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ORIGINAL ARTICLE

Evolutionary and dispersal history of Eurasian house mice *Mus musculus* clarified by more extensive geographic sampling of mitochondrial DNA

H Suzuki[1], M Nunome[1], G Kinoshita[1], KP Aplin[2], P Vogel[3], AP Kryukov[4], M-L Jin[5], S-H Han[6], I Maryanto[7], K Tsuchiya[8], H Ikeda[9], T Shiroishi[10], H Yonekawa[11] and K Moriwaki[12]

We examined the sequence variation of mitochondrial DNA control region and cytochrome *b* gene of the house mouse (*Mus musculus sensu lato*) drawn from *ca.* 200 localities, with 286 new samples drawn primarily from previously unsampled portions of their Eurasian distribution and with the objective of further clarifying evolutionary episodes of this species before and after the onset of human-mediated long-distance dispersals. Phylogenetic analysis of the expanded data detected five equally distinct clades, with geographic ranges of northern Eurasia (*musculus*, MUS), India and Southeast Asia (*castaneus*, CAS), Nepal (unspecified, NEP), western Europe (*domesticus*, DOM) and Yemen (*gentilulus*). Our results confirm previous suggestions of Southwestern Asia as the likely place of origin of *M. musculus* and the region of Iran, Afghanistan, Pakistan, and northern India, specifically as the ancestral homeland of CAS. The divergence of the subspecies lineages and of internal sublineage differentiation within CAS were estimated to be 0.37–0.47 and 0.14–0.23 million years ago (mya), respectively, assuming a split of *M. musculus* and *Mus spretus* at 1.7 mya. Of the four CAS sublineages detected, only one extends to eastern parts of India, Southeast Asia, Indonesia, Philippines, South China, Northeast China, Primorye, Sakhalin and Japan, implying a dramatic range expansion of CAS out of its homeland during an evolutionary short time, perhaps associated with the spread of agricultural practices. Multiple and non-coincident eastward dispersal events of MUS sublineages to distant geographic areas, such as northern China, Russia and Korea, are inferred, with the possibility of several different routes.
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INTRODUCTION

Despite the rapid rise of polygenic and genomic approaches to the analysis of population history (for example, Abe *et al.*, 2004; Stoneking and Delfin, 2010; Yang *et al.*, 2011), the study of mitochondrial DNA (mtDNA) continues to have a significant role in the investigation of many species. In the case of the house mouse complex (*Mus musculus* Complex), the availability of large numbers of mtDNA sequences derived from the European and other populations has facilitated detailed analysis of both prehistoric and historic range expansions (Rajabi-Maham *et al.*, 2008; Gabriel *et al.*, 2010, 2011; Jones *et al.*, 2010; Bonhomme *et al.*, 2011), often with significant implications for human history. By contrast, the other major lineages of the house mouse are known from far fewer sequences and this has hindered progress on even some of the most basic questions of phylogeography, such as their likely places of origin and the timing and routes of major dispersal episodes.

Early investigations of house mouse mtDNA, using the method of Restriction Fragment Length Polymorphism (for example, Yonekawa *et al.*, 1981, 1986), identified three major haplogroups (HGs) among wild house mice. These appeared to be associated with recognized subspecies and were designated accordingly: a DOM HG in *M. m. domesticus* from western Europe and North Africa (also southern Africa, Australia and the Americas, all as historical introductions); a MUS HG in *M. m. musculus* from the northern part of Eurasia excluding western Europe; and a CAS HG in *M. m. castaneus* from Southeast Asia. Later studies of mtDNA suggested a number of other possible divergent lineages: a BAC HG in *M. m. bactrianus* from Afghanistan and Pakistan (Boursot *et al.*, 1993, 1996; Yonekawa *et al.*, 1994); a GEN HG in *M. m. gentilulus* from Yemen (Prager *et al.*, 1998) and Madagascar (Duplantier *et al.*, 2002); and most recently, another divergent but as yet unnamed HG from Nepal (Terashima *et al.*, 2006). Broader genomic comparisons using microsatellites

[1]Laboratory of Ecology and Genetics, Graduate School of Environmental Earth Science, Hokkaido University, Sapporo, Japan; [2]Division of Mammals, National Museum of Natural History, Smithsonian Institution, Washington, DC, USA; [3]Department of Ecology and Evolution, University of Lausanne, Lausanne, Switzerland; [4]Institute of Biology and Soil Science, Russian Academy of Sciences, Vladivostok, Russia; [5]Shanghai Research Center of Biotechnology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, China; [6]National Institute of Biological Resources, Environmental Research Complex, Incheon, Korea; [7]Museum Zoologicum Bogoriense, Indonesian Institute of Sciences, Cibinong, Indonesia; [8]Laboratory of Bioresources, Applied Biology Co., Ltd, Minato-ku, Tokyo, Japan; [9]Department of Veterinary Public Health, Nippon Veterinary and Animal Science University, Musashino, Tokyo, Japan; [10]Mammalian Genetics Laboratory, National Institute of Genetics, Research Organization of Information and Systems, Mishima, Japan; [11]Department of Laboratory Animal Science, Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan and [12]RIKEN, Bioresource Center, Tsukuba, Japan

Correspondence: Dr H Suzuki, Laboratory of Ecology and Genetics, Graduate School of Environmental Earth Science, Hokkaido University, North 10, West 5, Kita-ku, Sapporo, Hokkaido 060-0810, Japan.

E-mail: htsuzuki@ees.hokudai.ac.jp

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(Sakai *et al.*, 2005), single-nucleotide polymorphic sites (Abe *et al.*, 2004) and whole-genome sequences (Frazer *et al.*, 2007) support the notion that each of the MUS, CAS and DOM mtDNA HGs represents a longstanding evolutionary lineage. However, the remaining mtDNA HGs (BAC and GEN) have not been subject to the same level of scrutiny, hence their status remains uncertain.

In this paper, we fill a number of the remaining gaps in geographic mtDNA coverage for the house mouse, with a particular emphasis on the Indian sub-continent, China and far eastern Russia. Addition of mtDNA sequences from these key areas sheds light on several issues, including (1) the likely ancestral range of each of the major evolutionary lineages; and (2) the direction and timing of range expansions, with a particular focus on East Asia, China and Japan, where multiple mtDNA lineages are known to regionally co-occur (Moriwaki *et al.*, 1984; Yonekawa *et al.*, 1986, 2003; Terashima *et al.*, 2006; Nunome *et al.*, 2010a).

MATERIALS AND METHODS

Materials

Our new sequencing effort is based chiefly on samples of house mouse genomic DNA stored in the National Institute of Genetics, Mishima, Japan. These were collected in China, India, Russia and a variety of other countries, on expeditions organized by KM during 1983–2003 (MG series, stored in the National Institute of Genetics; and BRC Series, stored in the RIKEN Bio-Resource Center), and by HI and KT during 1989–1992 (HI series, stored in Hokkaido University). We also used DNA samples stored at Hokkaido University (HS series), including mice collected by PV (IZEA series, vouchered in the Institut de Zoologie et d'Ecologie Animale of Lausanne University) and KA (ANWC series, vouchered in the Australian National Wildlife Collection). Some of the same samples have been used in previous studies (for example, Yonekawa *et al.*, 1988, 1994, 2003; Miyashita *et al.*, 1994; Nagamine *et al.*, 1994; Tsuchiya *et al.*, 1994; Munclinger *et al.*, 2002; Spiridonova *et al.*, 2004).

New sequences were generated for mtDNA control region (CR) from 212 house mouse individuals from 137 localities; the cytochrome *b* gene (*Cytb*) was also sequenced from a subset of 167 individuals from 106 localities (Supplementary Table S1). In our sampling, we strived to achieve maximum geographic coverage, at the cost of small sample sizes (frequently just one) for each locality. Although this reduced the scope for sophisticated analysis of population expansion scenarios, it increased the likelihood of detecting previously undiscovered components of mtDNA diversity. The geographic distribution of the new sequences is shown in Figure 1.

