



Patterns of morphological evolution in the mandible of the house mouse *Mus musculus* (Rodentia: Muridae)

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Received 23 August 2011; revised 28 September 2011; accepted for publication 28 September 2011

The worldwide distributed house mouse, *Mus musculus*, is subdivided into at least three lineages, *Mus musculus musculus*, *Mus musculus domesticus*, and *Mus musculus castaneus*. The subspecies occur parapatrically in a region considered to be the cradle of the species in Southern Asia ('central region'), as well as in the rest of the world ('peripheral region'). The morphological evolution of this species in a phylogeographical context is studied using a landmark-based approach on mandible morphology of different populations of the three lineages. The morphological variation increases from central to peripheral regions at the population and subspecific levels, confirming a centrifugal sub-speciation within this species. Furthermore, the outgroup comparison with sister species suggests that *M. musculus musculus* and populations of all subspecies inhabiting the Iranian plateau have retained a more ancestral mandible morphology, suggesting that this region may represent one of the relevant places of the origin of the species. *Mus musculus castaneus*, both from central and peripheral regions, is morphologically the most variable and divergent subspecies. Finally, the results obtained in the present study suggest that the independent evolution to commensalism in the three lineages is not accompanied by a convergence detectable on jaw morphology. © 2012 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2012, **105**, 635–647.

ADDITIONAL KEYWORDS: commensalism – *M. musculus castaneus* – *M. musculus domesticus* – *M. musculus musculus* – morphometrics – origin.

INTRODUCTION

Phylogeographical patterns are usually reconstructed by various computational approaches analyzing the patterns of differentiation of several molecular markers which, in certain cases, may have contrasted histories, yielding several possible phylogeographical scenarios (Edwards & Beerli, 2000; Riddle, 2009). In this respect, the study of phenotypes can be relevant because such complex structures are at least partly

determined by the combined effects of several genes and hence are likely to record an average history. Additionally, considering phenotypic variation in a phylogeographical context may help to better assess the evolutionary forces that may have acted on populations, with phenotypes being the direct target of natural selection (Losos *et al.*, 1998; Renaud, Chevret & Michaux, 2007).

The house mouse is a good candidate for understanding morphological evolution in a phylogeographical context. *Mus musculus* is indeed a model organism in biology for which phylogeography starts to be well established (Geraldts *et al.*, 2008;

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Rajabi-Maham, Orth & Bonhomme, 2008). Moreover, the recent evolutionary history of the species is directly related to its commensal ecology, and the recent pattern of worldwide colonization along with humans covers numerous and entangled evolutionary processes (Boursot *et al.*, 1993; Macholan *et al.*, in press). *Mus musculus* is the only species of the genus *Mus* having evolved a commensal way of life, whereas the other species of this genus are all wild (Auffray, Tchernov & Nevo, 1988; Sage, Atchley & Capanna, 1993; Prager, Orrego & Sage, 1998).

The house mouse is widely recognized as a polytypic species containing at least three distinctive lineages considered as subspecies: *Mus musculus musculus*, *Mus musculus domesticus* and *Mus musculus castaneus* (Bonhomme *et al.*, 1989, 1994; Boursot *et al.*, 1993, 1996; Sage *et al.*, 1993; Prager, Tichy & Sage, 1996; Prager *et al.*, 1998). *Mus musculus musculus* and *M. m. domesticus* have always been considered well differentiated lineages, whereas, from a molecular point of view, *M. m. castaneus* (the oriental lineage) appears to be much more polymorphic (Boursot *et al.*, 1996; Boissinot & Boursot, 1997; Darvish, Orth & Bonhomme, 2006; Baines & Harr, 2007; Bonhomme *et al.*, 2007; Darvish, 2008; Geraldes *et al.*, 2008). Because these three subspecies diverged more than 100 000 years ago (Bonhomme *et al.*, 1989; She *et al.*, 1990), and because commensalism in relation to humans is not considered to have begun before the Epipaleolithic time (time of the earliest human dwellings, 12 000 years BP; Auffray, Vanlerberghe & Britton-Davidian, 1990), the commensal way of life should have evolved independently in the three subspecies.

All hypotheses proposed for the cradle of this polytypic species state that it is located somewhere between the Iranian plateau (delimited to the west by the Zagros mountains, the Indus River to the east, the Kopet Dag mountains to the north, and the Persian gulf to the south) and the Indian peninsula (Bonhomme *et al.*, 1994; Boursot *et al.*, 1996; Prager *et al.*, 1998; Milishnikov, Lavrenchenko & Lebedev, 2004; Darvish *et al.*, 2006; Darvish, 2008). Currently, the three lineages are still present in this region (hereafter called the 'central region') from where they are considered to have colonized the rest of the world (hereafter called the 'peripheral regions') by active or passive transport as a result of their commensal relationship with humans (Bonhomme *et al.*, 1989, 1994; Auffray *et al.*, 1990; Boursot *et al.*, 1993, 1996; Sage *et al.*, 1993; Prager *et al.*, 1996; Cucchi, Auffray & Vigne, in press).

