ROYAL SOCIETY OPEN SCIENCE

royalsocietypublishing.org/journal/rsos

Research

Cite this article: Renaud S *et al*. 2026 Bioarchaeological evidence for hybridization between house mouse subspecies in early Neolithic Iran. *R. Soc. Open Sci.* **13**: 251645.
https://doi.org/10.1098/rsos.251645

Received: 28 August 2025
Accepted: 16 January 2026

Subject Category:
Ecology, conservation, and global change biology

Subject Areas:
evolution, environmental science

Keywords:
Geometric morphometrics, *Mus musculus*, bioarchaeology, commensalism

Author for correspondence:
Sabrina Renaud
e-mail: sabrina.renaud@univ-lyon1.fr

Supplementary material is available online at https://doi.org/10.6084/m9.figshare.c.8317562.

THE ROYAL SOCIETY PUBLISHING

Bioarchaeological evidence for hybridization between house mouse subspecies in early Neolithic Iran

Sabrina Renaud[1], Paul Clarkson[2], Katerina Papayianni[3], Emilie A. Hardouin[2], Lisa Yeomans[4,6], Pernille Bangsgaard[4], Hojjat Darabi[7], Tobias Richter[5], Ellen Hambleton[2], Paul Alibert[8], Jean-Christophe Auffray[9], Thomas Cucchi[10] and Emma Jenkins[2]

[1]UMR 5558 CNRS, Universite Claude Bernard Lyon 1, Villeurbanne, Auvergne-Rhône-Alpes, France
[2]School of Life and Environmental Sciences, Bournemouth University, Poole, UK
[3]The Malcolm H. Wiener Laboratory for Archaeological Science, American School of Classical Studies at Athens, Athens, Attica, Greece
[4]Globe Institute, and [5]Department of Cross-Cultural and Regional Studies, Faculty of Humanities, University of Copenhagen, Copenhagen, Denmark
[6]Institute of Archaeology, University College London, London, UK
[7]Department of Archaeology, Razi University, Kermanshah, Kermanshah Province, Iran
[8]Biogéosciences, Université de Bourgogne, Dijon, Bourgogne-Franche-Comté, France
[9]Institut des Sciences de l'Evolution, Université de Montpellier, Montpellier, Occitanie, France
[10]BioArch—BioArchéologie, Interactions Sociétés environnements, Museum National d'Histoire Naturelle, Paris, Île-de-France, France

SR, 0000-0002-8730-3113; PC, 0009-0002-5751-2448; KP, 0000-0002-3132-1945; EAH, 0000-0002-2031-5160; LY, 0000-0002-5180-8902; PB, 0000-0002-8517-6165; HD, 0000-0003-1628-5090; TR, 0000-0001-9902-8852; EH, 0000-0002-6480-437X; PA, 0000-0003-2961-2855; J-CA, 0000-0002-5184-0507; TC, 0000-0001-6021-5001; EJ, 0000-0002-3483-5749

House mice (*Mus musculus*) have been associated with humans since the beginning of sedentism, enabling them to become successful global colonisers. Three main subspecies originated approximately 0.5 Ma in a region extending from Southwest Asia to northern India. Molecular data suggest that a complex scenario of secondary admixture occurred thereafter in the Iranian region, leading to the formation of a Central Iranian lineage, but this evidence was overlooked in previous bioarchaeological analysis. The early Neolithic settlement of Ganj Dareh is located in this cradle area.

It delivered remains of commensal house mice formerly attributed to *M. m. domesticus*. A geometric morphometric analysis of the first lower molars is used here to characterize the signature of hybridization between *M. m. musculus* and *M. m. domesticus*. The subspecific attribution of the Ganj Dareh mouse remains is re-evaluated through the inclusion of modern specimens from Central Iran as a separate group in the reference dataset. The results indicate that, contrary to what was previously thought, the Ganj Dareh specimens are likely related to the Central Iranian lineage. Their idiosyncrasy compared with modern representatives, however, suggests a complex temporal dynamic of admixture, which may have been influenced by early human settlements and movements.

1. Introduction

House mice (*Mus musculus*) have been associated with humans since the beginning of sedentism, adapting to anthropogenic environments where they outcompete other species [1–5]. This close relationship enabled them to act as stowaways on human-mediated transport [6] and to become second only to humans as global colonisers [7]. The process left genetic signatures in modern mouse populations, thereby providing insights into former patterns of human-mediated travel and exchange [8–13]. For example, the evidence of a Northern European mitochondrial signature in house mice from Madeira suggested that the Vikings visited this island, unintentionally transporting mice [11,14,15]. The hypothesis was subsequently tested with radiocarbon dating, confirming that house mice were present on the island during the Viking period, thus preceding the Portuguese colonization [16]. This example illustrates the potential of integrating archaeological and natural science approaches to unravel the dynamics of human–mouse interactions [17,18].

While ancient DNA provides critical insights, morphological analysis remains a fundamental source of information about past animals, allowing researchers to trace complex evolutionary history, including rapid responses to human-driven changes [19–21]. When studying small mammals, particularly rodents, molars are the main element used for taxonomic identification. The development of geometric morphometric (GMM) methods applied to the outline of the first lower molar (m1) has proved efficient in distinguishing between wild and commensal mice within the genus *Mus*, and even in separating the three subspecies of commensal *M. musculus* [2]. The application of GMM on the m1, relying on modern reference datasets and applied to archaeological material, allowed researchers to trace the dynamics of house mouse colonization of Southwest Asia and Europe in association with the progression of sedentism and agriculture [2]. In Eurasia, the Western subspecies, *M. m. domesticus*, which today occupies West and Central Europe and the entire Mediterranean area, expanded from Southwest Asia northwards and westwards, via Cyprus as early as *ca* 9100–8600 BC, accompanying the first agropastoral societies migrating from the mainland [22,23]. The subspecies *M. m. musculus*, nowadays occupying Eastern Europe to Northern Asia, followed a route of progression along the Danube and thus colonized northern and eastern areas of Europe [2,24]. These subspecies eventually came into secondary contact, creating a hybrid zone extending from Denmark to Bulgaria [25–29]. This narrow tension zone is considered to be maintained by a balance between selection against hybrids and dispersal [30,31]. The third subspecies, *M. m. castaneus*, accompanied human populations in their eastward progression, leading to its current distribution in Southeast Asia. Although less well understood due to limited archaeological research in this region, recent genetic data provide evidence for an association between *M. m. castaneus* and the emergence of agricultural societies in Southeast Asia [12].

These three subspecies are believed to have originated *ca* 0.5 Ma in a 'cradle' region extending from Southwest Asia to northern India [32,33], where they still coexist today [8,34,35]. From this cradle, each subspecies diverged successively during interglacial stages, starting approximately 300 ka [36–38], evolving into 'peripheral' populations along their respective dispersal route [39]. However, the evolutionary history of *M. musculus* appears even more complex: recent whole-genome studies revealed an additional lineage in the Himalaya, now assigned to the new subspecies *M. m. gyirongus*, alongside intricate hybridization patterns, e.g. in the Nepalese population from Sudurpashchim [36] (cf. figure 1). These findings underscore that admixture and introgression have been recurrent processes in house mouse evolution, shaped by both natural dispersal and human-mediated expansion.