We downloaded a further 571 CR sequences and 41 *Cytb* sequences of *M. musculus* from public databases, drawn primarily from the work of Prager *et al.* (1996, 1998), Gündüz *et al.* (2005), Rajabi-Maham *et al.* (2008) and Bonhomme *et al.* (2011), along with representative sequences of closely related species for use as outgroups. Our sequence alignments for each mtDNA region are provided in Dryad repository: doi:10.5061/dryad.rf161.

Sequence analyses

The PCR and direct sequencing of the CR (around 800 bp; Yasuda *et al.*, 2005) and *Cytb* (1140 bp; Suzuki *et al.*, 2004) were performed according to previously described methods. Two primers were used for sequence determination of CR in *M. musculus*; CR1: 5′-CATGCCTTGACGGCTATGTT-3′ and CR2: 5′-ATC GCCCATACGTTCCCCTT-3′. The double-stranded PCR product was sequenced utilizing the ABI PRISM Ready Reaction DyeDeoxy Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) and an ABI3130 automated sequencer. Sequences of *M. cypriacus*, *M. macedonicus*, *M. spicilegus* and *M. spretus* were obtained from the databases and used as outgroups in the phylogenetic inference.

Phylogeny and divergence time estimation

Sequences were aligned by eye using MEGA5 (Tamura *et al.*, 2011). Before further analyses, we deleted tandem repeat sequences of 75–76 bp in CR of some MUS and some CAS haplotypes (identified by Prager *et al.*, 1996, 1998) and an 11-bp insertion in CR of some DOM sequences, while encoding the occurrence of these repeats into the taxon name to check for conformation with phyletic lineages.

To obtain a general impression of clustering topology, we constructed Neighbor-Net (NN) networks (Bryant and Moulton, 2004) for reduced data sets of 399 unique CR haplotypes and 98 unique *Cytb* haplotypes, and using the default parameters of uncorrected *P* distance and the EqualAngle algorithm, as implemented in SplitsTree 4.10 software (http://www.splitstree.org). The principal advantage of this hypothesis-poor method over others that generate dichotomous branching networks or trees is that NN networks illustrates all potentially supported splits among a group of sequences as a reticulation. The potential complexity of a data set is thus represented rather than reduced by this method, while any predominant network topology remains visible. Further insights into the structure of each of the CAS, MUS and DOM mtDNA lineages was obtained by constructing NN networks, together with Median-Joining (MJ) networks (Bandelt *et al.* 1999), as implemented in SplitsTree 4.10.

Maximum likelihood (ML) phylogenies were constructed for each of the CR and *Cytb* data sets and for a concatenate data set for 30 individuals. We used the PhylML algorithm (Guindon and Gascuel, 2003) with the HKY substitution model, as implemented on the ATGC website (http://www.atgc-montpellier.fr/). A maximum parsimony (MP) method and the neighbor-joining (NJ; Saitou and Nei, 1987) method were taken for phylogenetic inference with concatenate sequences using PAUP 4.0b10 (Swofford, 2001). Bootstrap analysis was carried out with 1000 pseudoreplicates in the ML and NJ analyses and 100 pseudoreplicates in the MP analysis. Sub-groups are designated within each of the major mtDNA lineages only if there was moderate-to-good bootstrap support ($=0.7$–0.9) from the ML analysis, combined with concordant structure in the NN networks.

We estimated the age of the most recent common ancestors (TMRCAs) for mtDNA clades using the *Cytb* sequences and a relaxed Bayesian molecular clock with uncorrelated rates (Bayesian evolutionary analysis by sampling trees (BEAST) v1.6.1, Drummond and Rambaut, 2007), as described previously (Nunome *et al.*, 2010b). For this analysis, we used *M. cypriacus*, *M. macedonicus*, *M. spicilegus* and *M. spretus*, the remaining members of the *M. musculus* Species Group, as outgroup taxa. For the root node of the *M. musculus* Species Group, we assigned a previous value of 1.7 mya (95% highest posterior density: 1.45–1.95), which is based on molecular divergences of single-copy nuclear gene sequences (*Irbp* and *Rag1*), calibrated against the known fossil record of the genus *Mus* and other Murinae (Suzuki *et al.*, 2004; Shimada *et al.*, 2010). The monophyletic setting was applied for clades of the lineages of the four subspecies groups (CAS, MUS, DOM and NEP) and *M. m.* subspecies. Then TMRCAs were estimated by the Bayesian Markov-chain Monte-Carlo (MCMC) method, using the HKY substitution model as selected under the Akaike Information Criterion in MrModeltest version 2.2 (Nylander, 2004). Analyses were run for 50 million generations from a UPGMA (Unweighted Pair Group Method with Arithmetic Mean) starting tree with sampling at every 5000 generations following 5 million burn-in generations. The convergence of MCMC chains and the effective sample size values >200 for all parameters were assessed using the software Tracer version 1.5 (Rambaut and Drummond, 2009; http://beast.bio.ed.ac.uk/Tracer). BEAST analysis was not performed with the CR sequences due to the greater inequality in branch lengths observed on the CR ML trees, compared with the *Cytb* ML trees, suggestive of less regular substitution fixation rates over evolutionary time.

Assessment of historical demographical processes

The DnaSP programme, version 5.00.7 (Librado and Rozas, 2009), was used to estimate haplotype diversity (*Hd*), nucleotide diversity (π), mean number of pairwise differences among sequences (k) and Tajima's *D* value. The same software was used for the analysis of mtDNA sequence mismatch distributions, measured as substitutional differences between pairs of haplotypes. Estimates of the expansion parameter tau (τ) were calculated using Arlequin version 3.5 (Excoffier and Lischer, 2010). Population expansion times were estimated under the assumption of a constant molecular clock and using mutation rates 2.5, 10 and 20% using the online tool developed by Schenekar and Weiss (2011); available at http://www.uni-graz.at/zoowww/mismatchca lc/mmc1.php. The goodness-of-fit of the observed distribution to the expected distribution