In the present study, we investigate the mandible morphology of the three subspecies of house mice from both the central and the peripheral regions aiming to appraise the patterns of phenotypic differ-

entiation among the subspecies. More precisely, three main topics of investigation are addressed:

1. What are the patterns of phenotypic variation among different lineages in *M. musculus*? It has been shown that *M. m. musculus* and *M. m. domesticus* are morphologically distinguishable on some characters in Europe (i.e. in the peripheral regions) (Gerasimov *et al.*, 1990; Auffray *et al.*, 1996; Macholan, Krystufek & Vohralik, 2003; Macholan, 2006; Darvish, 2008), although the question of whether this is also the case for *M. m. castaneus* and for the subspecies in central region has not yet been addressed.
2. It is assumed that the three subspecies of house mice have appeared in the central region and that they have dispersed from there through centrifugal diffusion to result in the existent distribution (Boursot *et al.*, 1993, 1996; Bonhomme *et al.*, 1994; Din *et al.*, 1996). Diffusion processes of the house mouse are likely accompanied by founder effects (Gyllensten & Wilson, 1987; Prager *et al.*, 1993) or selective pressures related to niche shift or to the environment of the different regions colonized. Therefore, one could expect that the morphological distance among the three subspecies should increase from the central to the peripheral regions. Alternatively, convergence as a result of commensalism may have played a role and reduced morphological variation between the subspecies. Commensalism with humans for the house mouse is supposed to buffer the environment (Tchernov, 1984), homogenizing adaptive processes in the populations regardless of their geographical location. It has already been proven that some functional characters related to commensalism like aggressive behaviour (Corti & Rohlf, 2001) and diet shift (Renaud, Auffray & de la Porte, 2010) can change the phenotype of the mandible. Therefore, is the diffusion capacity of the three subspecies related to common selective processes (e.g. 'strengthened' commensalism, ability for better social acceptance, smaller size, etc.) that would have involved parallel or convergent evolution from the central to the peripheral regions?
3. We investigated whether the central populations retained an ancestral morphology by comparing these populations to the sister species of the house mouse (*Mus macedonicus*, *Mus spretus*, and *Mus spicilegus*; Lundrigan, Jansa & Tucker, 2002; Guenet & Bonhomme, 2003; Chevret, Veyrunes & Britton-Davidian, 2005). Ancestral character reconstruction predicts that the common ancestor of the house mouse lineages should be morphologically more or less similar to the average morphology of its closest related wild sister species because

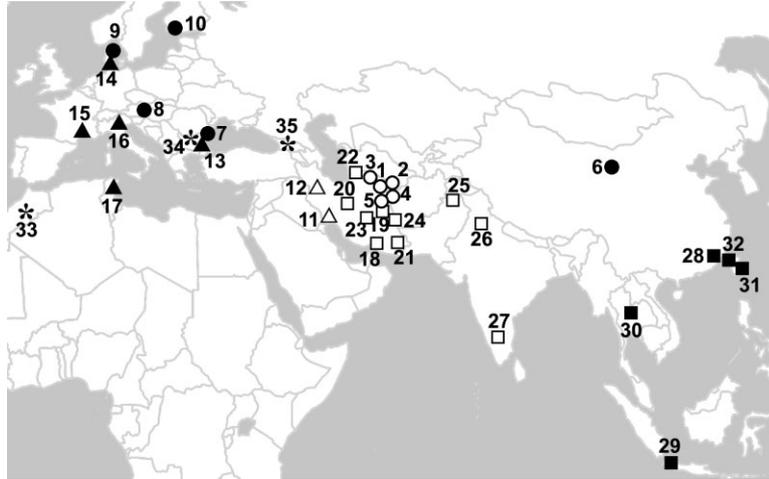


Figure 1. Map representing the localities of the populations studied. The numbers of the localities refer to those in Table 1. Solid circle: peripheral *Mus musculus musculus*; open circle: central *M. musculus musculus*; solid triangle: peripheral *Mus musculus domesticus*; open triangle: central *M. musculus domesticus*; solid square: peripheral *Mus musculus castaneus*; open square: central *M. musculus castaneus*; asterisk: three sister species of the house mouse.

this ancestor was a wild form, whereas *Mus musculus* is mostly known today as commensal populations (Prager *et al.*, 1998). The precise location of the origin of the house mouse in the central region is not yet clearly established (Bonhomme *et al.*, 1994; Boursot *et al.*, 1996; Prager *et al.*, 1998; Milishnikov *et al.*, 2004; Darvish *et al.*, 2006). Therefore, the appraisal of the morphological proximity of the central populations to a reconstructed ancestor may provide some clues in this respect.

MATERIAL AND METHODS

The specimens examined are deposited in the mice collection of ISE-M at the University of Montpellier, France; the Zoology Museum of Ferdowsi University of Mashhad, Iran; the National Museum of Natural Science of Taiwan, and the Natural History Museum of Besançon, France. A total of 808 individuals representing 32 geographical groups (hereafter referred as populations) of house mice from Eurasia and North Africa were studied.

We chose to study the mandible because it is related to diet (Renaud, Auffray & De La Porte, 2010), and because of its relative simplicity as compared to the skull (Klingenberg, Mebus & Auffray, 2003). Only adult specimens with the third molar erupted were considered. Because there is evidence for sexual dimorphism not being significant in murine rodents for such morphological characters (Davis, 1983; Renaud, 2005), both sexes were pooled for the present dataset. The specimens were chosen in such a way as

to represent populations of the three house mouse subspecies from both the central and the peripheral regions (Fig. 1, Table 1). Although the number of populations studied from central region face with an unbalanced sampling (ten for *M. m. castaneus* compared to five for *M. m. musculus* and two for *M. m. domesticus*), we consider that they are sufficiently representative. This is because the two central populations of *M. m. domesticus* originate from the two sides of the Zagros Mountains and may fairly cover the geographical variation in this group. Similarly, the five populations of *M. m. musculus* studied cover the distribution range of this subspecies in Iran. To polarize phenotypic evolution, the average jaw morphology of the three closest relatives of *M. musculus* was estimated from a sample of 15 wild caught individuals for *M. spretus*, *M. spicilegus*, and *M. macedonicus*.

Subspecific attribution of the individuals, regardless of their central or peripheral origin, was based on the mitochondrial (mt)DNA molecular analysis of the previous studies (Ferris *et al.*, 1983; Vanlerberghe *et al.*, 1988a, b; Boursot *et al.*, 1996; Boissinot & Boursot, 1997; Darvish *et al.*, 2006; Rajabi-Maham *et al.*, 2008). The mtDNA sub-lineages assignment of the specimens not previously published will be described elsewhere.