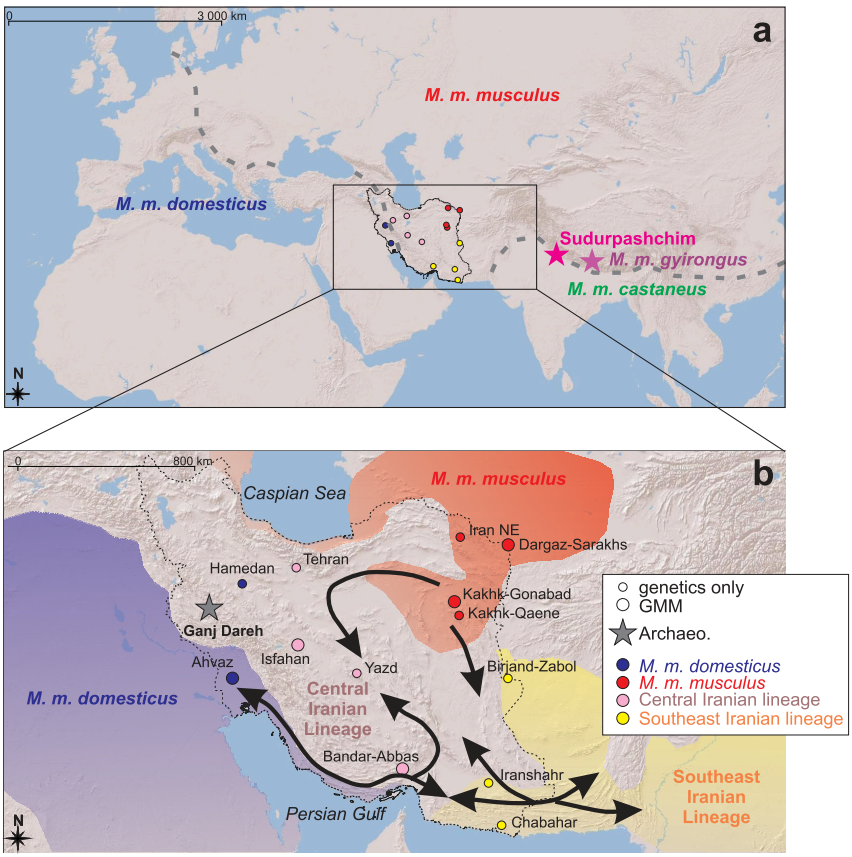

Figure 1. Map of the Iranian region, with the extension of the *M. musculus* subspecies and lineages, and the location of the archaeological site of Ganj Dareh. (a) General location and distribution of the four house mouse subspecies and of the hybrid Nepalese population from Sudurpashchim [36]. Thick grey dashed lines show the boundaries between subspecies. (b) The Iranian region with the presumed extension of the different subspecies and lineages. Genetic identification of each modern locality is based on microsatellite DNA data [34]. Arrows represent migration routes during Pleistocene interglacial periods [40]. The dotted line shows the current Iranian border.

A complex scenario of secondary admixture also occurred in the Iranian region [40]. This phase of gene flow between the subspecies is estimated to have occurred approximately 200 000 generations ago, thus clearly predating the onset of house mouse commensalism evidenced from 15 000 BP in the Levant [5] and was probably due to natural dispersal during interglacial periods [2,40]. This has left a complex genetic signature in the Iranian region. Mouse populations from Central Iran exhibit mitochondrial DNA (mtDNA) that is related to *M. m. castaneus* [41,42]. In terms of microsatellite variation, mouse populations from Central Iran and Southeast Iran possess distinct signatures. The Central Iranian population, referred to here as the 'Central Iranian lineage', is intermediate between the three subspecies [34] and is likely to be the result of an admixture between the *domesticus* and the *musculus* lineages [40,43], possibly with an early input of *M. m. castaneus*, which provided the aforementioned mitochondrial signature [42].

The evolutionary history of the Central Iranian lineage is thus based only on molecular analyses of modern populations. Archaeological data may provide an original insight into the temporal dynamics of admixture, provided that hybridization between the house mouse subspecies left a traceable phenotypic signature. Understanding past spatial and temporal distributions of these hybrids could potentially be used as a proxy for human trade and migration networks, expanding our understanding of past human connectivity.

Indeed, one of the most important archaeological sites for understanding house mouse commensalism—Ganj Dareh—is located in the Central Zagros of Western Iran, close to the distribution area of both *M. m. domesticus* and the Central Iranian lineage (figure 1). This site is a sedentary settlement dated to the Neolithic (8200–7600 cal. BC) [44] and comprised tightly packed structures made from clay, mud-brick and wood [45]. It is renowned for having the earliest evidence for goat domestication in the world [46,47] and is one of the first sites where it was suggested that the degree of sedentism

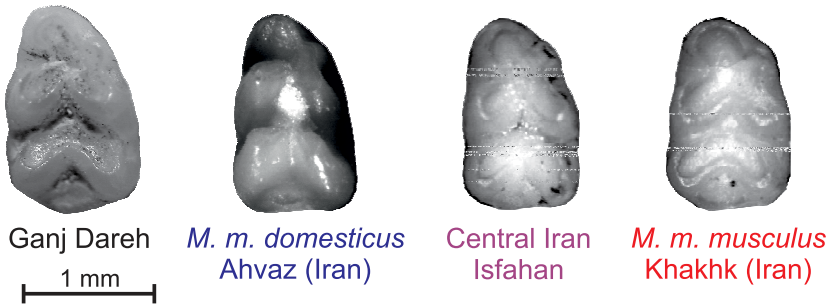

Figure 2. Examples of right first lower molars (m1) from the archaeological deposit of Ganj Dareh, and modern specimens from Iranian localities attributed to the two subspecies *M. m. domesticus* and *M. m. musculus*, and to the Central Iranian lineage.

could be inferred from the density of house mice [3]. Previous GMM analysis of house mice m1s from Ganj Dareh confirmed the presence of commensal *M. musculus* [2], making this assemblage a good candidate to trace early phases of admixtures between Iranian clades of house mice, even though such events may predate human-mediated dispersal.

The aforementioned GMM analysis further identified the Ganj Dareh m1s as *M. m. domesticus* [2]. However, this study did not consider the Central Iranian lineage as a separate group in the comparative dataset, potentially impacting identification. Only a few localities with genetic data inform about the current distribution of the house mouse subspecies and lineages in Iran [34,41], and Ganj Dareh appears to be located close to the occurrence of both *M. m. domesticus* and the Central Iranian lineage (figure 1) [34,43]. Therefore, it is unclear whether the Ganj Dareh mice really correspond to 'true' *M. m. domesticus* or if they are in fact part of the Central Iranian lineage.

To capture this complex process in the bioarchaeological record, it is necessary to characterize and understand the morphological signature of hybridization. The characteristics of hybrids between *M. m. domesticus* and *M. m. musculus* have been described for the mandible [48,49] and the first upper molar (M1) [50] based on a cross between wild-derived strains. In both cases, the hybrids exhibit an intermediate phenotype between the two parental groups, but with a 'transgressive component', setting them apart from the expected midway shape between the parental groups. While the dominance towards the larger parental size is consistent across all traits considered, there is no clear pattern of dominance in shape. The mandible, for instance, displays a mosaic of traits that can show a dominance towards the *domesticus* or the *musculus* parental strain [49]. The signature of hybridization on the m1 has not yet been described.

The aim of this study is to re-evaluate the subspecific identification of the Ganj Dareh mouse remains using GMM analysis of the m1 (figure 2), incorporating *M. m. musculus*, *M. m. domesticus* and the Central Iranian lineage [34] as reference groups. The research hypotheses were twofold. Firstly, hybrid molars from wild-derived strains should be morphologically intermediate between the parental groups, with a transgressive component. Secondly, if Ganj Dareh house mice are part of the Central Iranian lineage, they should be morphologically intermediate between *M. m. domesticus* and *M. m. musculus* and closer to the Central Iranian lineage than to either of these subspecies.

2. Material and methods

2.1. Material

2.1.1. Hybridization between wild-derived strains

The two parental strains were bred from wild-trapped animals. The western European house mouse *M. m. domesticus* was represented by the so-called WLA strain, derived from mice caught near Toulouse (France) in 1976. The Eastern European subspecies *M. m. musculus* was represented by the so-called PWK strain, derived from mice trapped in Prague (Czech Republic) in 1982. Both strains have been established and maintained by brother/sister matings at the Institut Pasteur (Paris, France) to obtain inbred wild-derived mouse strains, which were later housed at the Conservatoire de la Souris Sauvage (ISE-M, France). The first-generation (F1) hybrids were bred

Table 1. *Mus musculus* first lower molars (m1) included in the study.

dataset	subspecies/lineages	code	strain/locality	*N* m1
WLA x PWK cross	*M. m. domesticus*		WLA	33
	M. m. musculus		PWK	24
	F1 hybrids		F1	38
reference	Central Iran	CEI	Bandar Abbas	16
	Central Iran	CEI	Isfahan	34
	M. m. domesticus	DOM	Ahvaz	22
	M. m. musculus	MUS	Dargaz+Sarakhs	38
	M. m. musculus	MUS	Kakhk+Gonabad	11
archaeological		GD	Ganj Dareh	48

from the WLA and PWK parental groups. Outlines of the m1 of 33 WLA, 24 PWK and 38 hybrids were included in the present analysis.

2.1.2. Ganj Dareh and the Iranian region

As outlined above, Ganj Dareh is an internationally renowned early Neolithic site that was originally excavated in the 1960s and 1970s under the direction of Canadian archaeologist Smith [45] and was re-excavated in 2017 and 2018 by an Iranian-Danish team led by Darabi & Richter [44]. A former morphometric study on *Mus* m1s from Smith's excavation showed that the m1 shape was typical of a commensal species and closer to *M. m. domesticus* than to the other subspecies [2]. The present analysis was focused on comparing Ganj Dareh specimens from the excavations led by Darabi and Richter to modern referentials restricted to commensal mice from Iran (see electronic supplementary material, table 1, for details on the archaeological specimens).

