of the mandible outline. In the present

case, we found a significant linear correlation between both variables: $r^2 = 0.990$; $P < 0.001$; $a_0 = 0.59 + 0.888 P_m$ [mm].

A reconstruction of the outline corresponding to any set of Fourier coefficients can be obtained using the inverse Fourier transform, following the inverse process to that used to calculate the Fourier coefficients (Rohlf & Archie, 1984). A characteristic of the Fourier harmonics is that the higher the rank of the harmonic, the more details of the outline are described. This property can be used to filter measurement noise, which increases with harmonic rank (Renaud, 1999). Measurement error was estimated by ten repeated measurements of one randomly chosen specimen, and expressed as the coefficient of variation of the harmonic amplitude ($= \sqrt{a_n^2 + b_n^2}$). Results indicated an important increase of the measurement error above the seventh harmonic (Fig. 3). Above this harmonic rank, the measurement error reached values greater than 20% of the signal, which may render the Fourier coefficients unreliable. The content of information added by each harmonic has also been estimated using the cumulative power (Crampton, 1995). More than 95% of the total power is reached at the seventh harmonic, and the subsequent increase is only very

Table 2. Climatological stations from the online NOAA NCDC GCPS library (Baker *et al.*, 1994) corresponding to the nearest rodent localities. Lat: latitude (°E); Long: longitude (°N); Alt: altitude (m); Pm: mean monthly precipitation (mm); Pmax (Pmin): mean monthly maximal (minimal) precipitation (mm); DM: seasonal precipitation difference; Tm: mean monthly temperature (°C); Tmax (Tmin): mean monthly maximal (minimal) temperature (°C); DT: seasonal temperature difference; Tdd: mean daily temperature difference (°C)

Station	Code	Rodent	Lat	Long	Alt	Pm	Pmax	Pmin	DM	Tm	Tmax	Tmin	DT	Tdd
Ishinomaki	ISH	HH	38.4	141.3	43	92	194	16	177	11.4	23.4	-0.2	23.6	7.5
Izuhara	IZU	TS	34.2	129.3	21	183	515	30	485	15.2	26.3	4.5	21.8	6.8
Kofu	KOFU	HF	35.7	138.6	272	129	315	22	292	15.0	27.4	2.6	24.7	10.9
Matsue	MAT	HK	35.5	133.1	17	160	331	64	267	14.9	26.9	3.9	23.0	8.5
Miyako	MIY	HG,HH	39.7	142.0	43	106	272	14	258	10.4	22.2	-0.4	22.6	9.2
Morioka	MOR	HG,HH	39.7	141.2	155	147	282	62	221	10.6	24.6	-2.5	27.1	9.4
Obihiro	OBI	KK	42.9	143.2	38	94	216	31	185	7.0	21.3	-8.7	30.0	10.8
Osaka	OSA		34.7	135.5	23	114	293	22	271	16.1	28.2	4.8	23.4	7.8
Owase	OWA	HO	34.1	136.2	15	120	295	21	274	16.2	26.7	5.9	20.9	8.9
Saigo	SAI	OK	36.2	133.3	27	152	296	52	244	14.3	26.4	3.2	23.1	8.1
Takada	TAKA	SD	37.1	138.3	13	213	412	83	330	13.8	26.9	1.7	25.2	9.2
Tanegashima	TANE	TA	30.7	131.0	17	335	819	92	727	19.8	28.1	11.3	16.8	5.6
Tokyo	TOK		35.7	139.8	5	120	295	21	274	15.6	27.0	5.0	22.1	7.3
Urakawa	URA	KK	42.2	142.8	33	96	224	27	197	7.7	19.9	-3.6	23.5	6.8
Wakayama	WAK		34.2	135.2	14	114	294	22	272	16.7	28.3	5.7	22.6	8.3
Yakushima	YAKU	TA	30.9	130.7	36	339	851	95	756	19.4	27.6	11.1	16.6	6.3
Yokohama	YOKO		35.4	139.7	39	120	295	21	274	15.6	27.2	5.1	22.1	7.3
Yonago	YON	HK	35.4	133.4	6	155	344	59	285	14.4	26.4	3.5	22.9	8.7

limited (Fig. 3). Both approaches give congruent results, and Fourier coefficients have been considered up to the seventh harmonic for the subsequent analyses.