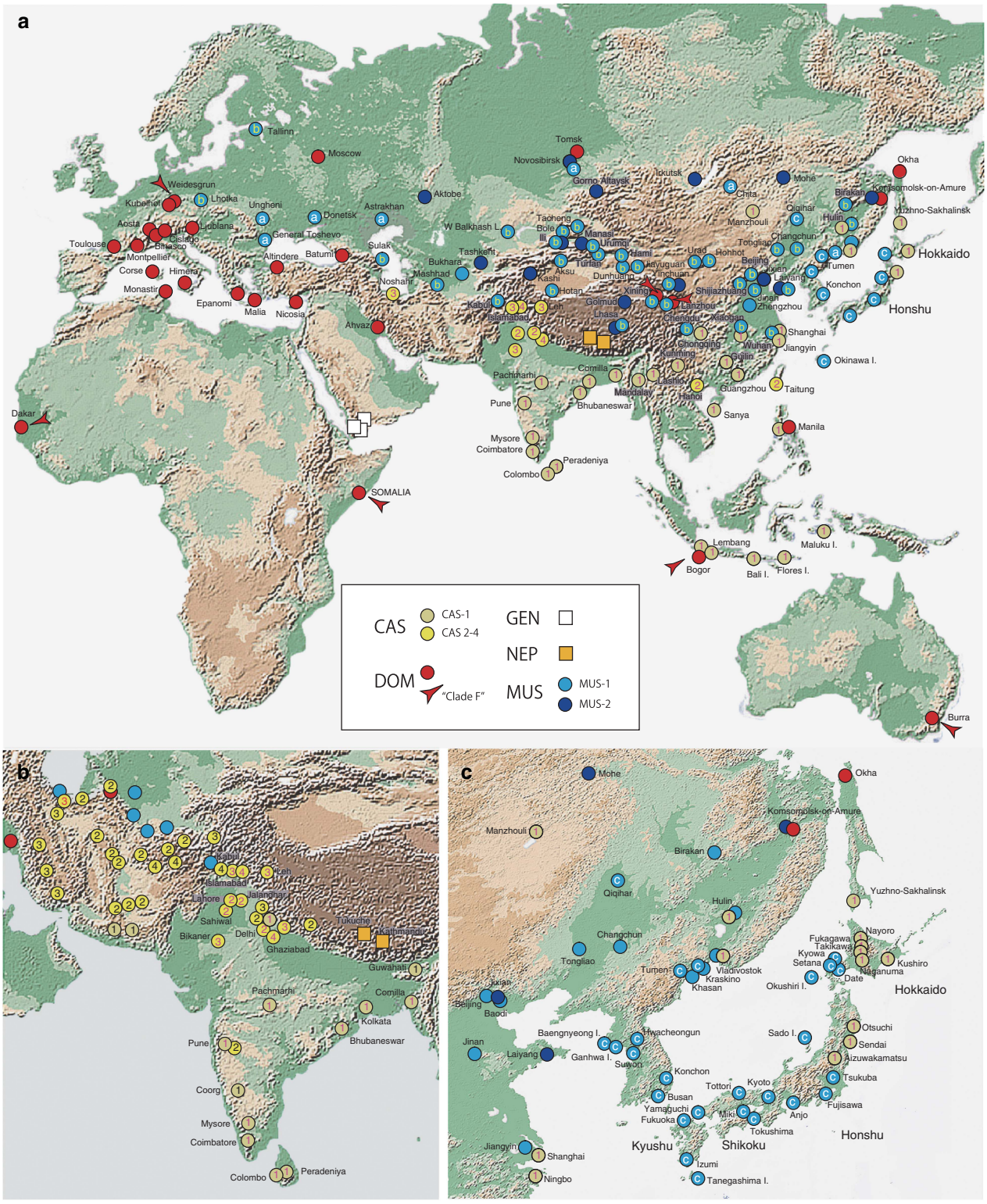

Figure 1 Collection of localities and mitochondrial genotypes in Eurasia of *M. musculus* samples examined in this study (**a**). New samples genotyped for this study are shown. Detailed locality names and sample codes are listed in Supplementary Table 1. Five major mitochondrial groups representing five subspecies groups, *M. m. musculus* (blue: MUS), *M. m. domesticus* (red: DOM), and *M. m. castaneus* (yellow: CAS), *M. m. gentilulus* (white: GEN) and the divergent lineage occurring in Nepal (orange: NEP) are differentially shown. The specific haplotype group of DOM that broadly dispersed to a variety of countries (Australia, Canada, China, Germany, Indonesia, Senegal, Somalia) are marked with arrowheads. Together with those from Prager *et al.* (1998), spatial patterns for the mitochondrial genotypes are shown for mice from Central Asia based on combination of new and previously published sequences (sources) (**b**), where further sub-division of the CAS lineage into four (CAS-1, CAS-2, CAS-3, CAS-4) are detected. The types of the four sub-groups of CAS are shown in circle with numerical numbers (black, Prager *et al.*, 1998; red, in this study). Further sub-division of the MUS lineages into two, MUS-1 (light blue) and MUS-2 (dark blue), and the MUS-1 sublineage into three (MUS-1a, MUS-1b, MUS-1c) is suggested in this study (**a** and **c**).

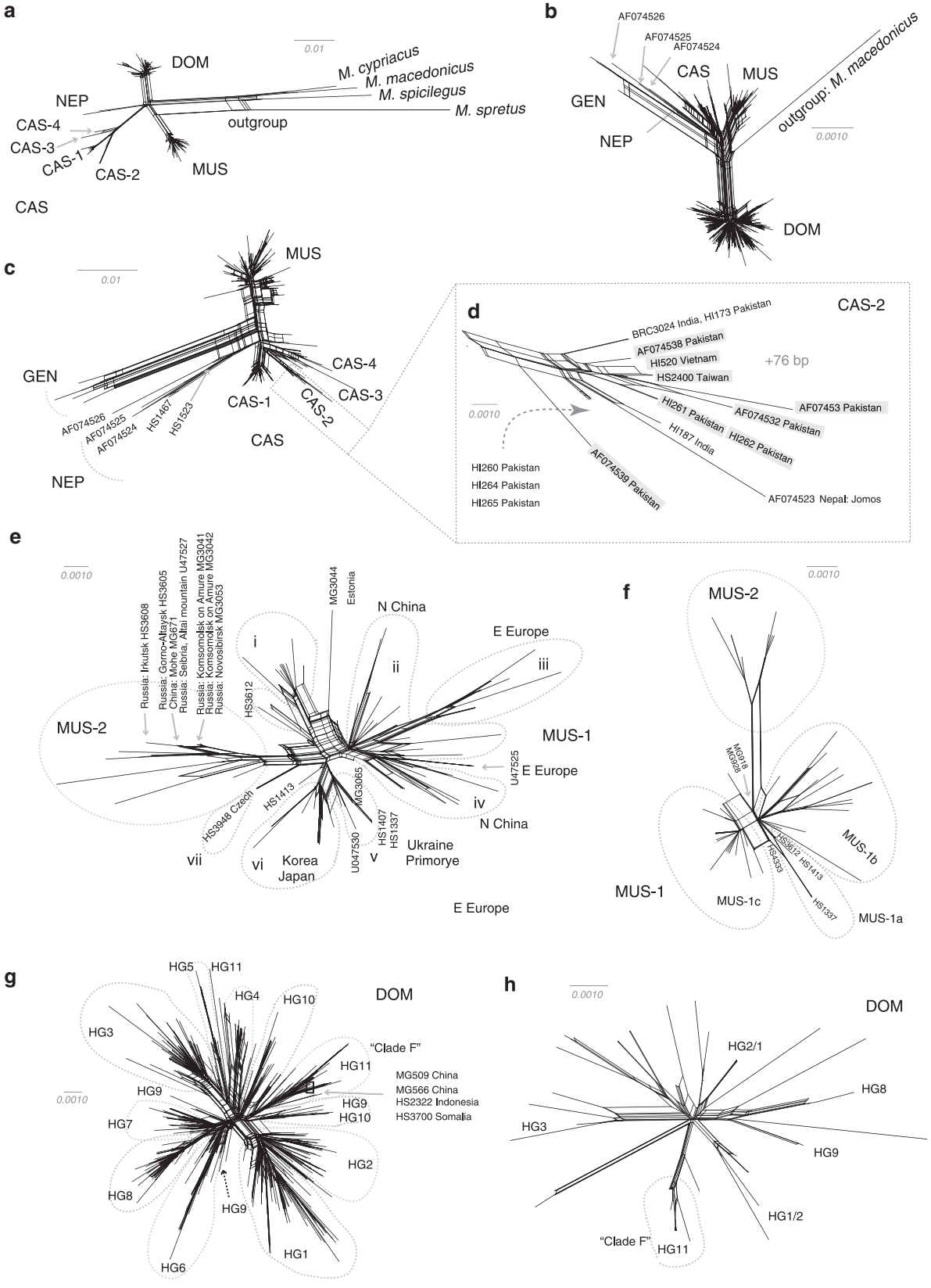

Figure 2 NN networks tree based on the cytochrome *b* gene (*Cytb*; **a**, **f** and **h**) and control region (CR; **b**–**d**, **e** and **g**) of the mitochondrial DNA, with tip labels for the three major subspecies groups, *M. m. musculus* (MUS), *M. m. castaneus* (CAS) and *M. m. domesticus* (DOM) and two rather geographically confined groups of *M. m. gentilulus* (GEN) and Nepalease mice (NEP). The portion of the CR network was enlarged to show the details of the branching patterns for CAS-2, in which most of members possess a 75-bp repeat (**d**). The codes for the HGs in the CR (**g**) and *Cytb* (**h**) network for DOM were taken from those used in Bonhomme *et al.* (2011).