The modern reference specimens are derived from the MouseTrack project dataset [2], and only modern localities from Iran have been retained (table 1; figure 1). Given its geographic location, Bandar Abbas and Isfahan, previously considered as *M. m. castaneus*, have been attributed to the 'Central Iranian lineage' based on microsatellite DNA data [34]. According to the same reasoning, the locality of Birjand, previously considered as *M. m. musculus*, should be considered as belonging to the 'Southeast Iran' lineage (see [34] for a discussion about this lineage). However, as this lineage was only represented by two specimens, it has been removed from the analyses.

The sampling comprised 95 m1s corresponding to the WLA × PWK cross, and 48 archaeological specimens from the Ganj Dareh excavations led by Darabi and Richter. These archaeological specimens were compared to 121 modern m1s, which were all wild-trapped and genetically identified [2,8]. For modern material, one m1 per skull was measured, preferably the right one, where available. All available m1s were included in the analysis of the archaeological material. Pictures of left m1s were mirrored for comparability.

2.2. Methods

2.2.1. Geometric morphometrics

The set of 64 points delineating the occlusal surface of the m1 was analysed as sliding semi-landmarks [24]. The outline points were adjusted using a general Procrustes analysis (GPA) procedure, while during the superimposition, semi-landmarks were allowed to slide along their tangent vectors until their positions minimized the shape difference between specimens, the criterion being bending energy following recommendations of a previous study [2]. Only the starting point of the outline, located at the anterior part of the tooth, was treated as a true landmark. The GPA was performed using the R package geomorph [51]. The size of the molar was estimated by the centroid size (i.e. the square root of the sum of the squared distances from the points to their centroid).

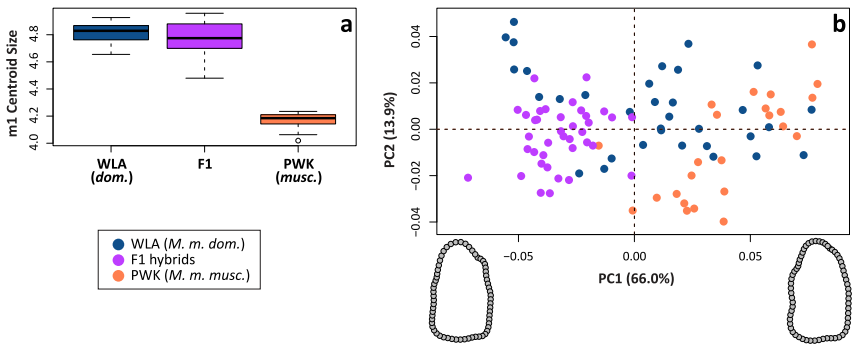

Figure 3. Size and shape of the m1 in the WLA x PWK cross. (a) m1 size, estimated by the centroid size of the molar outline. (b) m1 shape, based on a PCA on the aligned coordinates of the molar outline. Shapes corresponding to scores at the extreme ends of PC1 are shown.

2.2.2. Statistical analyses

The two datasets were measured by different operators (SR for the hybridization dataset, KP for the Iranian dataset). Inter-operator differences can occur, especially since the second lower molar (m2) overlaps the m1 for modern, intact tooth rows *in situ* within the mandible, thus making some interpolation necessary. The two datasets were therefore analysed separately, but following similar procedures as far as possible, in order to deliver comparable results.

A principal component analysis (PCA) was first performed on the aligned coordinates, followed by a between-group PCA. While PCA is an eigenanalysis of the total variance—covariance of the dataset, the bgPCA analyses the variance—covariance between group means weighted by the sample size of each group. A canonical variate analysis (CVA), also known as discriminant analysis (DA), was also performed, because this method is extensively used in the context of GMM, when looking at between-group differences (e.g. [52,53]). The CVA aims at separating the groups by looking for linear combinations of variables that maximize the between-group to within-group variance ratio, while the bgPCA does not standardize the within-group variance (e.g. [54]). The CVA also allows a reclassification of the original specimens and/or additional ones to the original groups, using a leave-one-out procedure. Both methods are sensitive to over-fitting issues when the number of variables exceeds the number of individuals, leading to 'spurious groups' appearing more differentiated than they really are based on CVA [55,56] as well as on bgPCA [57,58]. An approach to dimensionality reduction was applied for the CVA, retaining only the 'optimal' number of PC axes maximising cross-validated correct classification [59]. Regarding the bgPCA, the over-fitting issue was assessed using a cross-validation approach [58].

Firstly, the bgPCA and CVA were used to visualize the relationships between groups. In this context, the F1 hybrids in the WLA × PWK analysis, and the Ganj Dareh m1s in the Iranian populations study, were considered as 'active groups' that participated in the bgPCA and CVA computations. The modalities of the grouping factor were therefore WLA, PWK and F1 for the hybrid strains analysis, and *M. m. domesticus*, *M. musculus*, the Central Iranian lineage and Ganj Dareh for the Iranian populations study. To assess how the hybrids or the Ganj Dareh m1s would be classified if their status were unknown, two additional CVAs were performed. In this, the F1 and Ganj Dareh m1s were considered as 'supplementary' specimens and did not participate in the computation. They were then classified according to the reference groups only: WLA and PWK, and *M. m. domesticus*, *M. musculus* and Central Iranian lineages, respectively.

Shape differences between groups were tested using Procrustes ANOVA including pairwise tests, as well as by a randomization procedure implemented with the bgPCA [60]. Differences in centroid size were tested using non-parametric Kruskal–Wallis (KW) tests complemented by pairwise Wilcoxon tests.

The degree of transgression was assessed as the degree of deviation of the hybrids from the theoretical expectation of being midway between the parents, based on distances between group means in the multivariate space, hence: d(F1, WLA) + d(F1, PWK) – d(WLA, PWK) expressed as a percentage of the inter-parental strains distance d(WLA, PWK) [49].

The degree of closeness to a parental strain, pointing to a dominance-like pattern, was estimated by assessing the difference in the distance to one parental strain with respect to the average distance between the hybrids and the two parental strains, hence for F1 hybrids: [d(F1,WLA) + d(F1, PWK)]/2

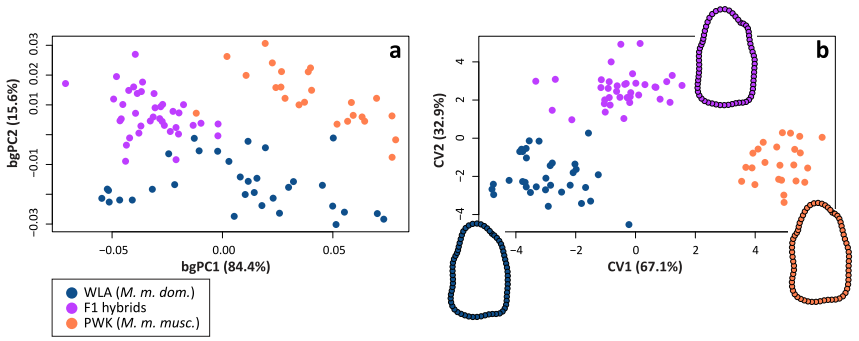

Figure 4. Patterns of between-group differentiation in m1 shape between WLA, PKW and their hybrids. (a) Morphospace corresponding to a bgPCA. (b) Morphospace corresponding to a CVA, with mean shapes of the three groups. WLA (*M. m. domesticus*) in dark blue, PWK (*M. m. musculus*) in dark orange, F1 hybrids in violet.

– d(F1, WLA), expressed as the percentage of the average distance of hybrids to the parental strains [d(F1, WLA) + d(F1, PWK)]/2 [49]. Positive values then indicate closeness to WLA, and negative values indicate closeness to PWK. Dominance and transgression were assessed based on two different between-group distances. Euclidean distances corresponded to the geometry of the bgPCA, while Mahalanobis distances correspond to the geometry of the CVA.

Similar indices were computed for the Central Iran population and Ganj Dareh, considering them as potential 'hybrids' between the modern reference groups *M. m. domesticus* and *M. m. musculus*.