MANOVA ON THE FOURIER COEFFICIENTS

A set of 14 Fourier coefficients (i.e. seven harmonics) for each mandible was obtained. Multivariate analyses of variance (MANOVA) were performed on these variables to evaluate the pattern of morphological differentiation and the importance of the among-group differentiation relative to within-group variation. Separate analyses were performed for *A. argenteus* and *A. speciosus*. Additionally, a combined analysis was performed to compare the patterns of variation of both species. Therefore, the groups correspond to the sampling localities of each species, or to the different species within each locality. Associated with the MANOVA, canonical discriminant functions were estimated, that are usually sufficient to account for almost all important group differences (Manly, 1994). The scores of the group means can be plotted on these canonical axes to visualize the patterns of morphological differentiation. A test of significance for among-group differences (Wilks' lambda) is also given (Marcus, 1993).

ANALYSIS OF CLIMATOLOGICAL DATA

Climatological data were obtained from the online library of NOAA NCDC Global Climate Perspective System (Baker *et al.*, 1994; <http://ingrid.ldeo.columbia.edu/>). Daily and monthly data from several stations were available and permitted the calculation of mean, maximum, and minimum monthly precipitation and temperature, seasonal precipitation and temperature difference, and mean daily difference in temperature. Eighteen stations (Fig. 1, Table 2) were selected, covering the climatic range of the Japanese archipelago, and close to the rodent-sampling localities. A PCA (principal components analysis) was performed on these data in order to obtain a few synthetic principal axes expressing most of the climatic variability. Comparison with morphological data was finally performed by linking rodent-sampling localities to the closest climatological stations (Table 2).

RESULTS

INTRASPECIFIC SIZE DIFFERENTIATION

Size patterns are different for both species (Fig. 4). *A. argenteus* shows only a small differentiation in size, possibly due to the reduced amount of material in

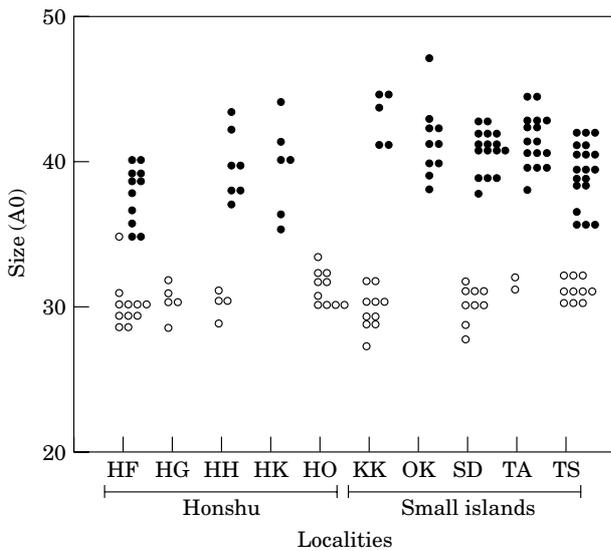


Figure 4. The size distribution of *A. argenteus* (○) and *A. speciosus* (●) for the different localities. The size of the mandibles is estimated by a_0 , the zeroth harmonic of the Fourier analysis, proportional to the diameter of the best-fit circle to the mandible outline. Each dot corresponds to an individual.

some samples. No differences exist between populations from Honshu vs. small islands, or among Honshu populations (Fig 4; Table 3), whereas differences are significant among small island populations.

Size variation is greater within *A. speciosus* (Fig. 4, Table 3). Size in small islands populations is significantly larger than in Honshu populations. Further differentiation exists among small island populations, but not within Honshu.

INTRASPECIFIC SHAPE DIFFERENTIATION

Both *A. argenteus* and *A. speciosus* show significant geographic differentiation in shape (Fig. 5; Table 3). Canonical axes based on the analysis of the Fourier

coefficients as a function of the different localities have been chosen to visualize the patterns of differentiation. *A. argenteus* is characterized along the first and the second canonical axes by a central position of the Honshu populations, surrounded by strongly differentiated small-island populations. The central position of the Honshu populations compared to the small islands illustrates the absence of significant differences between Honshu and small-island samples, and within Honshu (Table 3). Reconstructed outlines permit the visualization of the weak shape changes involved in this intraspecific variability. Variation along CA1, from the negative to the positive side, corresponds to a backward shift of the angular process (cf. Fig. 2) and a straighter lower edge of the symphysis, while variation along CA2 corresponds to a more pronounced coronoid process, a downward shift of the condylar process, and a forward movement of the angular process, together with a more curved lower edge of the symphysis, with a more prominent, forward-shifted molar row.