Thirteen two-dimensional landmarks, assumed to be homologous, were defined and digitized on the lateral view of the mandible (Fig. 2). To estimate the measurement error and to avoid the accumulation of measurement error in each population, two replicates of the complete series of images were randomly mixed before the digitization and then re-ordered. Images

Table 1. Sampling localities of the house mouse populations considered in the present study

Taxa	Country/locality	<i>N</i>	Mitochondrial DNA lineage	Sample number
<i>Mus musculus musculus</i>				
Central	Iran/Mashhad (MAS)	24	M (5, 6)	1
	Iran/Sarakhs (SAR)	22	M (5)	2
Peripheral	Iran/Shirvan (SHI)	26	M (6)	3
	Iran/TorbatJam (TOR)	26	M (*)	4
	Iran/Kakhk (KAK)	19	M (5, 6)	5
	China/Ningxia (CH-M)	41	M (*)	6
	Bulgaria/Sokolovo, Kranevo (BU-M)	31	M (3)	7
	Austria/Eisenstadt, Illmitz, Podersdorf (AUS)	20	M (*)	8
	Denmark/Gossmer, Klank, Foulum (DE-M)	53	M (2, *)	9
	Finland/Karjalohja (FIN)	17	D (1)	10
	<i>Mus musculus domesticus</i>			
Central	Iran/Ahvaz (AHV)	30	D (7)	11
	Iran/Hamedan (HAM)	27	D, O (7)	12
Peripheral	Bulgaria/Jitarovo, Prilep, Lozarevo, Karnobat, Bratovo (BU-D)	27	D (7, 3)	13
	Denmark/Egtved, Jerlev (DE-D)	54	D (2, *)	14
	France/ Montpellier, Carnon (FRA)	25	D (*)	15
	Italy/Binasco (ITA)	22	D (*)	16
	Tunisia/Munastir, Teboulba (TUN)	47	D (5)	17
<i>Mus musculus castaneus</i>				
Central	Iran/BandarAbbas (BAN)	8	O (*)	18
	Iran/Birjand (BIR)	41	O (5, 6)	19
	Iran/Espahan (ESP)	13	O (*)	20
	Iran/Iranshahr (IRA)	36	O (*)	21
	Iran/Raz (RAZ)	9	O (*)	22
	Iran/Taft (TAF)	22	O (*)	23
	Iran/Zabol (ZAB)	13	O (5)	24
	Pakistan/Gujarkhan, Rawalpindi, Islamabad (PAK)	15	O (6, 4)	25
	India/Delhi (IN-N)	17	O (6, 4)	26
	India/Masinagudi (IN-S)	8	O (6, 4)	27
Peripheral	China/Jinmen (Fukien Province) (CH-C)	13	O (+)	28
	Indonesia/Djakarta, Bogor (INDO)	8	O (4)	29
	Thailand/Bangkok (THA)	2	O (*)	30
	Taiwan/Chrshang (Taitung) (TW-E)	10	O (+)	31
	Taiwan/Tsaotun (Nantou), Shijou (Changhua) (TW-W)	37	O (+)	32
<i>Mus spretus</i>	Morocco (SPR)	15	–	33
<i>Mus spicilegus</i>	Bulgaria (SPI)	15	–	34
<i>Mus macedonicus</i>	Georgia (MAC)	15	–	35

Sample number and *N* refer to the number of localities as shown in Figure 1 and to the number of specimens studied, respectively. In the mitochondrial (mt)DNA lineage column, ‘M’, ‘D’ and ‘O’ indicate *M. musculus musculus*, *M. musculus domesticus*, and *orientalis* lineages, respectively. It should be noted that there are different mtDNA lineages of *M. musculus castaneus* considered as oriental type. These lineages are all considered as *M. musculus castaneus* in the present study. In this column, values in parenthesis indicate the reference in which these lineages have been presented: 1, Ferris *et al.* (1983); 2, Vanlerberghe *et al.* (1988a); 3, Vanlerberghe *et al.* (1988b); 4, Boursot *et al.* (1996); 5, Boissinot & Boursot (1997); 6, Darvish *et al.* (2006); 7, Rajabi-Maham *et al.* (2008); *unpublished data of F. Bonhomme, A. Orth, and H. Rajabi-Maham; +, unpublished data of H.-T. Yu.

were captured using a charge-coupled device camera installed on a stereomicroscope and connected to a personal computer. The coordinates of each landmark were captured using TPSDIG2.12 (Rohlf, 2008).

Landmarks configurations (two replicates of all individuals) were scaled to unit centroid size and superimposed using the partial Procrustes generalized least-squares method (Gower, 1975; Rohlf, 1990;

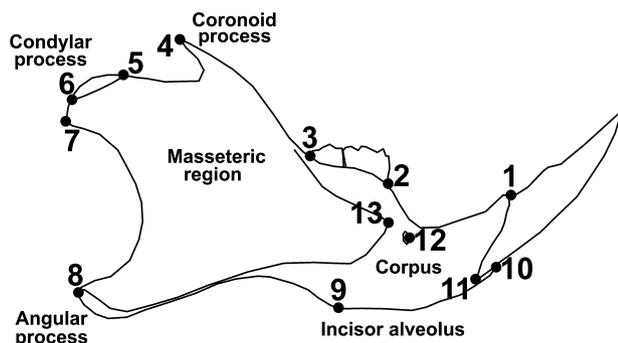


Figure 2. Position of 13 landmarks captured on the lateral view of the mandible.