All permutation tests were performed with 9999 permutations. The analyses were conducted in R [61]. The GPA, Permutation-based Procrustes ANOVA and the initial PCAs were performed using the R package geomorph [51]. The bgPCAs were performed using the package ade4 [60]. The CVAs were performed with Morpho [62] and the cross-validated reattribution with MASS [63].

Centroid size and aligned coordinates are provided as electronic supplementary material 2 for the WLA × PWK cross and electronic supplementary material 3 for the analysis of the Iranian and Ganj Dareh house mice.

3. Results

3.1. Signature of hybridization between *M. m. domesticus and M. m. musculus*: the WLA × PWK cross

There was an important size difference between the two parental strains (p-value = 5.3×10^{-16}), with the WLA strain having a much larger m1 than PWK (figure 3a). The F1 hybrids displayed a clear dominance towards the largest parental size (F1-WLA, p-value = 0.470), leading to a marked difference with the PWK parental strain (p-value < 2×10^{-16}).

Regarding the m1 shape (figure 3b), the hybrids appear to be associated with WLA (*M. m. domesticus*) along the first axis, shifting towards even more negative values along PC1, hence showing a transgressive signature. They are, however, intermediate between the two parental strains along PC2. Consequently, the three groups were highly different (pairwise tests of the Procrustes ANOVA, p-value = 1×10^{-4}).

The impression varies when considering the scores on the bgPCA axes (figure 4a). The groups explained 43.1% of the variance (randtest p-value = 1×10^{-4}). The leave-one-out procedure did not evidence over-fitting issues (delta Oij = 0). On the resulting morphospace, the hybrids appear in the prolongation of the variation of the WLA parents (*M. m. domesticus*), but with positive scores along the second axis, a trait shared with the PWK (*M. m. musculus*) parents.

When considering the CVA (figure 4b), 14 PC axes were deemed sufficient to maximize the cross-validated reclassification; they were retained for the following analysis. The hybrids appear more clearly intermediate between both parental strains, since the important within-group variance, expressed along PC1 and bgPC1, has been standardized. Hybrids are not, however, midway between parental strains, but shifted along the second axis, thus clearly showing a transgressive pattern.

The transgression of the F1 hybrids (table 2) was assessed as 162% based on the Euclidean distances and 50% based on the Mahalanobis distances. In both cases, the F1 hybrids were closer to the WLA (*M.*

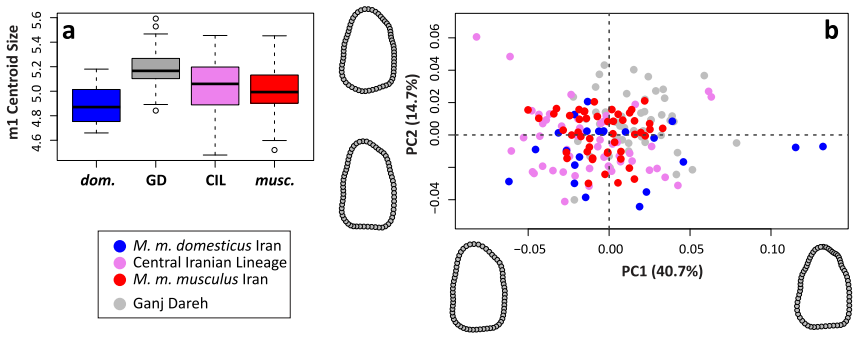

Figure 5. First lower molar (m1) size and shape in Iranian populations. (a) Centroid size. (b) Shape variation, based on a PCA on the aligned coordinates of the molar outline. Shapes corresponding to scores at the extreme ends of PC1 and PC2 are shown.

Table 2. Transgression and dominance characterizing the hybrid status of the F1, Central Iranian lineage and Ganj Dareh specimens. Transgression and dominance refer to the shape of the related *M. m. domesticus* and *M. m. musculus* (WLA and PWK parental strains or Iranian representatives, respectively).

		transgression		dominance → *M. m. dom.*	
	distances	Euclidean	Mahalanobis	Euclidean	Mahalanobis
data set	group				
WLA x PWK cross	F1	162	50	23	11
Iran (modern + archaeo.)	Central Iran	24	17	−31	−28
	Ganj Dareh	77	59	4	−2

m. domesticus) parental strain (dominance based on Euclidean distances 23%, based on Mahalanobis distances 11%).

This pattern impacted the cross-validated classification rates based on CVAs. When the three groups are considered, the correct classification rate was close to 100% (99%; one WLA classified as hybrid). If the hybrids are considered as supplementary specimens, they are attributed to both parental groups, but mostly to the WLA strain, due to the dominance effect (25 F1 hybrids classified as WLA, 13 as PWK).

3.2. Reconsidering the m1s from Ganj Dareh when including Central Iran as a separate lineage

The modern Iranian groups are slightly different in m1 centroid size (figure 5a), with the *M. m. domesticus* population from Ahvaz having smaller m1s than the *M. m. musculus* group and the Central Iranian lineage group (table 3). Archaeological m1s from Ganj Dareh were larger than those in the modern groups.

The shape differences among groups were all significant, despite a large overlap in the morphospace defined by the first and second PC axes (figure 5b). The less differentiated groups were Central Iran from *M. m. musculus* ($p = 0.0208$), followed by Central Iran from *M. m. domesticus* ($p = 0.0002$) (table 3). The archaeological specimens from Ganj Dareh plotted partly outside the range of modern variation (figure 5b). The shape difference involved the posterior part of the tooth, which is, as mentioned above, overlapped by the second molar in modern, intact tooth rows. However, the posterior part can be apparent in archaeological specimens if m1s are isolated (not within the mandible), or if, as was the case in some of the Ganj Dareh specimens, they are slightly loose within the mandible, or if the m2 is missing.

Considering a bgPCA (figure 6a), the groups explained 16.7% of variance (p-value = 0.0001), with little over-fitting effect (deltaOij = 0.02). The Iranian populations of *M. m. domesticus* and *M. m. musculus* were opposed along the second axis (28.2% of between-group variance). The mice from Central Iran and from Ganj Dareh were intermediate along bgPC2 but varied in position along bgPC1, Ganj Dareh being largely outside the range of modern variation.

A CVA was then performed on the 14 first PC axes (figure 6b), as for the analysis of the WLA × PWK cross. The resulting CV1 axis demonstrated the differentiation between *M. m. domesticus* and *M.*

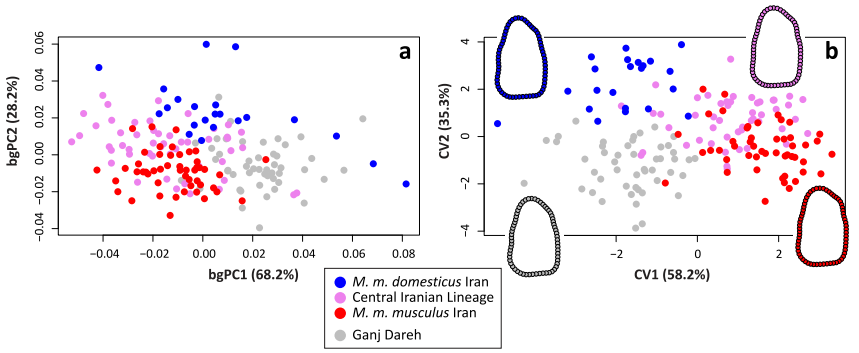

Figure 6. Patterns of between-group differentiation in m1 shape between Iranian populations, including the archaeological specimens from Ganj Dareh. (a) Morphospace corresponding to a bgPCA. (b) Morphospace corresponding to a CVA on the 14 first PC axes, with mean shapes of the four groups.

m. musculus. The Central Iran population was found to be intermediate but slightly overlapping with *M. m. musculus*. The archaeological specimens from Ganj Dareh were also intermediate between *M. m. musculus* and *M. m. domesticus*, but clearly shifted from Central Iran.

The cross-validated correct classification rate was moderate (76.9%), with Central Iran being most frequently misclassified as *M. m. musculus*, and *M. m. musculus* being frequently attributed to Central Iran (table 4). When the molars from Ganj Dareh were considered as supplementary specimens, they were attributed to all three reference groups, but mostly to the Central Iranian lineage (*M. m. domesticus* = 10; Central Iran = 21; *M. m. musculus* = 17).

Transgression and dominance levels were assessed for Central Iran and Ganj Dareh (table 2), using the same approach applied to the WLA × PWK hybrids. In the corresponding formulas, F1 was replaced either by the mean shape of Ganj Dareh or Central Iran, WLA by Iranian *M. m. domesticus* and PWK by Iranian *M. m. musculus*.