The pattern of shape differentiation of *A. speciosus* is roughly similar but even more pronounced. Here again, most Honshu populations (HH and HF) are characterized by a central position compared to small-island populations differentiated around HH and HF along CA1 and CA2. However, one population of Honshu (HK) strongly departs from this general pattern and is separated from all other populations, which leads to a significant morphological differentiation within Honshu. However, the strongest pattern is the differentiation among small-island populations (Table 3). Reconstructed outlines show that variation along CA1 corresponds to a forward shift of the angular process and a more curved lower edge of the symphysis, while CA2 expresses a forward movement of the molar row and a downward shift of the condylar process.

INTERSPECIFIC SIZE AND SHAPE DIFFERENTIATION

The mandibles of the two *Apodemus* species greatly differ in size (Fig. 4, Table 3). When present in sympatry, the mandible size ratio between the two species

Table 3. Size and shape differentiation of the mandible among the Japanese field mice within *A. argenteus*, *A. speciosus*, and between the two species. Size: probabilities of ANOVA on a_0 . Shape: probabilities of MANOVA on Fourier coefficients (a_1 to b_7). The factors considered in the different analyses are defined as follows: (1) Total: all localities, with locality as the group factor; (2) H-I: all localities, with type (Honshu vs. small-island) as the group factor; (3) H: Honshu localities, with locality as the group factor; (4) I: small-islands localities, with locality as the group factor; (5) A-S: all localities, with species (*A. argenteus* vs. *A. speciosus*) as the group factor

	<i>A. argenteus</i>				<i>A. speciosus</i>				<i>arg. vs. spe.</i>	
	Total	H-I	H	I	Total	H-I	H	I	A-S	Total
Size	0.093	0.853	0.427	0.019	0.000	0.00	0.207	0.001	0.000	0.000
Shape	0.001	0.110	0.375	0.001	0.000	0.021	0.003	0.000	0.000	0.000

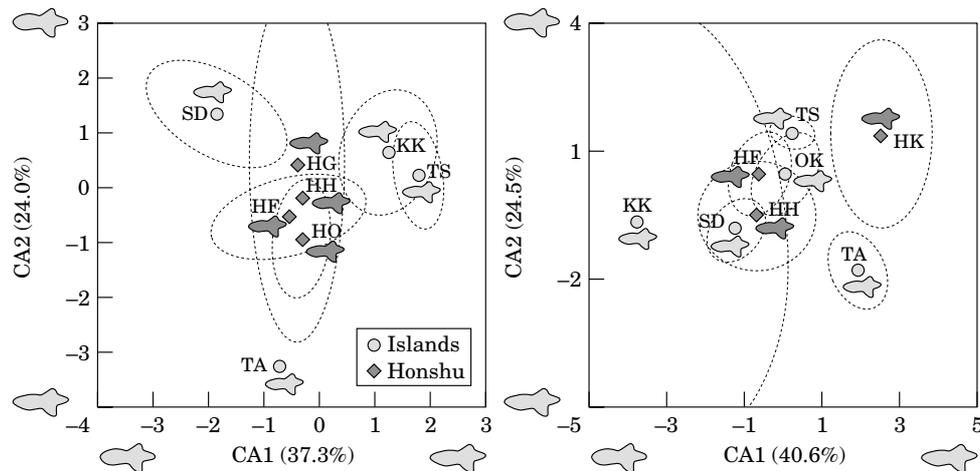


Figure 5. Shape differentiation within *A. argenteus* (left) and *A. speciosus* (right) mandibles, with the mean values of the different populations plotted on the first two canonical axes. Reconstructed outlines correspond to the mean values of the localities, and to variations along the canonical axes (magnification of the shape variations ~ 10 times). The intrapopulation variability is represented by the 95% confidence ellipse around the group mean.

ranges from 1.25 (HF, TS), 1.31 (HH, TA), 1.34 (SD) up to 1.44 (KK). From these results, no differences emerge between Honshu and the small-islands, and the amplitude of the size difference between the two species appears to be random. However, considering other rodent species, present in sympatry with these two *Apodemus* species, reveals such significant pattern (Millien-Parra & Loreau, 2000).