Rohlf & Slice, 1990) using R software (R Development Core Team, 2009) in accordance with the routines of Claude (2008). Size was computed as the centroid size, which is the square root of the sum of squares of the distances between each landmark and the centroid of the configuration of landmarks (Bookstein, 1991). The coordinates of the superimposed configurations were projected on the Euclidean tangent space and used as shape data for subsequent analyses. A one-way analysis of variance (ANOVA) using the specimen as factor was performed on the centroid size of both replicates to estimate the measurement error on size (Yezerinac, Loogheed & Handford, 1992). In a similar way, a Procrustes ANOVA (Klingenberg & McIntyre, 1998; Klingenberg, Bultuenga & Meyer, 2002) was performed on the shape data taking the specimen effect into account to calculate the percent of measurement error relative to individual variation.

To remove the potential effect of asymmetry, when possible, both the left and right hemi-mandibles of each individual were digitized and, after superimposition, the mean configurations of both sides were calculated. For each individual, the consensus configuration of the landmarks was therefore computed as the mean shape of replicates and sides (Rohlf, 1996) and used for subsequent morphometric analyses.

Shape variables were computed as the non-null principal components of a principal components analysis performed on the individual shapes variance-covariance matrix (Claude *et al.*, 2004). The inter-subspecific and interpopulation shape variations were tested through a nested multivariate analysis of variance (MANOVA) on the first ten components of shape variation (giving more than 87% of the total shape variation) for the central and peripheral populations, separately. These analyses were carried out to test whether morphological differences between subspecies or populations can still be detected even among the central populations that we expect to be morphologically closer to the house mouse ancestor.

In addition, we examined whether the peripheral populations and subspecies differed one from another aiming to investigate the possible effect of convergence or divergence. Variation among subspecies was compared with variation among populations within a given subspecies, and the latter variation was compared with residual inter-individual variation within a given population. Size variation was explored in a similar way (i.e. using nested ANOVAs on centroid size). To test whether the peripheral subspecies were different or not from the central subspecies, we also performed a type II two-way ANOVA and MANOVA on size and shape, using, respectively, the factors of region (central versus peripheral) and subspecies, and considering populations as a random effect.

To assess the geographical effect on mandibular shape variation, the correlation between interpopulation Mahalanobis and geographical distance matrices were compared using a Mantel test (Mantel, 1967) with 10 000 Monte Carlo permutations. This analysis was performed for all the populations of *M. musculus* together and for each subspecies separately.

We tested whether the inter-subspecific or interpopulation morphological differentiations were higher in the peripheral regions or in the central one. For this purpose, variance was decomposed using the Procrustes ANOVA procedure using subspecies and populations as factors (Klingenberg & McIntyre, 1998). The interpopulation variances (mean squares) were then estimated and compared between the central and peripheral populations, as well as between subspecies from the central and peripheral subspecies. The traces of variance-covariance matrices for peripheral and central regions were compared by an *F*-test of the equality of variance, using modified degrees of freedom *sensu* Klingenberg & McIntyre (1998). In this procedure, the usual degrees of freedom were multiplied by 22 for shape (26 coordinates; four dimensions lost as a result of the superimposition process). Size variation was analyzed similarly using a classic *F*-test.

A canonical discriminant analysis was performed on the individual shapes using the population factor to explore the relationship between phylogeography and morphology. Because size variation could possibly explain shape differences, this analysis was performed using two strategies, including and excluding allometry. The Burnaby method was used to remove allometry from the shape data based on the allometric vector of the largest sample for each subspecies (Burnaby, 1966; Claude, 2008). The phenotypic similarity among the populations was analyzed via the ordination of populations on the first two canonical axes and via a minimum spanning tree on the Mahalanobis distances between populations (*sensu* Claude, 2008). The jaw configurations of *M. spretus*,

M. spicilegus, and *M. macedonicus*, which are the closest relatives of *M. musculus*, were also included in these ordination analyses to polarize phenotypic evolution on the basis of multiple outgroup comparisons.

RESULTS

The measurement error is 1.1% for size (ANOVA) and 4.8% for shape variation (Procrustes ANOVA), and is significantly lower than inter-individual variation.

In the central and the peripheral regions (Table 2), inter-subspecific size differentiation is significantly larger than interpopulation size variation within a given subspecies (marginally significant for the central region). Size differences among populations are also significantly larger than inter-individual variation. This is not the case for shape variation for which the inter-subspecific differentiation is not significantly higher than the variation among populations in the central region, whereas it remains significant for the peripheral ones. Interpopulation shape variation, however, is significant for both the central and peripheral regions (Table 3).

The size variation among subspecies in the peripheral regions is approximately ten-fold greater than

that in the central region, although it is not statistically significant (Table 4). This nonsignificance can be partly artefactual because the degrees of freedom in the *F*-test for both numerator and denominator are as small as two (i.e. three subspecies). Shape variation between taxa is also larger in the peripheral regions than in the central region. This suggests that differentiation in size and shape between subspecies increased from the central to the peripheral regions. The interpopulation variation is also significantly larger among the peripheral populations than among the central ones for both size and shape (Table 4).

The type II two-way ANOVA and MANOVA subspecies \times region (central versus peripheral) on size and shape variables, respectively, suggest that the three subspecies are significantly different for both size and shape (Tables 5, 6). However, the significant differentiation of the central and peripheral groups and the significant interaction between subspecies and region show that the size and shape evolution from the central to the peripheral groups is different between subspecies. In other words, the three subspecies evolved differently from the central to the peripheral regions.