Transgression was moderate for Central Iran (Euclidean distances 24%, Mahalanobis distances 17%) with a clear dominance towards *M. m. musculus* (Euclidean distances 31%, Mahalanobis distances 28%). Little dominance was found for Ganj Dareh (4% towards *M. m. domesticus* based on Euclidean distances and 2% towards *M. m. musculus* based on Mahalanobis distances). Transgression, however, was consistently important (77% based on Euclidean distances and 59% based on Mahalanobis distances).

4. Discussion

4.1. A morphological signature of hybridization

The signature of hybridization observed between the wild-derived strains WLA (*M. m. domesticus*) and PWK (*M. m. musculus*) for the m1 was consistent with previous observations regarding the existence of transgression and dominance of the larger parental size for both the mandible [48,49] and the M1 [50]. Discrepancies however exist, with a clearer dominance towards the *domesticus* parental strain WLA than observed for the mandible [48,49] or the M1 [50], despite strong morphological integration between both occluding molars [64]. This underlines the mosaic signature of hybridization [49] in such highly polygenic characters [65]. The occurrence of transgression and dominance would make it difficult, if the hybrid nature of the specimens is ignored, to easily identify them as such, because they are not simply an intermediate between both parental groups, with their position depending on the degree of introgression [66,67].

4.2. Central Iran, a morphological signature of a hybrid lineage?

The house mouse as wild *M. musculus* originated in the region extending from Southwest Asia to northern India, in Mediterranean-like or drier environments (savannah, steppes) with rocky places and crevices presumably being its original biotope before commensalism [68]. The three subspecies *M. m. domesticus*, *musculus* and *castaneus* diverged in allopatry within this cradle area [32,33], which appears as a hotspot of genetic diversity within *M. musculus* [34,40,42,43].

Table 3. Pairwise comparison in m1 size and shape between groups of the modern referential and Ganj Dareh. Pairwise size differences have been tested using pairwise Wilcoxon tests (*p*-values below the diagonal); shape differences using pairwise Procrustes ANOVA (*p*-value above the diagonal, pairwise distances below the diagonal).

	distance/ *p*-value	*M. m. d.* Iran	Central Iran	Ganj Dareh	*M. m. m.* Iran
centroid size	*M. m. d.* Iran	–			
	Central Iran	0.0257	–		
	Ganj Dareh	<0.0001	0.0008	–	
	M. m. m. Iran	0.0245	0.6416	<0.0001	–
shape	*M. m. d.* Iran	–	0.0002	0.0001	0.0001
	Central Iran	0.031	–	0.0001	0.0208
	Ganj Dareh	0.032	0.038	–	0.0001
	M. m. m. Iran	0.038	0.016	0.035	–

Table 4. Reclassification of the m1 to the original groups based on a CVA performed on the 14 first PC axes. 'GD as group': percentages of cross-validated attribution between the four groups, Ganj Dareh being considered as an 'active' group in the analysis (see figure 5b). 'GD as supp' (below): attribution of Ganj Dareh m1s considered as supplementary specimens, the three active groups being the Iranian *M. m. Domesticus*, *M. m. musculus* and the Central Iranian lineage.

		M. m. d. Iran	Central Iran	*M. m. m.* Iran	Ganj Dareh
cross-validated %					
GD as group	*M. m. d.* Iran	18	1	0	3
	Central Iran	5	32	9	4
	M. m. m. Iran	1	11	36	1
	Ganj Dareh	0	2	2	44
GD as supp.	Ganj Dareh	10	21	17	–

The Central Iranian lineage has been identified as an entity issued from an early secondary admixture between the subspecies *M. m. musculus* and *M. m. domesticus* [40]. Based on autosomal genomic data, its ancestry could be traced as 60% to *musculus* and 40% to *domesticus* [43]. Its mitochondrial DNA however displays a *castaneus* signature [42], probably due to an ancestral *musculus* population predating allele sorting with the sister subspecies *castaneus* [40]. The proposed scenario leading to this Central Iranian lineage is a first phase of divergence in allopatry, with the current range of the Central Iranian lineage being occupied by a *musculus*-like ancestor, followed by the phase of male-biased *domesticus* migration [43], that would have occurred approximately 200 000 generations ago [40]. It remains challenging to translate this estimation into absolute chronological dating, as the generation time of *M. musculus* is poorly constrained and varies between one and two generations per year [69]. Observations indicate that commensal mice can breed year-round under favourable environmental conditions (e.g. stable food), with direct estimates of generation time averaging 1.4 generations per year [70]. However, as feral populations breed seasonally, the generation time of pre-commensal house mice was probably shorter. Published studies have used various estimates of generation time—1 [67], 1.5 [68] or even 2 generations yr^{-1} [36]—to model evolutionary dynamics. This introduces substantial uncertainty when attempting to correlate divergence times and admixture events with paleoclimatic fluctuations. Despite these challenges, it has been hypothesized that interglacial periods facilitated lineages' dispersal, divergence and admixture [36–38], as the warmer and wetter conditions during these phases potentially opened dispersal corridors between eastern/western and southern/northern Iran [71]. Conversely, glacial periods, which were characterized by arid conditions, likely restricted gene flow, confining incipient lineages to isolated refugia [41].

The morphometric signature of the Central Iranian Lineage fits this scenario of a 'hybrid lineage' based on genetic analyses [34,40,43]. Central Iranian mice have been reported to display a larger skull [41,72] and molar size [41] than other Iranian clades, in line with *M. m. domesticus* × *M. m. musculus*

hybrids having mandibles larger than both parents [48]. This trend was not, however, confirmed here. Central Iranian mice had m1s of similar size to the related group with the largest m1s (i.e. *M. m. musculus*). This finding is consistent with previous research, showing that hybrid M1s are comparable in size to the largest M1s in the parental groups [50]. In contrast, the archaeological Ganj Dareh m1s are larger than those of any modern Iranian population. This may indicate a hybrid signature, or a general trend of decreasing size over time, possibly related to human-driven environmental changes [73]. Indeed, archaeological commensal house mouse populations are often larger than modern ones [18,74], and this trend is also commonly seen in non-commensal rodent populations [19,75].

As for the m1 shape, Central Iranian mice display an intermediate morphology between *M. m. domesticus* and *M. m. musculus* with a transgressive component. The dominance towards *M. m. musculus* fits the genetic analyses, suggesting a majority *musculus* ancestry, while the transgressive component agrees with the strong transgression observed in the m1 shape of F1 hybrids. Transgression however appears moderate compared to the F1 hybrids of the WLA × PWK cross, a fact that might be explained by the presumably ancient hybridization, leading to many generations of backcrosses that tend to decrease transgression [50].

4.3. Ganj Dareh archaeological remains: a witness to complex hybridization dynamics in the Central Iranian region

Remains from Ganj Dareh had already been analysed using the GMM of the m1 shape. They were clearly identified as belonging to the commensal species *M. musculus*, and within the species, to the Western European subspecies *M. m. domesticus* [2], although they displayed some divergence from the modern sampling of this subspecies. In that study, however, the complexity of the genetic situation in the Iranian area [34,40,42] was overlooked. Consequently, in the modern reference dataset, Iranian populations from the range of the Central Iranian Lineage (Bandar Abbas, Isfahan) were considered as belonging to *M. m. domesticus*, while Eastern Iranian localities such as Birjand were attributed to *M. m. castaneus* instead of the Southeast Iranian Lineage [34].

In terms of m1 shape, when Central Iran is considered a distinct lineage, the relative majority of the Ganj Dareh molars are classified as belonging to it. As for modern populations in Central Iran, the m1 shape of Ganj Dareh mice is intermediate between *M. m. domesticus* and *M. m. musculus*. This supports the interpretation that Ganj Dareh mice are related to the Central Iranian lineage, contradicting earlier studies that attributed them to *M. m. domesticus* [2]. This highlights the potential difficulty of identifying the house mouse at the subspecies level in the biologically complex cradle area of the species, although this may remain valid for peripheral populations.

Putting aside the issue of subspecific identification, the archaeological population from Ganj Dareh displays idiosyncratic characteristics when compared to all modern genetic groups. Contrary to expectations for the Central Iranian Lineage [43], it does not display a dominance towards *M. m. musculus*, and transgression is much higher. The important transgressive component documented by the current morphometric analysis may be partly due to the posterior part being apparent in many m1s at Ganj Dareh, because they were slightly loose within their alveolar settings, or revealed by the loss of the m2 behind them. While such an effect might be negligible when considering morphological variation on a broad phylogeographic scale (e.g. [2]), it may be significant in the present case study that focuses on small-scale variation within the Iranian region.