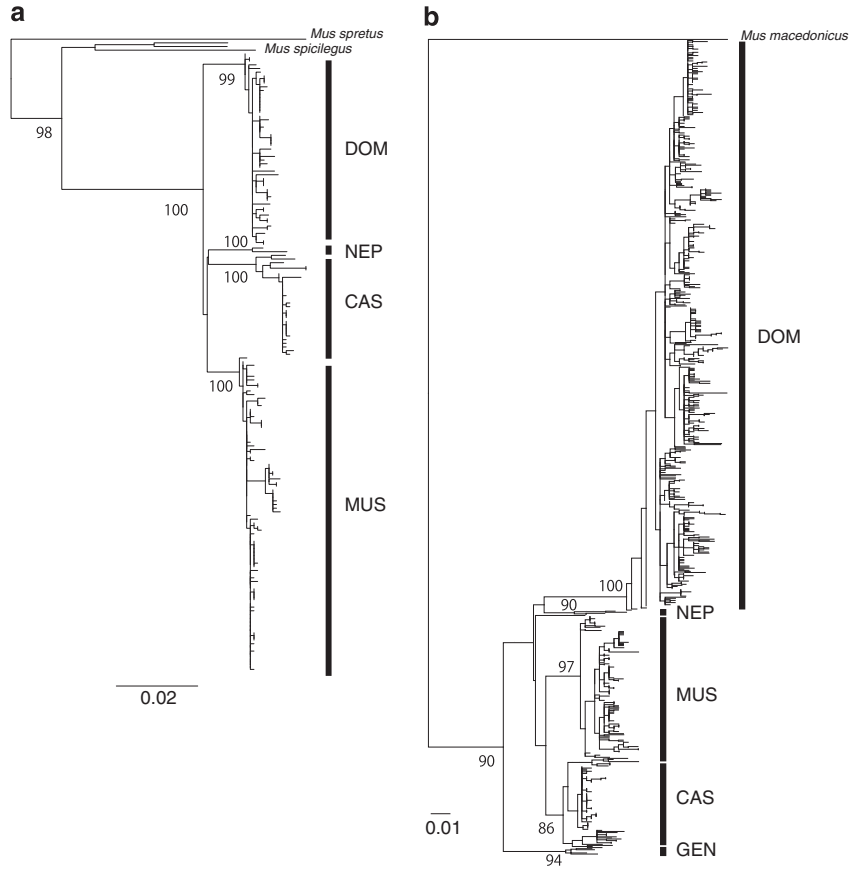

Figure 3 ML trees for mitochondrial DNA sequences of the cytochrome *b* gene (**a**) and control region (**b**). The PhyIML algorithm (Guindon and Gascuel, 2003) was used for the tree reconstruction and bootstrap analysis (100 replications). Bootstrap values (>50%) are shown under basal branches.

under the sudden-expansion model (Rogers, 1995) was tested by computing the sum of squares deviation (SSD).

RESULTS

Characteristics of major HGs

The NN network generated from the *Cytb* data set features four well-differentiated HGs (Figure 2a), three of them correspond to the previously identified DOM, CAS and MUS lineages. The fourth HG includes two sequences derived from Nepalese mice, one reported previously by Terashima *et al.* (2006; HS1467) and one new to this study (HS1523). For convenience, this HG is herein labeled NEP to indicate its geographic origin. No *Cytb* sequences are available for the GEN HG. The four *Cytb* HGs are equally divergent from a common central hub and outgroups either join this central hub or have an affinity with the MUS HG. The CAS HG appears to contain deeper lineage diversity than either the MUS or DOM HGs, both of which have distinctly brush-like terminal segments. Overall, the topology of the NN network for the *Cytb* data set is suggestive of a more or less simultaneous diversification of an ancestral *M. musculus* stock into multiple evolutionary lineages and also indicative of much recent diversification in each of the DOM and MUS HGs.

The ML phylogeny generated from the *Cytb* data set also features the same four clades with support values between 99% (DOM) and 100% (CAS) (Figure 3a). Monophyly of *M. musculus* (*sensu lato*) is well-supported relative to the outgroups, but there is no support for any special relationships among the four HGs.

The NN network generated from the CR data set (Figure 2b) shows a well-differentiated cluster of DOM sequences but less marked segregation among the other groups, which now includes GEN. A network generated without DOM (Figure 2c) shows well-differentiated HGs for GEN and MUS, and a less cohesive cluster of 5–6 HGs that includes 4–5 that might be regarded as 'CAS' (CAS-1, CAS-2, CAS-3, CAS-4 and AF074526) and one that includes the two NEP haplotypes (HS1467, Tukuche; HS1523, Kathmandu) as well as 'CAS' types 13 (AF074524, Kathmandu) and 14 (AF074525, Nuwakot) from Prager *et al.* (1998). The GEN and some CAS HGs are more divergent from the central hub than other HGs, but this may be due, in part, to missing data in some sequences obtained by Prager *et al.* (1998) from museum skins.

The ML phylogeny generated from the CR data set features five major clades with support values between 86% (CAS) and 100% (DOM) (Figure 3b). Monophyly of *M. musculus* (*sensu lato*) is well-supported, but there is no strong support for any special relationships among the HGs, as well as in the *Cytb* data set.

To further explore the phylogenetic relationships among the HGs, we constructed an ML phylogeny using concatenate sequences (CR + *Cytb*) for the 30 individuals represented in both the data sets. The resultant trees remain ambiguous for branching order among the four major lineages of CAS, DOM, NEP and MUS (Figure 4).

Genetic diversity in each of the main HGs is summarized according to a variety of standard parameters in Table 1. Excluding NEP where $n = 2$, for *Cytb* the highest nucleotide diversity (*Pi*) is observed in DOM, followed by CAS and MUS; while for CR nucleotide diversity