Both species also greatly differ in shape (Fig. 6A), and the interspecific difference corresponding to the variation along CA1 represents up to 62.3% of the among-group variance. This difference involves a flattening of the symphysis and a backward shift of both the coronoid and angular processes from *A. argenteus* to *A. speciosus*. Intraspecific variation of each species is then expressed on CA2 (only 11% of the variance), and corresponds to a more pronounced and forward-shifted molar row together with a downward shift of the condylar process. Displayed on orthogonal canonical axes, pattern of intraspecific variation (concerning mainly the molar row and condylar process) are therefore independent of interspecific patterns of differentiation (involving the coronoid and angular processes). Furthermore, intraspecific variation of both species seems to involve similar mandibular features since they are displayed in a parallel way along the same canonical axis (CA2).

COMPARISON WITH CLIMATOLOGICAL DATA

The localities considered in this study experience a wide range of climatic conditions (Table 2). A PCA on temperature, precipitation, and altitude data (Fig. 7) provides a synthetic view of these climatic variations,

mainly expressed along PC1 (63.5% of the total variance). The gradient of stations along PC1 corresponds to an increase in temperature and precipitation from the northernmost (OBI) to the southernmost stations (TANE, YAKU), together with a decreasing amplitude of daily and seasonal temperature variations. These variations along PC1 correspond to a latitudinal climatic gradient (correlation: $r^2=0.811$; $P<0.001$).

Some relationships exist between this climatic gradient and the size and shape of the mandible of both field mice species (Table 4, Fig. 8). *A. argenteus* size variation correlates well with the climatic latitudinal gradient, in particular among small islands. Although the differences are not significant ($P=0.427$), the same trend seems to exist within Honshu. Shape variations appear to be less related to the latitudinal gradient, since such a relation is only expressed along CA2, while CA1 mostly characterizes the random differentiation of the small islands around Honshu populations.

The pattern emerging from *A. speciosus* is quite different. Size differences are not significantly related to the climatic gradient, but to an increased size of the small-island populations. Shape variation, however, correlates well with the latitudinal gradient expressed along CA1. An influence on CA2 also exists, if the sample of TA, strongly departing from the general pattern, is removed from the analysis (correlation of CA2 with latitude with TA: $P=0.873$; without TA: $P=0.024$). However, differentiation on CA2 mainly corresponds to a random differentiation of the small islands and Honshu populations.

The climatic gradient present across the Japanese archipelago therefore appears to have an influence on the morphological differentiation of both field mouse

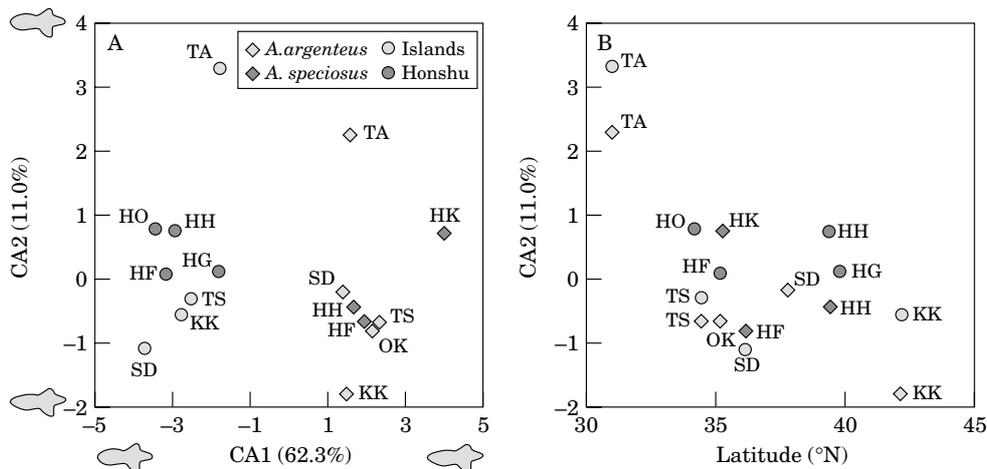


Figure 6. (A) Shape differentiation of the mandibles between populations of both species of field mice, represented by mean values of the different populations on the first two canonical axes. Reconstructed outlines correspond to variations along the canonical axes (magnification of the shape variations ~ 10 times). (B) The relation between the latitudinal gradient and morphological variation along CA2.