Table 2. Inter-subspecific and interpopulation size variation: nested analysis of variance of individual centroid size in central and peripheral populations (upper and lower parts of the table, respectively) of *Mus musculus* studied, taking the factor subspecies and populations into account

Effect	d.f	Sum of squares	Mean squares	<i>F</i>	<i>P</i>
Central populations					
Subspecies	2	0.0072	0.0180	3.8083	0.0478
Individual	338	2.6087	0.0077		
Peripheral populations					
Subspecies	2	0.0733	0.83830	10.445	< 0.0024
Individual	399	2.5355	0.00635		

Table 3. Inter-subspecific shape variation: multivariate analysis of variance of the shape principal components in central and peripheral populations (upper and lower parts of the table, respectively) of *Mus musculus* studied, taking the factor subspecies and populations into account

Effect	d.f. (num)	d.f. (den)	Pillai	Approximated <i>F</i>	Approximated d.f. (num)	Approximated d.f. (den)	<i>P</i>
Central populations of <i>Mus musculus</i>							
Subspecies population	2	14	1.539	2.004	20	12	> 0.1
Population intrapopulation	14	338	1.438	4.053	140	3380	< 10 ⁻¹⁵
Peripheral populations of <i>Mus musculus</i>							
Subspecies population	2	12	1.905	8.014	20	8	< 0.003
Population intrapopulation	12	394	2.867	11.311	120	3940	< 10 ⁻¹⁵

d.f. (num) and d.f. (den) refer to numerator and denominator degrees of freedom, respectively. Approximated *F* is based on inter- and intra-group variance.

Table 4. Comparison of inter-subspecies and interpopulation shape and size variances between central and peripheral taxa

<i>F</i> -test	MS (num)	MS (den)	Approximated <i>F</i>	d.f. (num)	d.f. (den)	<i>P</i>
Size						
Inter-subspecies	0.0733	0.0072	10.1800	2	2	0.089
Interpopulation	1.2275	0.2780	4.4142	14	12	< 0.006
Shape						
Inter-subspecies	0.0013	0.0006	2.1159	44	44	< 0.008
Interpopulation	0.0098	0.0054	1.8227	308	264	< 10 ⁻⁶

The numerator (num) and denominator (den) values are the values of peripheral and central taxa, respectively. MS, mean square.

Table 5. Type II analysis of variance of subspecies × region (central versus peripheral) on mandible centroid size in the house mouse

Effect	d.f. (effect)	d.f. (residual)	Sum of squares	MS	<i>F</i>	<i>P</i>
Subspecies	2	26	0.0435	0.0217	10.206	< 0.0006
Region	1	26	0.0495	0.0495	23.255	< 10 ⁻⁴
Subspecies × region	2	26	0.0371	0.0185	8.704	< 0.002

Table 6. Type II multivariate analysis of variance of subspecies × region (central versus peripheral) for the mandible shape in the house mouse

Effect	d.f. (effect)	d.f. (residual)	Pillai	Approximated <i>F</i>	Approximated d.f. (effect)	Approximated d.f. (residual)	<i>P</i>
Subspecies	2	26	1.672	9.178	20	36	< 10 ⁻⁸
Region	1	26	0.834	8.523	10	17	< 10 ⁻⁴
Subspecies × region	2	26	1.130	2.340	20	36	< 0.01

The largest mandibles correspond to the central populations of *M. m. domesticus*, and the smallest ones to the peripheral populations of *M. m. castaneus*. The central populations of *M. m. castaneus* show a significantly larger mandible than the peripheral ones [Tukey's honestly significant difference (HSD), $P < 10^{-7}$]. This rule is also observed, to a lesser although still significant extent, between the central and peripheral populations of *M. m. domesticus* (Tukey's HSD, $P < 0.001$). *Mus musculus musculus* of the central and peripheral regions show similar mandible sizes. In the central region, *M. m. musculus* displays a smaller mandible than *M. m. domesticus* (Tukey's HSD, $P < 0.0001$) but the same size as the one of *M. m. castaneus* (Fig. 3).

Based on the canonical discriminant analysis on the mandible shape, the three subspecies occupy different positions in the first canonical plan (explaining 36% of within population variation; Fig. 4). However,

it is not the case for central *M. m. castaneus*, which shows a high variation and overlaps with central and peripheral *M. m. domesticus* and peripheral *M. m. musculus*. As expected from the results reported above, the central populations are distinct from the peripheral ones for all three subspecies. The ordination of populations of *M. m. castaneus* is particularly interesting. The central populations of *M. m. castaneus* appear to occupy two positions in the shape space: one corresponding to the Indian populations, closer to the peripheral *M. m. castaneus* populations, and one corresponding to those on the Iranian plateau. The latter one, however, is still much variable and overlaps with peripheral *M. m. musculus* and central and peripheral *M. m. domesticus*.

The three sister species of house mice (*M. spretus*, *M. spicilegus*, and *M. macedonicus*) are close to the Iranian *M. m. castaneus* and the peripheral *M. m. musculus*. These results are well confirmed by