Discrepancies between Ganj Dareh and the modern Central Iranian Lineage may also point to the local divergence of a lineage, as observed in the Himalayan region today [36], and/or a dynamic process of admixture. The formation of the Central Iranian Lineage by admixture would date back to an interglacial period, when *M. musculus* was still a wild, non-commensal species, but evidence of this ancient admixture between *M. m. domesticus* and *M. m. musculus* does not exclude the occurrence of a much more recent introgression [76]. At the onset of commensalism, human population density was low, and mouse populations were presumably small and isolated. Early Neolithic human trade and exchange may have favoured translocations [77] and hybridization, as is suggested by the Nepalese population from Sudurpashchim nowadays [36]. The shape of the m1 in Ganj Dareh might therefore result from an *M. m. domesticus* input into a Central Iranian population, possibly as a consequence of eastward human-mediated translocation. Similarly, the clearer *musculus*-like dominance in modern Central Iran compared to Ganj Dareh may result from increased input from northern regions, as suggested by genetic relationships between people from Ganj Dareh and Caucasus hunter-gatherers [78].

Such transient changes in the distribution patterns of the Iranian house mouse lineages may represent the signature of discontinuous human occupation, leading to extinction and recolonization of mice [11,18]. As human and mouse populations increased in density by the Neolithic period, mouse populations would have become more stable and resilient to further introductions. This case thus exemplifies the complex interplay between humans and their fellow traveller, the house mouse. Further light on this possible process, where early human settlements and movements may have influenced admixture between Iranian house mouse lineages, might be shed by ancient DNA, to validate the hybridization signal, and/or GMM analysis of the faster evolving M1 [79].

5. Conclusion

GMM analysis of the lower first molar of *M. musculus* from the iconic Neolithic site of Ganj Dareh in Iran indicates that, contrary to previous studies, these specimens are not typical *M. m. domesticus* [2]. Instead, they are likely related to what is known as the 'Central Iran lineage', which is thought to be a hybrid population of *M. m. domesticus* and *M. m. musculus* that has so far only been documented in modern populations [34,41,43].

Evidence of admixture between the house mouse subspecies in their Iranian cradle calls for a re-evaluation of faunal archaeological assemblages from this region, highlighting the importance of interdisciplinary collaboration between archaeology and evolutionary biology. Although house mice are well known as proxies for tracing the historical dynamics of human exchange and movement, this case highlights a complementary dynamic: zooarchaeological data can also provide key insights into the complex evolutionary dynamics of the house mouse, which may have been driven by early human mobility and population dynamics.

Ethics. This work did not require ethical approval from a human subject or animal welfare committee.

Data accessibility. Original GMM data are provided as electronic supplementary material (S2 and S3) [80].

Declaration of AI use. We have not used AI-assisted technologies in creating this article.

Authors' contributions. S.R.: conceptualization, formal analysis, investigation, methodology, visualization, writing—original draft, writing—review and editing; P.C.: formal analysis, investigation, resources, writing—review and editing; K.P.: formal analysis, investigation, methodology, resources, writing—review and editing; E.A.H.: conceptualization, writing—review and editing; L.Y.: resources, writing—review and editing; P.B.: resources, writing—review and editing; H.D.: funding acquisition, investigation, resources, writing—review and editing; T.R.: funding acquisition, investigation, resources, writing—review and editing; E.H.: supervision, writing—review and editing; P.A.: investigation, resources, writing—review and editing; J.-C.A.: investigation, resources, writing—review and editing; T.C.: investigation, resources, writing—review and editing; E.J.: conceptualization, resources, supervision, writing—original draft, writing—review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Conflict of interest declaration. We declare we have no competing interests.

Funding. No funding has been received for this article.

Acknowledgements. We thank the Iranian Centre for Archaeological Research (ICAR) for the permission to carry out the excavations and allowed the export of the samples. The excavations at Ganj Dareh were funded by the C. L. Davids Foundation as part of the Tracking Cultural and Environmental Change project. We further thank Lior Weissbrod (The Israel Museum, Jerusalem, Israel) and one anonymous reviewer for their constructive remarks on the manuscript.

References

1. Bar-Yosef O, Tchernov E. 1966 Archaeological finds and the fossil faunas of the Natufian and microlithic industries at Hayonim Cave (Western Galilee, Israel): A preliminary report of the 1965, 1966 seasons. *Isr. J. Ecol. Evol.* **15**, 104–140. (doi:10.1080/00212210.1966.10688238)
2. Cucchi T *et al*. 2020 Tracking the Near Eastern origins and European dispersal of the western house mouse. *Sci. Rep*. **10**, 8276. (doi:10.1038/s41598-020-64939-9)
3. Hesse B. 1979 Rodent remains and sedentism in the Neolithic: evidence from Tepe Ganj Dareh, Western Iran. *J. Mammal*. **60**, 856–857. (doi:10.2307/1380212)
4. Jenkins EL. 2005 The Çatalhöyük microfauna: preliminary results and interpretations. In *Inhabiting Çatalhöyük: reports from the 1995–1999 seasons* (ed. I Hodder), pp. 111–116. Cambridge, UK: McDonald Institute for Archaeological Research and British Institute at Ankara.
5. Weissbrod L, Marshall FB, Valla FR, Khalaily H, Bar-Oz G, Auffray JC, Vigne JD, Cucchi T. 2017 Origins of house mice in ecological niches created by settled hunter-gatherers in the Levant 15,000 y ago. *Proc. Natl Acad. Sci. USA* **114**, 4099–4104. (doi:10.1073/pnas.1619137114)