species. This is confirmed by an analysis of the global set of samples, including both species (Fig. 6). If CA1, which clearly corresponds to the interspecific morphological differentiation, is not related to the latitudinal climatic gradient, CA2, related to intraspecific variability, is significantly correlated with climatic variations (Table 4). This relation is particularly well illustrated by the strong morphological divergence of the Tane island (TA) populations in both species, a location also corresponding to very different climatic conditions (see TANE and YAKU stations on Fig. 7).

DISCUSSION

INSULAR SYNDROME ON SMALL ISLANDS

The expected expression of the insular syndrome in rodents is a tendency towards gigantism, an effect particularly enhanced on small islands. Such a pattern has been observed in European species of *Apodemus* (Angerbjörn, 1986; Delany, 1970; Libois *et al.*, 1993; Michaux *et al.*, 1996). The size of the lower incisor is related to body size in rodents (Millien-Parra, 2000), and it is likely that the whole mandible, considered here as an indicator of body size, is also similarly related. Our results show contrasting effects with regards to the importance of the insular syndrome on *Apodemus* on the small islands of the Japanese archipelago. A trend towards larger size exists among the small-island populations, but only for the largest species *A. speciosus*, whilst the size of the smallest species *A. argenteus* seems to be affected by the environmental gradient.

The results for the shape differentiation of small-islands populations appear more consistent. Both

species display an intraspecific pattern of variation corresponding to a central position of the Honshu populations, from which the small islands populations have morphologically diverged in various directions (Fig. 5). This pattern is especially clear for *A. argenteus*, but also exists for *A. speciosus*. Such random differentiation of island populations when compared to the mainland has been observed for the Western European wood mouse *Apodemus sylvaticus* on British (Berry, 1969, 1973) and North Frisian islands (Murbach, 1979). This differentiation of island populations may have a genetic basis and be interpreted as the combined result of: (1) a founder effect, the island populations being founded by a reduced number of individuals representing only a random part of the genetic variability of the mainland population, and (2) a subsequent genetic drift, due to the reduction of gene flow through isolation. The relative importance of these two effects is linked to the historical background of the island populations: the founder effect is important when the island population has been founded by few

Table 4. Probabilities of linear correlations between size and shape variations of the mandible in the field mice and geographical and climatological data

		<i>A. argenteus</i>		<i>A. speciosus</i>		<i>Total</i>	
		<i>Lat</i>	<i>PC1</i>	<i>Lat</i>	<i>PC1</i>	<i>Lat</i>	<i>PC1</i>
Size	a_0	0.002	0.005	0.367	0.995	—	—
Shape	CA1	0.717	0.627	0.009	0.045	0.817	0.684
	CA2	0.029	0.039	0.873	0.528	0.006	0.001