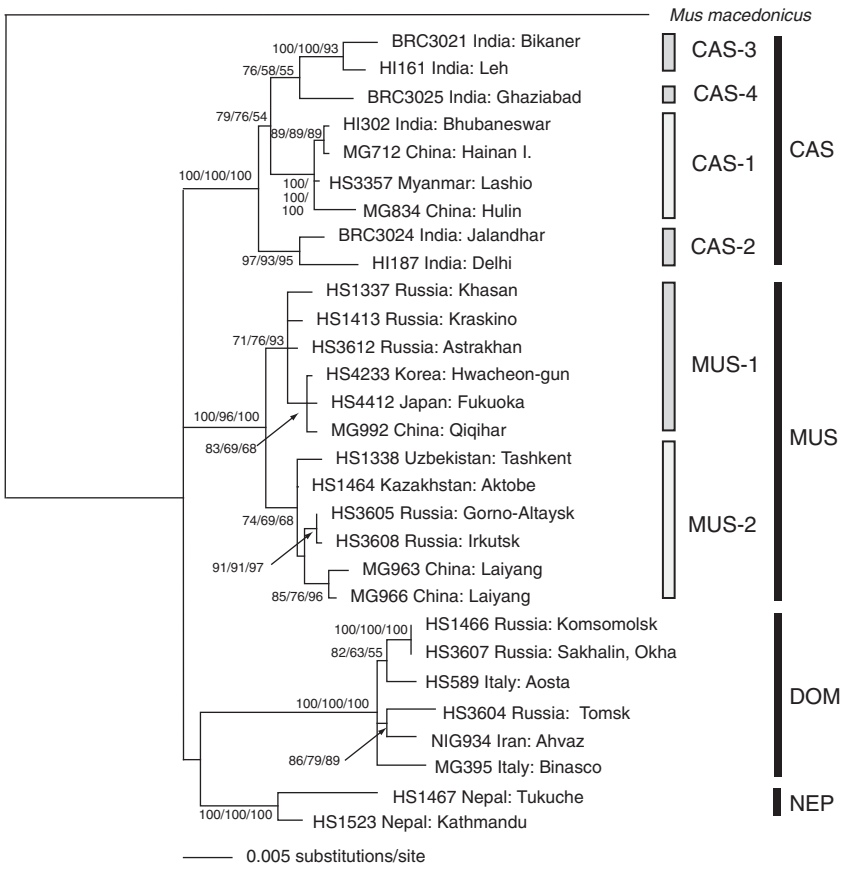

Figure 4 ML tree for concatenated mitochondrial DNA haplotypes (control region and cytochrome *b* gene) using representatives for the four major HGs of *M. musculus* and *M. macedonicus* as outgroup. Bootstrap values (>50%) are shown under basal branches (ML/MP/NJ).

is highest in CAS, followed by DOM and GEN, with MUS once again the lowest. The average number of nucleotide differences (*k*) is the highest for *Cytb* in DOM, followed by CAS and MUS; and the highest for CR in DOM, followed by CAS, GEN, and MUS. The number of distinct haplotypes (*H*) and number of polymorphic sites (*S*) both are clearly correlated with the total number of samples (*N*) in each of the *Cytb* and CR data sets (Table 1).

Geographic distribution of major HGs

The newly determined haplotypes show geographic distributions largely consistent with expectation based on previous findings (Figure 1a). DOM haplotypes are concentrated around the Mediterranean region but show numerous widely dispersed outliers, including localities within MUS territory in western and northeastern Russia and in China and within CAS territory in the Philippines and Indonesia; MUS haplotypes are predominant in northern part of Eurasia excluding western Europe; and CAS haplotypes are predominant across South and Southeast Asia but with outliers in Japan, the Middle East and eastern Russia.

A more detailed mapping of new and previously published sequences from South Asia through to the Middle East illustrates the concentration of mtDNA diversity in southwestern Asia for *M. musculus* as a whole and additionally for sub-groups within CAS (Figure 1b; identity of sub-groups discussed below).

Genetic and phylogeographic structure of individual HGs

CAS HG. Four well-differentiated sub-groups within CAS are clearly depicted in the NN network for the CR data set (Figure 2c), and they are also evident in the NN for the smaller *Cytb* data set (Figure 2a) and in the ML tree for the concatenated data set (Figure 4); they are herein designated as CAS-1, CAS-2, CAS-3 and CAS-4, as mentioned above. Two of these sub-groups were identified by Terashima *et al.* (2006) and labeled CAS-II (=CAS-1 of this study) and CAS-I (=CAS-2 of this study). An outlier CR sequence (AF074526=CAS type 15 of Prager *et al.*, 1998, from Ilam, western Nepal) may represent a fifth sub-group (Figure 2c), but this requires confirmation as it was obtained from a museum skin and contains several gaps. The CAS sub-groups emerge from a central hub on the NN networks and, with the exception of AF074526, show approximately equivalent degrees of divergence. Each of the main sub-groups also shows relatively deep haplotype diversity; uniquely in CAS-1, this includes a brush-like structure suggestive of recent radiation from a common ancestral haplotype.

The ML phylogeny for the concatenated data set (Figure 4) recovered monophyletic clades with good support (>90%) for CAS-1, CAS-2 and CAS-3, indicated a close relationships between CAS-3 and CAS-4 (BRC3025) with low or moderate support (>50%) and suggested a basal derivation of CAS-2 with low or moderate support (>50%).

The phylogeographic pattern for the CAS HG appears relatively uncomplicated. The greatest haplotype diversity is observed in Pakistan and northern India where all four sub-groups are present but CAS-2 and CAS-3 are dominant (Figure 1b). Approximately half of the sequences of CAS-2 share a 76-bp tandem repeat (reported by Prager *et al.*, 1996, 1998), which further supports the monophyly of this sub-group; these include mice from Taitung in Taiwan and Hanoi

Table 1 Data summary for populations, haplotypes and nucleotide diversity of *Mus muculus*

Marker	*Subspecies group*	N	H	*Hd*	S	*Pi (%)*	k
Control region (CR)							
	CAS	86	42	0.958	54	1.195	6.91
	MUS	132	66	0.981	58	0.842	5.16
	DOM	531	370	0.997	163	1.053	8.3
	GEN	6	6	1	16	1.029	6.26
	NEP[a]	2	2	1	12	1.376	12
	Nepal[b]	5	5	1	22	2.703	11
Cytochrome b (Cytb)							
	CAS	38	17	0.895	44	0.468	4.48
	MUS	88	49	0.956	67	0.407	4.62
	DOM	53	31	0.968	57	0.526	5.07
	NEP	2	1	1	12	1.053	12

Abbreviations: *H*, number of haplotypes; Hd, haplotype diversity; *N*, number of samples; *S*, number of polymorphic sites; *Pi*, nucleotide diversity; *k*, mean number of pairwise differences among sequences.
[a]NEP: HS1467, HS1523.
[b]Nepal: HS1467, HS1523, AF074524, AF074525, AF074526.

in Vietnam (Figure 2d), constituting the only occurrences of the CAS-2 HG outside of India and Pakistan.

Sub-groups CAS-1 to CAS-3 are represented in central India but CAS-1 alone is more widely distributed, with representation in southern India and Sri Lanka, and also across southeast Asia, China and eastern Russia to Japan (Figure 1). A NN analysis of using concatenate sequences (CR + *Cytb*) from 40 individuals of CAS-1 (Figure 5a) suggested the presence of a further sub-division that we recognize as CAS-1a and CAS-1b. CAS-1a haplotypes come from two localities in southern China (Guilin and Kunming), from northern Japan (northern Honshu and Hokkaido) and from southern Sakhalin. CAS-1b haplotypes come from a wider geographic area, including several parts of India, Bangladesh, Sri Lanka, Myanmar, southern China, Hainan Island, southern Sakhalin and Primorye, eastern Indonesia and Morocco.

MUS HG. The MUS HG appears to be comprised of two main sub-groups, which are herein designated MUS-1 and MUS-2. These are most clearly expressed in the NN network based on concatenated CR and *Cytb* data from 38 individuals (Figure 5b), but they are also evident in the networks generated from the individual data sets (CR, Figure 2e; *Cytb*, Figure 2f).

A total of seven clusters were identified within MUS-1 on the CR NN network (labeled i–vii on Figure 2e); the majority of these clusters show a high level of geographic fidelity. Based on relationships observed in the NN networks for *Cytb* (Figure 2f) and the concatenated data set (Figure 5b), we suggest that these CR phyletic groups can be revolved into three phyletic lineages that we herein designate as MUS-1a (CR clusters i, iii, v, vii), MUS-1b (CR clusters iii, v) and MUS-1c (CR cluster vi).

Sub-group MUS-1 as a whole is represented across the entire geographic range of MUS. However, its components show high fidelity to discrete geographic areas: MUS-1a is largely confined to eastern Europe (Ukraine, Moldova, south Siberia and Primorye in the Russian Far East; see Figure 1 for the geographic distribution); MUS-1b is predominantly Chinese, being represented at multiple localities spanning the entire breadth of China, from Xanjiang Uyghur Autonomous Region in the northwest to Shandong Province on the eastern seaboard, though there are several occurrences of this in Transcaucasia, Iran, eastern Europe and Russia adjacent to China; and MUS-1c is geographically restricted to far northeast China (Tumen, Qiqihar), Korea and Japan, with one outlier recorded from the coastal city of Kraskino, near the Russian–Korean border (Figure 1c).