6. Cucchi T. 2008 Uluburun shipwreck stowaway house mouse: molar shape analysis and indirect clues about the vessel's last journey. *J. Archaeol. Sci*. **35**, 2953–2959. (doi:10.1016/j.jas.2008.06.016)
7. Witmer GW, Jojola SM. 2006 What's up with house mice? A review. *Proc. of the Vertebrate Pest Conference* (eds M Timm, JM O'Brien), **22**, 124–130. (doi:10.5070/V422110126)
8. Bonhomme F, Orth A, Cucchi T, Rajabi-Maham H, Catalan J, Boursot P, Auffray JC, Britton-Davidian J. 2011 Genetic differentiation of the house mouse around the Mediterranean basin: matrilineal footprints of early and late colonization. *Proc. R. Soc. B Biol. Sci.* **278**, 1034–1043. (doi:10.1098/rspb.2010.1228)
9. Gabriel SI *et al*. 2024 House mice in the Atlantic region: genetic signals of their human transport. *Genes* **15**, 1645. (doi:10.3390/genes15121645)
10. García-Rodríguez O *et al*. 2018 Cyprus as an ancient hub for house mice and humans. *J. Biogeogr*. **45**, 2618–2630. (doi:10.1111/jbi.13458)
11. Jones EP, Skirnisson K, McGovern TH, Gilbert MTP, Willerslev E, Searle JB. 2012 Fellow travellers: a concordance of colonization patterns between mice and men in the North Atlantic region. *BMC Evol. Biol*. **12**, 35. (doi:10.1186/1471-2148-12-35)
12. Li Y *et al*. 2021 House mouse *Mus musculus* dispersal in East Eurasia inferred from 98 newly determined complete mitochondrial genome sequences. *Heredity* **126**, 132–147. (doi:10.1038/s41437-020-00364-y)
13. Searle JB *et al*. 2009 Of mice and (Viking?) men: phylogeography of British and Irish house mice. *Proc. R. Soc. B* **276**, 201–207. (doi:10.1098/rspb.2008.0958)
14. Förster DW, Gündüz I, Nunes AC, Gabriel S, Ramalhinho Mdg, Mathias ML, Britton-Davidian J, Searle JB. 2009 Molecular insights into the colonization and chromosomal diversification of Madeiran house mice. *Mol. Ecol*. **18**, 4477–4494. (doi:10.1111/j.1365-294X.2009.04344.x)
15. Gündüz I, Auffray JC, Britton-Davidian J, Catalan J, Ganem G, Ramalhinho Mdg, Mathias ML, Searle JB. 2001 Molecular studies on the colonization of the Madeiran archipelago by house mice. *Mol. Ecol*. **10**, 2023–2029. (doi:10.1046/j.0962-1083.2001.01346.x)
16. Rando JC, Pieper H, Alcover JA. 2014 Radiocarbon evidence for the presence of mice on Madeira Island (North Atlantic) one millennium ago. *Proc. R. Soc. B Biol. Sci.* **281**, 20133126. (doi:10.1098/rspb.2013.3126)
17. Brace S, Ruddy M, Miller R, Schreve DC, Stewart JR, Barnes I. 2016 The colonization history of British water vole (*Arvicola amphibius* (Linnaeus, 1758)): origins and development of the Celtic fringe. *Proc. R. Soc. B* **283**, 20160130. (doi:10.1098/rspb.2016.0130)
18. Romaniuk AA, Renaud S, Bendrey R, Searle JB, Owen O, Herman J. 2024 Insular evolution from an archaeological perspective: a case study of Orkney house mouse. *Biol. J. Linn. Soc*. **143**, blae005. (doi:10.1093/biolinnean/blae005)
19. Cucchi T *et al*. 2014 The changing pace of insular life: 5000 years of microevolution in the Orkney vole (*Microtus arvalis orcadensis*). *Evolution* **68**, 2804–2820. (doi:10.1111/evo.12476)
20. Cucchi T, Neaux D, Féral L, Goussard F, Adriensen H, Elleboudt F, Sansalone G, Schafberg R. 2024 How domestication, feralization and experience-dependent plasticity affect brain size variation in Sus scrofa. *R. Soc. Open Sci*. **11**, 240951. (doi:10.1098/rsos.240951)
21. Harbers H *et al*. 2020 The mark of captivity: plastic responses in the ankle bone of a wild ungulate (*Sus scrofa*). *R. Soc. Open Sci*. **7**, 192039. (doi:10.1098/rsos.192039)
22. Cucchi T, Papayianni K, Vigne J. 2023 Cat and mice: commensalism and shifting of continental connectivity. In *Klimonas. an early pre-pottery neolithic village in cyprus* (eds JD Vigne, F Briois, J Guilaine), pp. 467–476. Paris, France: CNRS Éditions. (doi:10.4000/129kr)
23. Cucchi T, Vigne JD, Auffray JC, Croft P, Peltenburg E. 2002 Introduction involontaire de la souris domestique (*Mus musculus domesticus*) à Chypre dès le Néolithique précéramique ancien (fin IXe et VIIIe millénaires av. J.-C.). *Comptes Rendus Palevol* **1**, 235–241. (doi:10.1016/S1631-0683(02)00033-7)
24. Cucchi T *et al*. 2013 On the trail of Neolithic mice and men towards Transcaucasia: zooarchaeological clues from Nakhchivan (Azerbaijan). *Biol. J. Linn. Soc*. **108**, 917–928. (doi:10.1111/bij.12004)
25. Alibert P, Renaud S, Dod B, Bonhomme F, Auffray JC. 1994 Fluctuating asymmetry in the *Mus musculus* hybrid zone: a heterotic effect in disrupted co-adapted genomes. *Proc. R. Soc. Lond. B* **258**, 53–59. (doi:10.1098/rspb.1994.0141)
26. Auffray JC, Marshall JT, Thaler L, Bonhomme F. 1990 Focus on the nomenclature of European species of *Mus*. *Mouse Genome* **88**, 7–8.
27. Bonhomme F. 1986 Evolutionary relationships in the G*enus Mus*. In *The wild mouse in immunology* (eds M Potter, JH Nadeau, MP Cancro), pp. 19–34. Berlin, Heidelberg: Springer.
28. Boursot P, Din W, Anand R, Darviche D, Dod B, Deimling F, Talwar GP, Bonhomme F. 1996 Origin and radiation of the house mouse: mitochondrial DNA phylogeny. *J. Evol. Biol*. **9**, 391–415.
29. Sage RD, Atchley WR, Capanna E. 1993 House mice as models in systematic biology. *Syst. Biol*. **42**, 523–561. (doi:10.1093/sysbio/42.4.523)
30. Dod B, Jermiin LS, Boursot P, Chapman VH, Nielsen JT, Bonhomme F. 1993 Counterselection on sex chromosomes in the *Mus musculus* European hybrid zone. *J. Evol. Biol*. **6**, 529–546. (doi:10.1046/j.1420-9101.1993.6040529.x)
31. Macholán M, Munclinger P, Sugerková M, Dufková P, Bímová B, Bozíková E, Zima J, Piálek J. 2007 Genetic analysis of autosomal and X-linked markers across a mouse hybrid zone. *Evolution* **61**, 746–771. (doi:10.1111/j.1558-5646.2007.00065.x)
32. Boursot P, Din W, Anand R, Darviche D, Dod B, Von Deimling F, Talwar GP, Bonhomme F. 1996 Origin and radiation of the house mouse: mitochondrial DNA phylogeny. *J. Evol. Biol*. **9**, 391–415. (doi:10.1046/j.1420-9101.1996.9040391.x)
33. Prager EM, Orrego C, Sage RD. 1998 Genetic variation and phylogeography of central Asian and other house mice, including a major new mitochondrial lineage in Yemen. *Genetics* **150**, 835–861. (doi:10.1093/genetics/150.2.835)
34. Hardouin EA, Orth A, Teschke M, Darvish J, Tautz D, Bonhomme F. 2015 Eurasian house mouse (*Mus musculus* L.) differentiation at microsatellite loci identifies the Iranian plateau as a phylogeographic hotspot. *BMC Evol. Biol*. **15**, 26. (doi:10.1186/s12862-015-0306-4)
35. Rajabi-Maham H, Orth A, Bonhomme F. 2008 Phylogeography and postglacial expansion of *Mus musculus* domesticus inferred from mitochondrial DNA coalescent, from Iran to Europe. *Mol. Ecol*. **17**, 627–641. (doi:10.1111/j.1365-294X.2007.03601.x)