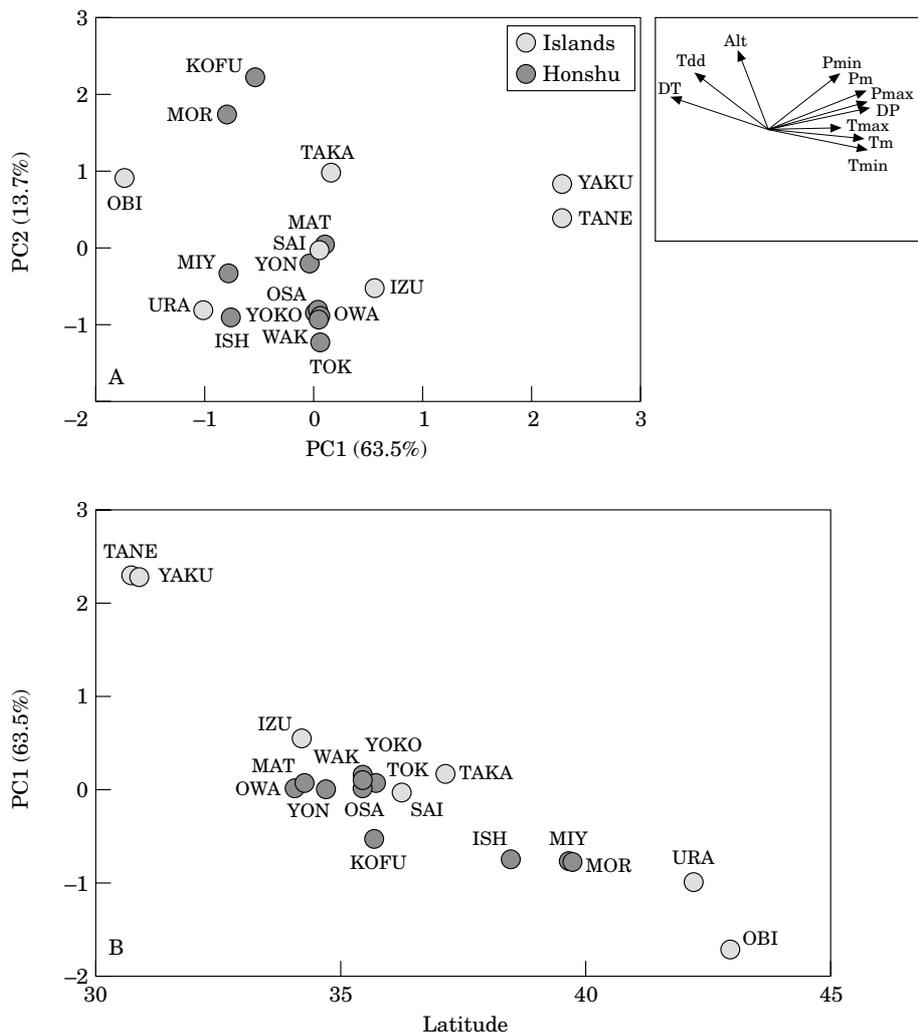


Figure 7. Climatic variations across the Japanese archipelago. (A) First principal plane of a PCA on climatic variables from NOAA NCDC monthly and daily stations, with contribution of each of the climatic variables to the first two principal axes. See Table 2 for abbreviations. (B) The relation between PC1 and latitude.

immigrants, while the genetic drift is a more important mechanism of differentiation when the isolation of an island population is the consequence of the fragmentation of a previously homogeneous habitat. This latter situation corresponds to the Japanese archipelago, which is the result of the fragmentation during the Late Quaternary of a large territory into several islands (review in Millien-Parra & Jaeger, 1999). Since an island relict population is expected to show similar initial levels of genetic variability to that of a mainland equivalent (Berry, 1986), the influence of an initial founder effect on the evolutionary trends in *Apodemus* is not likely compared to the subsequent genetic drift. Additionally, the morphological divergence of the small-island populations could have been favoured by strong selective pressure due to the environmental

conditions peculiar to small islands (i.e. reduced food availability, decreased competition and predation).

In comparison to the intraspecific variation in the small-islands populations, Honshu populations are characterized by little or no differentiation in either size or shape. This suggests that within this island, gene flow could be important enough to homogenise the size and shape characteristics of the different populations. However, one population of *A. speciosus* (HK) diverges strongly from the other Honshu populations. Such strong local morphological differentiation has also been reported in house mice (Auffray *et al.*, 1996) and these results underline the conclusion that interpretations regarding patterns of morphological differentiation should be based on as many populations as possible.