The MUS-2 sub-group is distributed across the eastern half of the range of MUS, with representation to the north and east of the Caspian Sea (Kazakhstan and Turkmenistan, respectively), south Siberia in the Altai Mountains, Novosibirsk and Irkutsk, Primorye, and across China, including localities in the far northwest (Ili Khazakh Autonomous Prefecture), the central region (Ningxia Hui Autonomous Region), the far north (Manasi), the Tibetan Plateau (Lhasa) and Shandong Province in the east (Liyang). Most of the MUS-2 haplotypes recorded to date are known from single localities and many differ by two or more nucleotide substitutions from the closest sequences (Figures 2e and f). Only two MUS-2 haplotypes were detected at multiple localities and neither appears to be an ancestral haplotype. In each case, the shared haplotypes are recorded from widely separated localities, suggestive of recent long-distance dispersal or translocation.

DOM HG. The NN network for DOM CR sequences is an explosively radiating structure, likened by Bonhomme *et al.* (2011): Figure 1b to a 'multiple-armed sea star' (Figure 2g). The additional 23 CR sequences added in this study do not disrupt the primary structure of the NN network with 11 HGs, though HGs 1 and 2 appear somewhat more mixed than in the presentation of Bonhomme *et al.* (2011): Figure 1b and the small HG9 appears to have disaggregated into basal positions within HGs 1, 2 and 7. Most of our new sequences fall into HG11, which corresponds with Clade F of Jones *et al.* (2010), including sequences from the novel (outlier) localities of Somalia (HS3700), central China (MG509, MG566) and Java, Indonesia (HS2322).

Most of the HGs are also evident in an ML tree (not shown) though supporting values were low (65% for HG5 and $<50\%$ for others). However, HG9 occupies a more diffuse central position consistent with its lack of unity in the NN network, and HGs 1 and 2 were not supported, though most members of these putative HGs associated correctly in smaller clades. An aggregation of HG7 with HG8, is also evident in both the NN network and the ML tree, albeit with no substantial support in the ML analysis.

The smaller *Cytb* data set presents a simpler picture (Figure 2h). The NN network shows 12 clusters, some of which are represented by single sequences. Six of the clusters can be correlated to CR HGs based on the subset of individuals represented in both the data sets. An MJ network (not shown) shows a completely stellar arrangement with minimal reticulation and with all terminal haplotypes similarly divergent (3–6 nucleotide substitutions) from a central node. This putative ancestral haplotype has not been detected. HGs 8 and 9 derive from a common primary branch on the MJ network.

The various analyses performed on DOM sequences do not suggest any grounds for its formal sub-division, as was suggested above for each of CAS and MUS. Rather, the topology appears to be genuinely explosive, involving differentiation of multiple, regionally- based matrilines, as concluded also by Rajabi-Maham *et al.* (2008, 2012) and Bonhomme *et al.* (2011).

Divergence time among and within the HGs

Divergence estimates generated by BEAST for each of the four major HGs have central values that range between 0.37 and 0.46 mya (Figure 6); in each case, the 95% highest probability density values have spans of around ± 0.16 mya (Table 2). The TMRCA of sub-group diversification within each of the CAS, MUS, DOM and NEP HGs

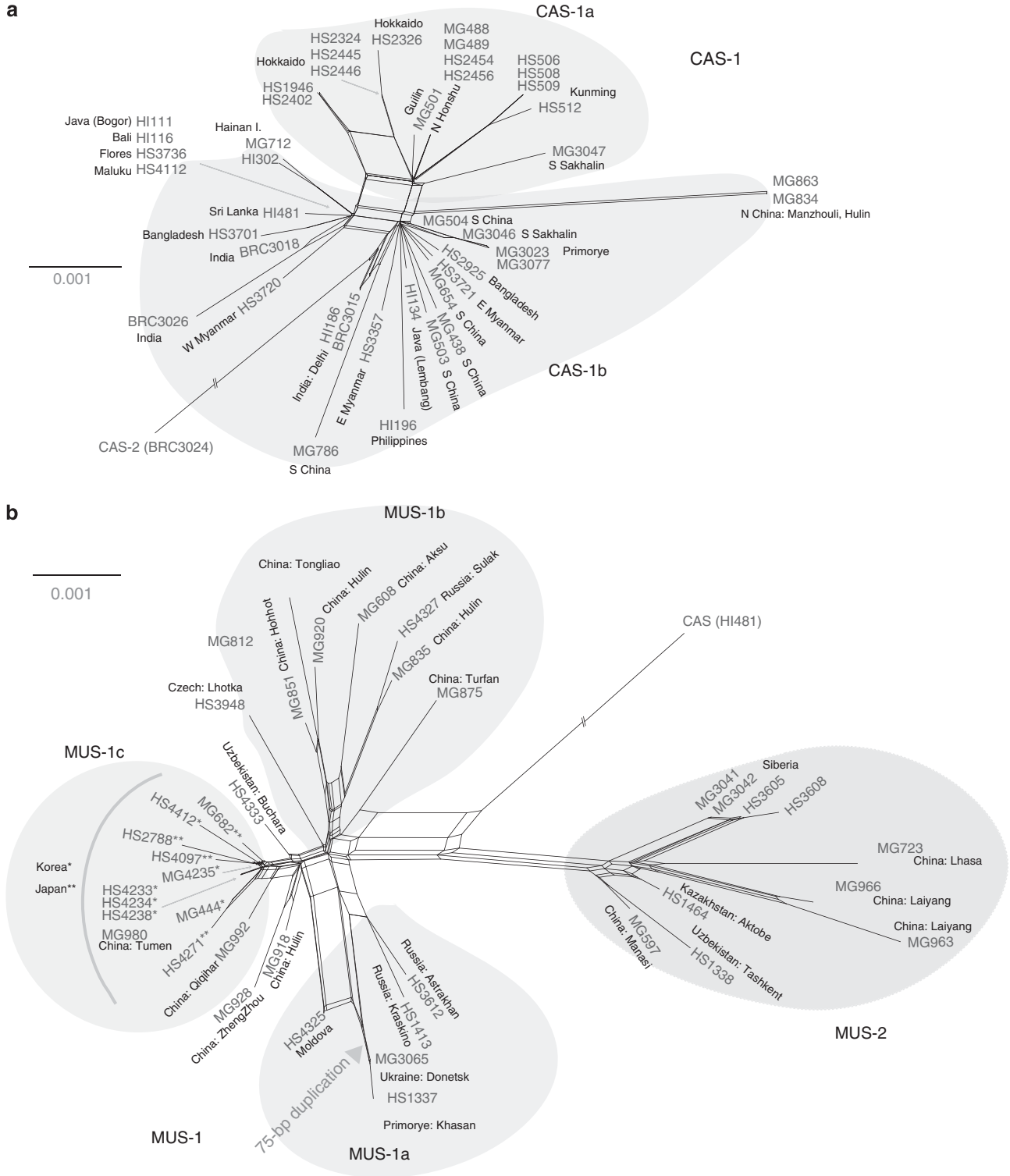

Figure 5 NN networks of concatenate sequences of control region and cytochrome *b* gene (*ca.* 2020 bp) from individuals representing the sublineage of CAS, CAS-1 (**a**) and MUS (**b**); individuals from Korea and Japan are marked with * and **, respectively. Prominent sub-groups appeared in the networks are indicated.

was estimated at 0.22 ± 0.08, 0.15 ± 0.07, 0.13 ± 0.04 and 0.14 ± 0.06 mya, respectively (Table 2).