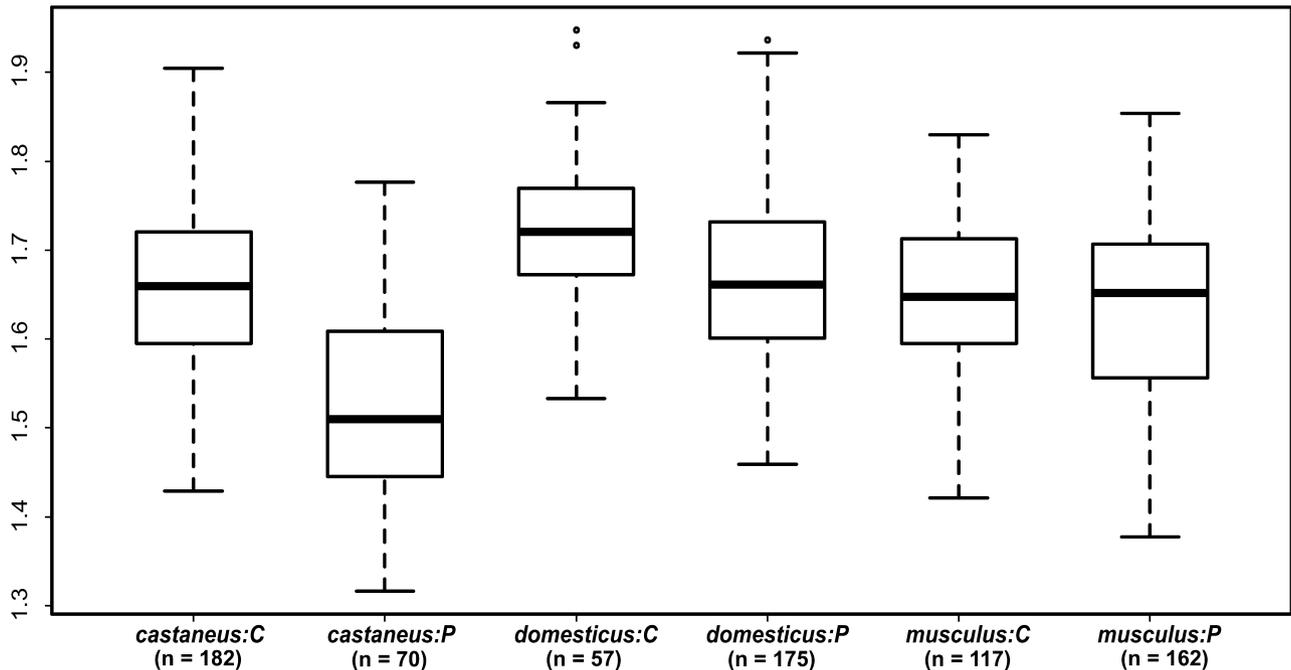


Figure 3. Box plots of the centroid size of the mandible for the three different subspecies in both the central (indicated by C) and peripheral (indicated by P) regions. *n*, number of specimens examined for each group.

the minimum spanning tree on the Mahalanobis distance among populations (Fig. 5). The tree also shows that *M. m. musculus* and *M. m. domesticus* represent two rather homogeneous taxa and that *M. m. castaneus* appears to be more polymorphic. The relationship between mandible shape distances and geographical distances among all populations of house mouse was significant ($r = 0.535$; Mantel test $P < 0.0001$). The same result was observed when the analysis was performed for each subspecies separately: *M. m. castaneus* ($r = 0.667$, Mantel test $P < 0.0001$), *M. m. domesticus* ($r = 0.513$, Mantel test $P < 0.01$), *M. m. musculus* ($r = 0.480$, Mantel test $P < 0.04$).

Removing allometry for the canonical discriminant analysis did not change the main pattern of distribution of the populations (results are not shown but are available upon request). Variation as a result of size is thus not responsible for ordination of populations according to mandible morphology.

Figure 4 shows the pattern of shape variation among populations. The major shape changes associated with the first canonical axis are localized in the angular and condylar processes, the corpus and the length of *incisor alveolus* (morphological terms of mandible *sensu* Atchley, [1993]). For all subspecies, the peripheral populations have superior values on LD1 than the central populations. This would suggest that the peripheral groups present a longer incisor alveolus, narrower corpus, shorter and wider condylar

process, and longer angular process than their respective central counterparts.

Interestingly, the second discriminant axis mainly distinguishes some populations located at the extreme of the colonization processes of two of the subspecies (i.e. the Scandinavian populations of *M. m. musculus* for positive values of LD2, and the Chinese and Taiwanese populations of *M. m. castaneus* for negative ones), which is also shown by the minimum spanning tree (Fig. 5). These shape variations take mostly place in the coronoid and condylar process, corpus width and the incisor alveolus position.

DISCUSSION

INTER-SUBSPECIFIC DIFFERENTIATION

Mus is a very conservative genus in terms of skull and teeth morphology and the different species are hardly distinguishable by simple characters (Auffray & Britton-Davidian, in press). The use of geometric morphometrics and related statistics has allowed establishing subtle but significant differences between species (Gerasimov *et al.*, 1990; Macholan *et al.*, 2003; Macholan, 2006). The distinction between some *M. musculus* subspecies was formerly established on cranial or dental structures by Gerasimov *et al.* (1990), followed by several other studies (Auffray *et al.*, 1996; Macholan *et al.*, 2003; Darvish, 2008). The present study shows that the three sub-