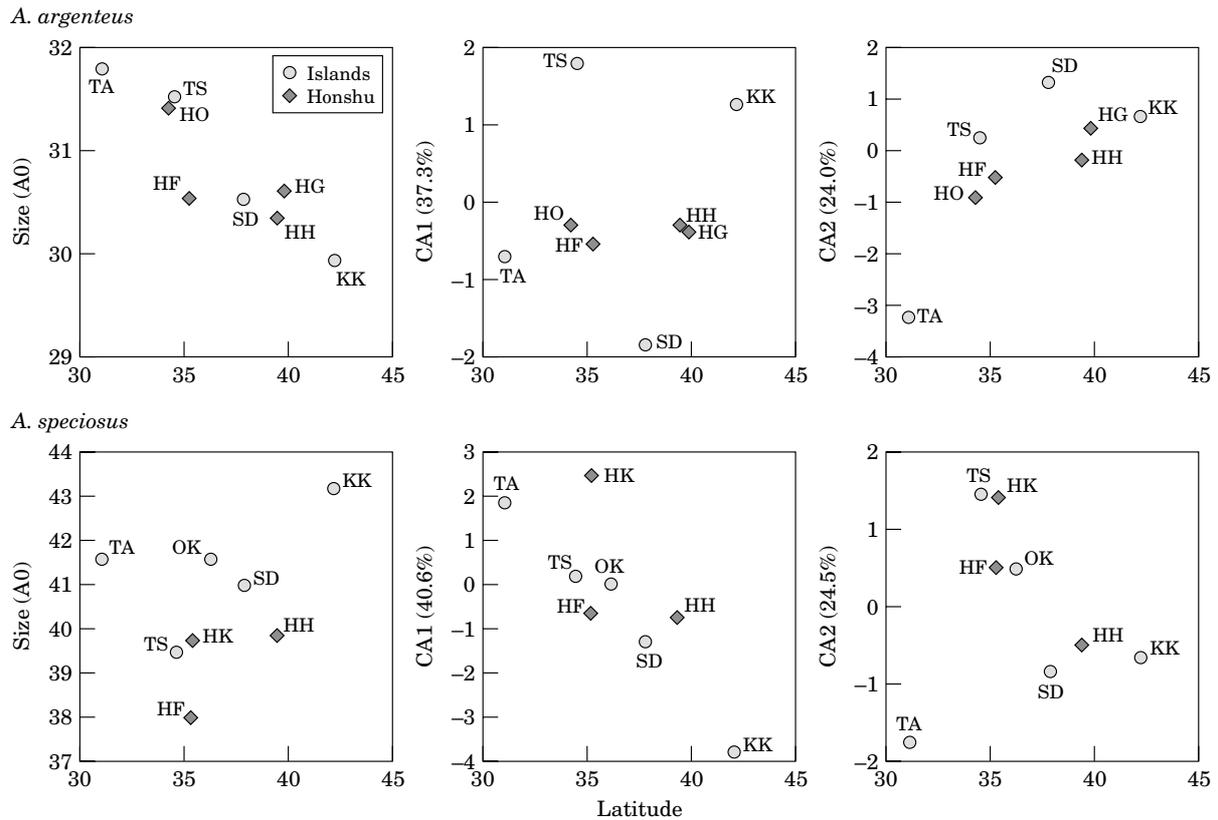


Figure 8. The relation between morphological and climatic variations, for *A. argenteus* (upper row) and *A. speciosus* (lower row). From left to right: relations of size, defined by a_0 , and shape, defined by CA1 and CA2, with latitude.

INFLUENCE OF THE CLIMATIC GRADIENT

Climatic gradients are known to have an influence on mammal body size, as in the case of the 'Bergmann's rule', which describes an increase in size with decreasing temperature (Bergmann, 1847). As a consequence, the evolution of size during the Quaternary of several rodent species has been interpreted as a response to the temperature change (Smith, Betancourt & Brown, 1995; Tchernov, 1979). Shape variations can also be related to geographic gradients (Renaud, 1999). We therefore examined whether the large climatic gradient existing between the northernmost and southernmost populations could have influenced the patterns of differentiation observed.

Size differences seem to be related to climatic gradient only in the case of *A. argenteus*, which displays a trend of increasing size with increasing temperature and precipitation. When considered alone, the Honshu populations, although not significantly different between each other, seem to exhibit the same trend. The gene flow within Honshu could prevent any strong morphological differentiation. In addition, the climatic gradient experienced across Honshu island is somewhat reduced when compared to the one existing across

the whole Japanese archipelago. The expression of the response to environmental gradients could therefore be enhanced across the small islands scattered across the whole Japanese archipelago, where insular isolation allows the development of local adaptation to fine scale environmental variations. The insular isolation, dating back to the Quaternary, is sufficiently old for such evolutionary processes to have taken place.

The climatic gradient appears to have influenced the shape differentiation as well. In contrast to the size pattern, both species seem to be influenced, although *A. speciosus* more strongly than *A. argenteus* (Fig. 8). The different ecological preferences of both species might explain these differences in response to the similar environmental variation. Here again, the trend is better expressed across the small-island populations. In both species, the patterns of morphological variation seem to show a combined effect of random differentiation due to insular isolation, and response to environmental gradients.