The Tajima's *D* values for all HGs and sub-groups were significantly negative, indicating various phases of rapid population growth involving mice with matrilines in CAS-1, CAS-1b, MUS-1, MUS-1b, MUS-1c and DOM (Table 3). We estimated the age of population growth in each of the phyletic groups under four different mutation rates of *Cytb*; 2.5, 10 and 20% (Table 3).

The mismatch distribution for the CAS-1 *Cytb* data set (Supplementary Figure S1) shows a multi-peaked distribution, which

is consistent with the notion of CAS-1 as a well-structured HG (Figure 5a). CAS-1b shows a good mismatch conformation to a model of recent population growth, with further support coming from a statistically significant negative value for Tajima's *D* (Table 3). Tajima's *D* was negative but not statistically significant for CAS-1a. In MUS, there is support for recent population expansion of MUS-1 as a whole and for each of MUS-1b and MUS-1c, each backed up by statistically significant negative values for Tajima's *D*, though the SSD value for MUS-1 was significant ($P<0.01$), as evidence for departure from the estimated model of population expansion (Table 3). In contrast, there is no support for recent population expansion of either MUS-1a and MUS-2. The mismatch distribution for both DOM CR (data not shown) and *Cytb* data sets (Supplementary Figure S1) shows near perfect conformation with the population growth and decline model provided by DNASP. Neutrality test statistics also point to a significant phase of population expansion in the recent history of DOM (Tajima's $D=-2.02$, $P<0.05$), as concluded previously by others (Rajabi-Maham *et al*., 2008; Bonhomme *et al*., 2011), though the SSD value was significant ($P=0.024$).

DISCUSSION

Much of our current understanding of *M. musculus* phylogeography remains little modified from the conclusions of early studies of mtDNA (for example, Boursot *et al*., 1993, 1996; Yonekawa *et al*., 1994; Prager *et al*., 1996, 1998; Boissinot and Boursot, 1997) and of allozymes and other nuclear markers (for example, Bonhomme *et al*., 1984; Miyashita *et al*., 1994; Din *et al*., 1996). Three of the most persistent notions to emerge from these early studies are: (1) the understanding that the common ancestor of all of the major *M. musculus* HGs arose in the region of western to central Eurasia, either somewhere in the mountainous terrain that extends from Transcaucasia through to northwest India (Boursot *et al*., 1993, 1996; Din *et al*., 1996) or possibly in the low-lying region of Mesopotamia (Prager *et al*., 1993, 1996); (2) the belief that the broader distribution of all major HGs is due to range expansions that occurred following the development of commensalism and thus within the last 10 000 years; and (3) the conclusion that the CAS lineage is genetically more diverse and probably older than either of DOM or MUS, with MUS probably trailing DOM in this regard.

To date, these notions have been subjected to detailed scrutiny only for the DOM HG (Gündüz *et al*., 2005; Darvish *et al*., 2006; Rajabi-Maham *et al*., 2008; Bonhomme *et al*., 2011; Duvaux *et al*., 2011). In this case, the majority of results uphold the general assumptions as outlined above.

For each of the MUS and CAS HGs, the most comprehensive phylogeographic analyses before this study were contained in the work of Prager *et al*. (1996, 1998). For their initial study of the *musculus* and *domesticus* lineages, geographic sampling was heavily biased toward Europe, with only a smattering of samples derived from the eastern range of *musculus*. In the later study, this was partially rectified through the laborious extraction of DNA from museum skin samples from eastern populations of *musculus* and *castaneus*. Despite this remarkable effort, major geographic gaps in sampling remained; and with such large geographic areas to cover, sample sizes were small for all the regions.

Our sampling has filled many of the gaps in geographic coverage, especially for the Indian subcontinent, Indochina and the Far East. However, the issue of small sample sizes remains and will not be solved without further field collection on a multi-regional scale. Nevertheless, our broader sampling produces new insights into the phylogeography of each of the CAS and MUS groups and allows us to challenge several key aspects of the current understanding.

A homeland for *M. musculus* in southwestern Asia

The ancestral homeland of *M. musculus* is most likely to coincide with a broad region of co-occurrence of the various phylogroups, and it should encompass or about the geographic range of the most restricted phylogroups, namely GEN and NEP of the Arabian

Table 2 Divergence time estimation using mitochondrial cytochrome *b* sequences (1140 bp)

Node[a]	*TMRCA estimated*	*Divergence time*[b]	*Confidence interval*[c]
A	DOM/NEP and CAS/MUS	0.4591	0.3250–0.4808
B	CAS and MUS	0.4179	0.2863–0.5432
C	DOM and NEP	0.3719	0.2385–0.4805
D	CAS	0.2173	0.1340–0.2947
E	MUS	0.1509	0.0812–0.2136
F	DOM	0.1333	0.0690–0.1478
G	NEP	0.1408	0.0623–0.1852

Abbreviation: TMRCA, the age of the most recent common ancestor.
Divergence times were calculated on the assumption of 1.7 mya for the divergence of *Mus muculus* and *Mus spretus*.
[a]See Figure 6 for detail.
[b]Mean divergence times (million years ago; mya) were obtained from the BEAST (Bayesian evolutionary analysis by sampling trees) analysis.
[c]Confidence interval values are 95% highest posterior density interval.

Table 3 Estimation of population expansion times (year before present) based on mitochondrial cytochrome *b* sequences (1140 bp)

Group	*Main range*	N	*Pi (%)*	*Tajima's* D	*Tau (confidence interval)*	*SSD*	*Subsitution rates (myr/site/lineage)*		
							2.5%	*10%*	*20%*
CAS-1	S and E Asia	34	0.195	−1.93*	1.835 (1.279–2.556)	0.00353	32 200	8000	4000
CAS-1a	Yunnan, N Japan	17	0.08	−1.237	—				
CAS-1b	India, SE Asia	17	0.202	−2.583*	1.734 (0.436–3.676)	0.01005	30 000	7600	3800
MUS-1	N Eurasia	76	0.304	−2.257**	1.725 (1.724–2.352)	0.07956**	30 200	7500	3800
MUS-1a	E Europe	9	0.244	−0.255	—				
MUS-1b	N China	26	0.392	−1.952*	4.920 (2.941–6.468)	0.00451	86 000	21 000	10 800
MUS-1c	Korea, Japan	41	0.12	−2.190*	1.527 (0.746–2.535)	0.01595	26 000	6600	3300
MUS-2	China, Siberia	12	0.293	−0.353	—				
DOM	W Europe	53	0.526	−2.058*	5.464 (3.998–6.089)	0.00891*	95 900	23 900	12 000

Abbreviations: *N*, number of samples; SSD, sum of squares deviation.
N, *Pi* and Tajima's *D* values were calculated using DNASP v5, and *Tau* and SSD values were obtained using Arlequin.
*$P<0.05$, **$P<0.01$.