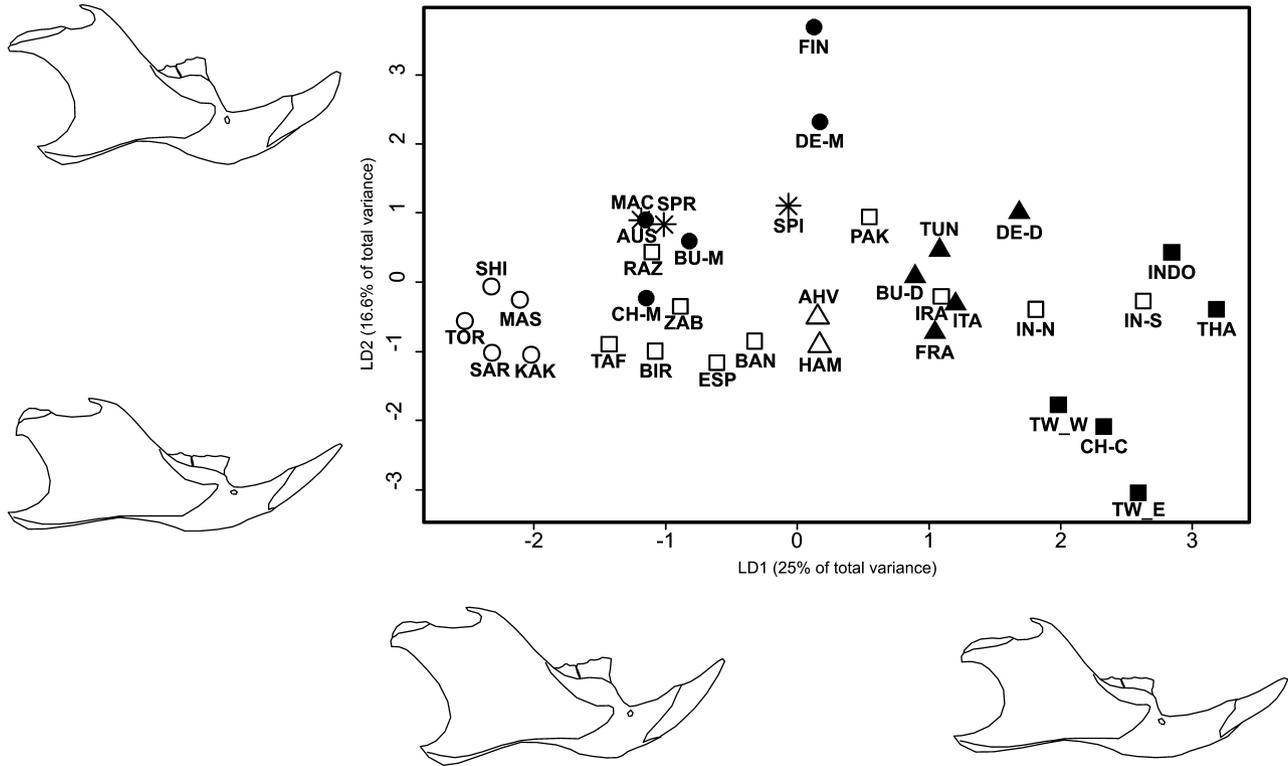


Figure 4. Ordination of the 35 populations of the house mouse (including the three sister species of *Mus musculus*) on the first two discriminant factors (LD1 and LD2) of canonical analysis of shape coordinates. Only the mean values for each population are plotted. Extreme shape changes associated with each axis were amplified twice. Solid circle: peripheral *Mus musculus musculus*; open circle: central *M. musculus musculus*; solid triangle: peripheral *Mus musculus domesticus*; open triangle: central *M. musculus domesticus*; solid square: peripheral *Mus musculus castaneus*; open square: central *M. musculus castaneus*; asterisk: three sister species of the house mouse.

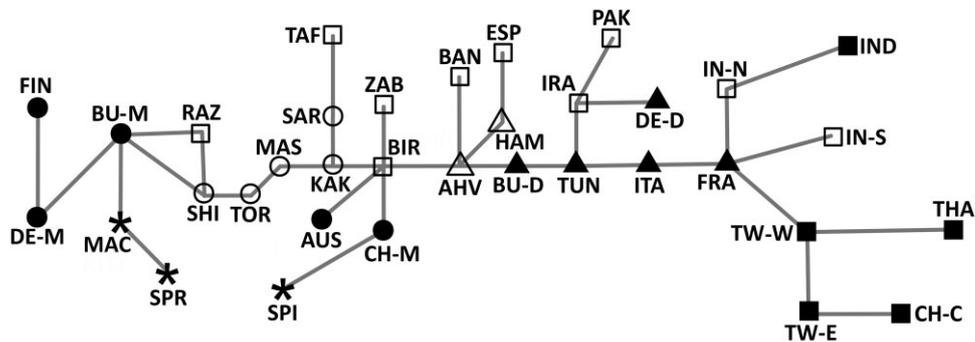


Figure 5. The minimum spanning tree based on the Mahalanobis distance between populations. The length of the connecting lines is proportional to Mahalanobis distance between the populations. Symbols are the same as in Figure 1 and abbreviations are as in Table 1.

species can be distinguished on the basis of their mandibular morphology. The discrimination is clearly shown in the peripheral populations for both size and shape, although it fails to be significant in the central populations where, even if size variation is shown to be marginally significant among subspecies,

shape variation remains nonsignificant. This lack of differentiation may be explained in different, although non-mutually exclusive, ways. First, the central region is the place of origin of the differentiation process. In comparison with the peripheral regions, sufficient gene flow could have been main-

tained there to prevent the discrimination of the taxa. Another explanation is that variation is high within the so-called *M. m. castaneus* in the central region (Figs 4, 5) and may prevent the discrimination between the three subspecies to be significant. Indeed, it appears that the central populations referred to *M. m. castaneus* likely represent at least two morphological groups. The first one encompasses the Indian and Southeast Asian populations whereas the second one includes the Iranian and Pakistani populations. However, the latter is not fully uniform and can be split into two different sub-clusters: one in the centre and east of the Iranian plateau, and the other one in Southeast Iran and at the border of the Persian Gulf. Although, some parts of this high variation in *M. m. castaneus* may be a result of the unbalanced geographical samples from central region (ten populations in comparison with five and two populations of *M. m. musculus* and *M. m. domesticus*, respectively), the difference between central and peripheral *M. m. castaneus* is highly significant to not be simply explained by this effect. This observation confirms that *M. m. castaneus* is a polyphyletic subspecies, as suggested by biochemical and molecular analyses demonstrating that *M. m. musculus* and *M. m. domesticus* are much more uniform than *M. m. castaneus* (Boursot *et al.*, 1993, 1996; Boissinot & Boursot, 1997; Darvish *et al.*, 2006; Baines & Harr, 2007; Bonhomme *et al.*, 2007; Darvish, 2008; Geraldès *et al.*, 2008).

From a morphological point of view, the peripheral populations of *M. m. musculus* are characterized by a short angular process, short incisor alveolus, wide corpus, long condylar process and intermediate size of the mandible. The peripheral populations of *M. m. castaneus* present a long angular process, long incisor alveolus, narrow corpus, short condylar process, and small mandible size. The peripheral *M. m. domesticus* populations are characterized by intermediate shape characters and the largest mandible size.