COMPARED INTER- AND INTRASPECIFIC VARIABILITY

Depending on the competitor present, interspecific competition has been evoked to explain gigantism of

Apodemus on some islands (Angerbjörn, 1986). We therefore assessed the case for this effect for the Japanese archipelago. Among the murid community, the two *Apodemus* species appear to be generalist and their occurrence is influenced neither by the occurrence of potential competitor species, or by particular environmental conditions (Millien-Parra & Loreau, 2000). A more direct effect of competition could exist between the two species of field mice. If size is playing an important role in interspecific competitive interactions, the size ratio between both species is expected to match a theoretical value, comprised between 1.1 and 1.4 and close to the mean value of 1.28 (Hutchinson, 1959). The size ratios observed between *A. speciosus* and *A. argenteus* fall within this interval. This result suggests that interspecific competition between the two species could explain the large difference in size between them. This result is in agreement with the conclusions of Sekijima & Soné (1994), who demonstrated by removal experiments the impact of *A. speciosus* on the ecological characteristics of *A. argenteus*. In addition, a reduction of interspecific competition between coexisting species may not only be realized through size differentiation, but may also be related to differences in shape, e.g. of the incisor (Parra, Loreau & Jaeger, 1999) or of the molars (Renaud *et al.*, 1999). A strong difference in the shape of the mandible between the two *Apodemus* species is the main feature that emerges from our morphometrical analysis (Fig. 6), and it appears to be independent of the environmental gradient. The shape of the mandible could thus play a role in reducing interspecific competition between the two *Apodemus* species. Unfortunately, a functional interpretation of the shape differences is very difficult. The coronoid and angular processes, reduced and shifted backward in *A. speciosus* compared to *A. argenteus*, are two main zones of attachment of the muscles involved in biting and mastication (Kessner, 1980; Satoh, 1997). Such variations between other families of rodents have been interpreted as linked to an increased efficiency of food grinding by enhancing the antero-posterior component of mandible movement (Kessner, 1980). The morphological differences between *A. speciosus* and *A. argenteus* could therefore have an adaptive value and be related to different diet and habitat preferences.

The second main common morphological feature of the two species is the geographic differentiation in the shape of the mandible, according to the latitudinal gradient (Fig. 6B). The patterns of intraspecific shape variability appear to be similar for both species, and independent of the pattern of interspecific differentiation, since these two patterns are expressed on two orthogonal, and therefore independent, canonical axes. These results suggest that the inter- and intraspecific patterns of variability are under quite different and

independent constraints. The different patterns of variation could be due to different selective pressures acting on fine morphological features. Although difficult to identify, the shape differences involved could have an adaptive significance, for example, avoidance of interspecific competition on the one hand and adaptation to the local climatic conditions on the other hand. In addition, different morphological features of the mandible could be canalized during the development differently. Independent patterns of morphological variations could then be expressed in response to different factors, i.e. interspecific ecological differences, random insular differentiation, and response to environmental gradients. The strength of the response to these various factors would be determined by ecological characteristics of the species. Finally, the similar intraspecific patterns of morphological variation of each species suggest either a common canalization pattern, most probably inherited from a common ancestor, or the effect of similar environmental pressures on both species, in spite of their differences in ecological preferences.

CONCLUSION

Our morphometric analyses showed that the two species of field mouse *A. argenteus* and *A. speciosus* display both size and shape differentiation of the mandible across the Japanese archipelago. Interspecific differences are very apparent for both size and shape, and suggest that interspecific competition between both species is effectively reduced via niche partitioning. Intraspecific patterns of differentiation are more complex and depend on both insular isolation and response to geographic gradients. In both species, the shape of the mandible of the small-islands populations seems to differentiate from the Honshu-like basic morphological pattern. A part of the morphological differentiation is also related to the latitudinal climatic gradient. Isolation on small islands could have favoured such a response to environmental factors by lowering the gene flow that appear important for preventing almost any significant differentiation within Honshu populations. The similar morphological response of both species to these environmental variations suggests a common selective pressure or a conserved pattern of canalization.

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