36. Chen Y, Wang R, Zhu Z, Subedi N, Jiang X, Jing M, Huang L. 2025 Phylogenomic analyses revealed a new lineage of house mouse (*Mus musculus*) in Gyirong Basin of Xizang Autonomous Region, China. *Mol. Phylogenetics Evol*. **209**, 108370. (doi:10.1016/j.ympev.2025.108370)
37. Fujiwara K, Ranorosoa MC, Ohdachi SD, Arai S, Sakuma Y, Suzuki H, Osada N. 2022 Whole-genome sequencing analysis of wild house mice (*Mus musculus*) captured in Madagascar. *Genes Genet. Syst*. **97**, 193–207. (doi:10.1266/ggs.22-00090)
38. Phifer-Rixey M, Harr B, Hey J. 2020 Further resolution of the house mouse (*Mus musculus*) phylogeny by integration over isolation-with-migration histories. *BMC Evol. Biol*. **20**, 120. (doi:10.1186/s12862-020-01666-9)
39. Siahsarvie R, Auffray JC, Darvish J, Rajabi-maham H, Yu HT, Agret S, Bonhomme F, Claude J. 2012 Patterns of morphological evolution in the mandible of the house mouse *Mus musculus* (Rodentia: Muridae). *Biol. J. Linn. Soc*. **105**, 635–647. (doi:10.1111/j.1095-8312.2011.01821.x)
40. Duvaux L, Belkhir K, Boulesteix M, Boursot P. 2011 Isolation and gene flow: inferring the speciation history of European house mice. *Mol. Ecol*. **20**, 5248–5264. (doi:10.1111/j.1365-294X.2011.05343.x)
41. Haddadian Shad H, Darvish J, Rastegar-Pouyani E, Mahmoudi A. 2017 Subspecies differentiation of the house mouse *Mus musculus* Linnaeus, 1758 in the center and east of the Iranian plateau and Afghanistan. *Mammalia* **81**, 147–168. (doi:10.1515/mammalia-2015-0041)
42. Rajabi-Maham H, Orth A, Siahsarvie R, Boursot P, Darvish J, Bonhomme F. 2012 The south-eastern house mouse *Mus musculus* castaneus (Rodentia: Muridae) is a polytypic subspecies. *Biol. J. Linn. Soc*. **107**, 295–306. (doi:10.1111/j.1095-8312.2012.01957.x)
43. Marques JPN. 2022 Using genomic tools to understand species differentiation and admixture in hares and mice. Thesis, Université de Montpellier, France.
44. Darabi H, Richter T, Mortensen P. 2019 Neolithization process in the central Zagros: Asiab and Ganj Dareh revisited. *Doc. Praehist*. **46**, 44–57. (doi:10.4312/dp.46.3)
45. Smith PEL. 1990 Architectural innovation and experimentation at Ganj Dareh, Iran. *World Archaeol*. **21**, 323–335. (doi:10.1080/00438243.1990.9980111)
46. Daly KG *et al*. 2021 Herded and hunted goat genomes from the dawn of domestication in the Zagros Mountains. *Proc. Natl Acad. Sci. USA* **118**, e2100901118. (doi:10.1073/pnas.2100901118)
47. Zeder MA, Hesse B. 2000 The initial domestication of goats (*Capra hircus*) in the Zagros mountains 10,000 years ago. *Science* **287**, 2254–2257. (doi:10.1126/science.287.5461.2254)
48. Renaud S, Alibert P, Auffray JC. 2009 Mandible shape in hybrid mice. *Die Naturwissenschaften* **96**, 1043–1050. (doi:10.1007/s00114-009-0563-4)
49. Renaud S, Alibert P, Auffray JC. 2012 Modularity as a source of new morphological variation in the mandible of hybrid mice. *BMC Evol. Biol*. **12**, 141. (doi:10.1186/1471-2148-12-141)
50. Renaud S, Alibert P, Auffray JC. 2017 Impact of hybridization on shape, variation and covariation of the mouse molar. *Evol. Biol*. **44**, 69–81. (doi:10.1007/s11692-016-9391-6)
51. Adams DC, Otárola-Castillo E. 2013 geomorph: an r package for the collection and analysis of geometric morphometric shape data. *Methods Ecol. Evol*. **4**, 393–399. (doi:10.1111/2041-210X.12035)
52. Leinonen T, Cano JM, Mäkinen H, Merilä J. 2006 Contrasting patterns of body shape and neutral genetic divergence in marine and lake populations of threespine sticklebacks. *J. Evol. Biol*. **19**, 1803–1812. (doi:10.1111/j.1420-9101.2006.01182.x)
53. Valenzuela-lamas S, Baylac M, Cucchi T, Vigne JD. 2011 House mouse dispersal in Iron Age Spain: a geometric morphometrics appraisal. *Biol. J. Linn. Soc*. **102**, 483–497. (doi:10.1111/j.1095-8312.2010.01603.x)
54. Renaud S, Dufour AB, Hardouin EA, Ledevin R, Auffray JC. 2015 Once upon multivariate analyses: when they tell several stories about biological evolution. *PLoS One* **10**, e0132801. (doi:10.1371/journal.pone.0132801)
55. Kovarovic K, Aiello LC, Cardini A, Lockwood CA. 2011 Discriminant function analyses in archaeology: are classification rates too good to be true? *J. Archaeol. Sci*. **38**, 3006–3018. (doi:10.1016/j.jas.2011.06.028)
56. Mitteroecker P, Bookstein F. 2011 Linear discrimination, ordination, and the visualization of selection gradients in modern morphometrics. *Evol. Biol*. **38**, 100–114. (doi:10.1007/s11692-011-9109-8)
57. Cardini A, O'Higgins P, Rohlf FJ. 2019 Seeing distinct groups where there are none: spurious patterns from between-group PCA. *Evol. Biol*. **46**, 303–316. (doi:10.1007/s11692-019-09487-5)
58. Thioulouse J, Renaud S, Dufour AB, Dray S. 2021 Overcoming the spurious groups problem in between-group PCA. *Evol. Biol*. **48**, 458–471. (doi:10.1007/s11692-021-09550-0)
59. Evin A, Cucchi T, Cardini A, Strand Vidarsdottir U, Larson G, Dobney K. 2013 The long and winding road: identifying pig domestication through molar size and shape. *J. Archaeol. Sci*. **40**, 735–743. (doi:10.1016/j.jas.2012.08.005)
60. Thioulouse J, Dray S, Dufour AB, Siberchicot A, Jombart T, Pavoine S. 2018 *Multivariate analysis of ecological data with ade4*. New York, NY: Springer.
61. R Core Team. 2020 R: a langage for environment and statistical computing. Vienna, Austria. R Foundation for Statistical Computing.
62. Schlager S. 2017 Chapter 9. Morpho and Rvcg – Shape analysis in R: R-packages for geometric morphometrics, shape analysis and surface manipulations. In *Statistical shape and deformation analysis* (eds G Zheng, S Li, G Székely), pp. 217–256. Academic Press.
63. Venables WN, Ripley BD. 2002 *Modern applied statistics with S*, 4th edition. New York, NY: Springer.
64. Renaud S, Pantalacci S, Quéré JP, Laudet V, Auffray JC. 2009 Developmental constraints revealed by co-variation within and among molar rows in two murine rodents. *Evol. Dev*. **11**, 590–602. (doi:10.1111/j.1525-142X.2009.00365.x)
65. Pallares LF, Ledevin R, Pantalacci S, Turner LM, Steingrimsson E, Renaud S. 2017 Genomic regions controlling shape variation in the first upper molar of the house mouse. *eLife* **6**, e29510. (doi:10.7554/elife.29510)

66. Auffray J -C., Alibert P, Latieule C, Dod B. 1996 Relative warp analysis of skull shape across the hybrid zone of the house mouse (*Mus musculus*) in Denmark. *J. Zool*. **240**, 441–455. (doi:10.1111/j.1469-7998.1996.tb05297.x)
67. Loy A, Capula M, Palombi A, Capanna E. 2001 Genetic and morphometric evidence of introgression between two species of moles (Insectivora: *Talpa europaea* and *Talpa romana*) in central Italy. *J. Zool*. **254**, 229–238. (doi:10.1017/s0952836901000747)
68. Patnaik R, Auffray JC, Jaeger JJ, Sahni A. 1996 House mouse ancestor from late Pliocene Siwalik sediments of India. *C. R. Acad. Sci. III*. **319**, 431–434.
69. Bronson FH. 1979 The reproductive ecology of the house mouse. *Q. Rev. Biol*. **54**, 265–299. (doi:10.1086/411295)
70. Geiger M, Sánchez-Villagra MR, Lindholm AK. 2018 A longitudinal study of phenotypic changes in early domestication of house mice. *R. Soc. Open Sci*. **5**, 172099. (doi:10.1098/rsos.172099)
71. Shoaee MJ, Breeze PS, Drake NA, Hashemi SM, Vahdati Nasab H, Breitenbach SFM, Stevens T, Boivin N, Petraglia MD. 2023 Defining paleoclimatic routes and opportunities for hominin dispersals across Iran. *PLoS One* **18**, e0281872. (doi:10.1371/journal.pone.0281872)
72. Alibert P. 2025 Size and shape changes in relation to the phylogeography of West Eurasian and North African house mice (*Mus musculus*). Biol. J. Linn. Soc. **145**, blaf047. (doi:10.1093/biolinnean/blaf047)
73. Guralnick R, Hantak MM, Li D, McLean BS. 2020 Body size trends in response to climate and urbanization in the widespread North American deer mouse, Peromyscus maniculatus. *Sci. Rep*. **10**, 8882. (doi:10.1038/s41598-020-65755-x)
74. Cassaing J, Sénégas F, Claude J, Le Proux de la Rivière B. 2011 A spatio-temporal decrease in molar size in the western European house mouse. *Mammal. Biol.* **76**, 51–57. (doi:10.1016/j.mambio.2010.02.002)
75. Stoetzel E, Denys C, Michaux J, Renaud S. 2013 *Mus* in Morocco: a Quaternary sequence of intraspecific evolution. *Biol. J. Linn. Soc*. **109**, 599–621. (doi:10.1111/bij.12065)
76. Pool JE, Nielsen R. 2009 Inference of historical changes in migration rate from the lengths of migrant tracts. *Genetics* **181**, 711–719. (doi:10.1534/genetics.108.098095)
77. Vaiglova P *et al*. 2025 Transport of animals underpinned ritual feasting at the onset of the Neolithic in southwestern Asia. *Commun. Earth Environ*. **6**, 519. (doi:10.1038/s43247-025-02501-z)
78. Gallego-Llorente M *et al*. 2016 The genetics of an early Neolithic pastoralist from the Zagros, Iran. *Sci. Rep*. **6**, 31326. (doi:10.1038/srep31326)
79. Renaud S, Pantalacci S, Auffray JC. 2011 Differential evolvability along lines of least resistance of upper and lower molars in island house mice. *PLoS One* **6**, e18951. (doi:10.1371/journal.pone.0018951)
80. Renaud S, Clarkson P, Papayianni K, Hardouin EA, Yeomans L, Bangsgaard P *et al*. 2026 Supplementary material from: Bioarchaeological evidence for hybridization between house mouse subspecies in early Neolithic Iran. FigShare (doi:10.6084/m9.figshare.c.8317562)