DISPERSION AND MORPHOLOGICAL DIVERGENCE

As stated above, variation among subspecies and among populations within subspecies is much higher for both size and shape in the peripheral regions than in the central one. The variation of mandible size among subspecies in the peripheral region is ten-fold greater than the one of the central region, and four-fold greater when considering interpopulation variation. For shape, inter-subspecies and interpopulation variances are approximately two-fold greater in the peripheral regions than in the central one. In other words, the morphological differentiation of subspecies has much increased during the dispersion from the

central region to the peripheral ones. Mantel tests show that, in the house mouse and for each subspecies, the morphological differentiation is paralleled with geographical distances, and therefore that it likely increased during the dispersion of the house mouse. The divergent evolution of the three subspecies confirms a centrifugal sub-speciation within this species in which the three subspecies originated somewhere in the central region from where they expanded to the rest of the world through different pathways (Boursot *et al.*, 1993, 1996; Din *et al.*, 1996). This may be a result of the diversity of the peripheral regions colonized by the house mouse or to the successive founder effects that occurred and accumulated during the house mouse expansion. Increased phenotypic variance or transgressive segregation in hybrid populations between subspecies (Auffray *et al.*, 1996; Renaud, Alibert & Auffray, 2009) may also partly explain an increase in morphological variation among peripheral populations within subspecies. This effect, however, should be limited in the present study, because the four populations that come from secondary contact zones (populations 7, 9, 13, and 14 as used in the present study) do not exhibit more than 6% of diagnostic alleles of other subspecies, suggesting a low level of introgression among subspecies for these populations.

The significant interaction between subspecies and region on both size and shape demonstrates that the morphological changes from central to peripheral populations are different among subspecies and do not favour any hypotheses of convergence among subspecies. Furthermore, the increasing variation within and among subspecies in the peripheral regions compared to populations of the same subspecies in the centre of the range demonstrates that commensalism did not involve a phenomenon of convergence in mandible shape variation. In the same respect, the positions of the outgroups in the canonical discriminant plan (Fig. 4) are not found outside of the morphological variation of the three peripheral subspecies, confirming that no convergence occurred. Therefore, the evolution of mandible morphology during the diffusion of each subspecies may be related either to different selective pressures or, more simply, to the effects of genetic drift, which lead to the morphology of each subspecies distinguishing itself from the other ones. Founder effects have already been recognized for the colonization of the Scandinavian house mice (Gyllensten & Wilson, 1987; Prager *et al.*, 1993). For these Scandinavian populations in particular, we did indeed find different morphology than what was expected (differentiation along LD2 rather than LD1). In our sample, a divergent pattern has also been observed for the Taiwanese and Chinese *M. m. castaneus* that could probably be related to local selection or founder effects.

ORIGIN

In the present study, the central populations of *M. musculus* display mandible morphologies close to those of the three sister species, suggesting that the central populations would retain more ancestral traits than the ones in the peripheral ones. Moreover, the assumed ancestral morphology is closer to that of *M. m. musculus* (either central or peripheral) compared to other subspecies. The most derived morphology appears to be that of the peripheral *M. m. castaneus*.

For a long time, the Indian subcontinent has been considered as the cradle of the house mouse as a whole (Boursot *et al.*, 1993, 1996; Bonhomme *et al.*, 1994; Din *et al.*, 1996) or as the place of origin of the subspecies *M. m. castaneus* and *M. m. musculus* (Prager *et al.*, 1998). In the present study, the house mice from India present a different mandible morphology than the other samples of the central region, although they are comparable to the *M. m. castaneus* from the peripheral regions. It may suggest that either Indian house mice have not retained the ancestral morphology or that ancestral-like mice did not exist in India, or that they may have existed in the past but subsequently disappeared from this area; further sampling is however needed in this region. By contrast, the populations from Iran are closer to the ancestral morphology, advocating that the origin of the house mouse could be located on the Iranian plateau or, at least, that this region is a place where the ancestral morphology was preserved.

The Iranian plateau is also the only geographical place where the three subspecies are found, a condition that could have been preserved after the centrifugal divergence from the ancestral populations. However, it may also result from a recent re-admixture of some of the subspecies, as shown by Darvish *et al.* (2006) and Rajabi-Maham (2007). It remains difficult, on the basis of morphological differentiation, to appraise the nature of the contact: primary, secondary or a mosaic of both. Indeed, the complex topographic features of the Iranian plateau (Misonne, 1959) could provide a relevant geographical frame for a differentiation process: the Zagros Mountains in West Iran could have acted as a barrier between *M. m. domesticus* and *M. m. castaneus* (Prager *et al.*, 1998); a past great lake having covered the central desert of Iran until approximately 10 000 years ago (Nazari *et al.*, 2010) and the mountain chains in Northeast Iran (Darvish *et al.*, 2006) could act as partial geographical barriers between *M. m. musculus* and the *M. m. castaneus* populations of the Iranian plateau.

The findings of the study do not make it possible to decide whether India or the Iranian plateau is the

place of the origin of the house mouse differentiation process, although the proximity of the Iranian populations and the putative ancestral morphology, as well as the apparent derived morphology of the Indian *M. m. castaneus*, should not allow us to exclude the Iranian plateau as a candidate cradle for the house mouse.

ACKNOWLEDGEMENTS

The material of *M. m. musculus* of China was loaned by Jean-Pierre Quéré and was collected during the research supported by grant number ROI TW001565 from the Fogarty International Center, European Union (TSC-CT94-0270). We thank Yen-Jean Chen from National Museum of Natural Science in Taiwan who kindly sent us some of the specimens under her care for examination. Ylenia Chiari is gratefully acknowledged for her comments that improved the manuscript. We also thank Annie Orth and Jean-Marc Duplantier for their help in providing some of the specimens and also for their advice during the study. This is the publication number 2011-157 of Institut des Sciences de l'Evolution de Montpellier (ISE-M).

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