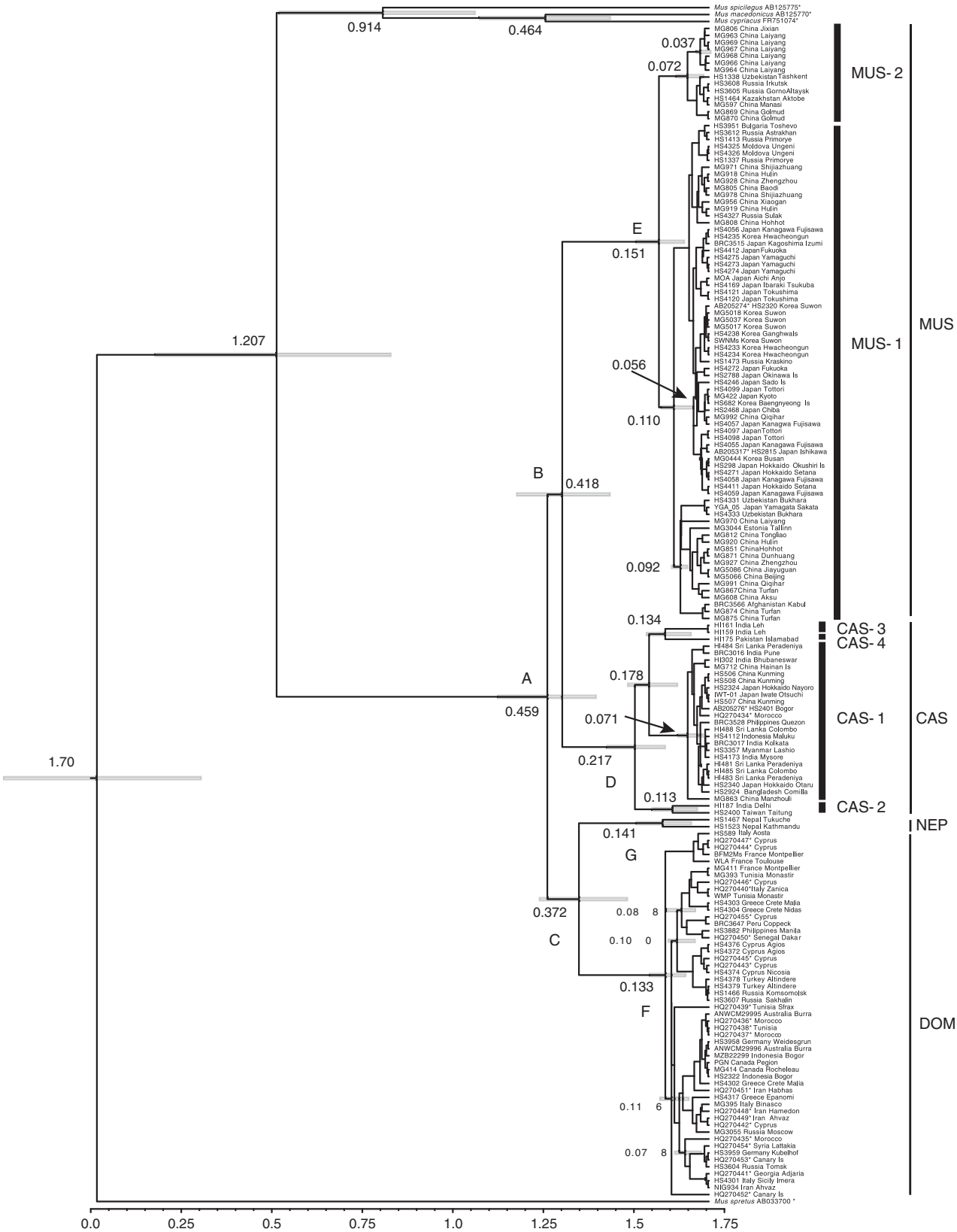

Figure 6 Divergence time estimates (million years ago, mya) of *M. musculus* phylogroups and its closely related species, based on a Bayesian-relaxed molecular clock applied to the mitochondrial cytochrome *b* sequences (1140 bp). The posterior probability and 95% highest posterior density intervals of node ages in mya (gray bars) are shown in particular nodes with ancient divergent. The time estimates of 1.7 mya for the root node of the divergence of *M. spretus* and the other species of *M. musculus* Species Group (Suzuki *et al*., 2004) was used as calibration point. Sequences obtained from the databases are marked with their accession numbers and asterisks.

Peninsula and Himalayan region, respectively. Under these criteria, the region of southwestern Asia, encompassing modern day Iraq, Iran, Afghanistan, Pakistan and northwestern India stands out as the most likely candidate area. Bonhomme *et al.* (1984) reached the same conclusion on different evidence, namely the higher levels of variation in nuclear genes among mice in this area compared with peripheral regions (see also Suzuki *et al.*, 1986; Boursot *et al.*, 1996; Boissinot and Boursot, 1997; Prager *et al.*, 1998; Darvish *et al.*, 2006; Duvaux *et al.*, 2011). As discussed at length by Prager *et al.* (1998), mtDNA lineage boundaries in this area show general association with major geographic barriers (see also Duvaux *et al.*, 2011). In particular, the Zagros Mountains divide DOM in the west from CAS in the east, while the Elburz Mountains divide MUS in the north from CAS in the south. Similarly, the mountain chains of the Hindu Kush separate populations of MUS and CAS in northern Afghanistan, though the present day distribution of the mtDNA haplotypes is not always associated with the mountainous range (for example, MUS in Kabul, Afghanistan, Figure 1a). A process of allopatric differentiation is indicated, as also suggested by the lack of overt ecological differentiation among the divergent populations.

Our divergence estimates of 0.37–0.47 mya (see Table 2 for confidence interval) for the major mitochondrial phylogroups are in good accord with previous determinations (Rajabi-Maham *et al.*, 2008; Terashima *et al.*, 2006). Initial lineage diversification evidently predates the dispersal of modern humans out of Africa; hence it is likely that initial phases of range expansion were not mediated by human activity, unless of course the impact of early human populations on the environment was much greater than currently understood.

An interesting biogeographic observation is that the inferred place of origin of *M. musculus* (i.e. southwestern Asia) lacks any other co-occurring mouse species belong to subgenus *Mus*. In this regard, it differs from each of those found in peninsular India, where *M. booduga* and *M. terricolor* of the *M. booduga* Species Groups are both found (Musser and Carleton, 2005); Indochina, which hosts a variety of species in the *M. booduga* and *M. cervicolor* Species Groups (Suzuki and Aplin, 2012); and eastern Europe, where other species of the *M. musculus* species group are present. It is tempting to speculate that the presence of these ecologically similar native species in surrounding areas formerly served to constrain the geographic distribution of *M. musculus*.

The distribution and ecology of contemporary *castaneus* populations in Asia provides further clues to its regional history. As summarized by Marshall (1977): 205–206 the only 'outdoor commensal' (that is, agricultural field) populations of CAS mice are found in the semi-arid habitats of Pakistan (the *bactrianus* morphotype). Elsewhere on the Indian subcontinent and through into Southeast Asia, house mice are found only as 'indoor commensals'; furthermore, across Southeast Asia, they are generally confined to larger towns and absent in rural villages (see also Aplin *et al.*, 2006). Marshall (1977) attributed the absence of house mice from agricultural contexts in these areas to competitive exclusion by other species of *Mus*, notably members of the *Mus booduga* Species Group on the Indian subcontinent and members of the *Mus cervicolor* Species Group in South East Asia; and he attributed the absence of house mice in rural villages in Southeast Asia to the presence of commensal species of *Rattus* such as *R. exulans*.

Phylogeography of the CAS lineage

The CAS lineage has been subject to two different phylogeographic interpretations. Boursot *et al.* (1993, 1996) proposed that the northern Indian subcontinent was both the place of origin of *M. musculus* and the cradle of genetic diversity within this group. This model was based on the discovery in this area of numerous highly divergent mtDNA lineages (Boursot *et al.*, 1996) and levels of nuclear diversity (as determined by allozyme electrophoresis) that exceeded those found in European populations of *domesticus* and *musculus* (Din *et al.*, 1996). To explain these dual observations, Boursot *et al.* (1993, 1996) proposed a 'centrifugal' model of differentiation in which the ancestors of each of the *domesticus*, *musculus* and *castaneus* lineages dispersed to the west, east and north, each carrying a subset of the mtDNA and nuclear diversity, and subsequently undergoing local differentiation. They referred to populations in the ancestral area as '*Mus musculus* subsp.' and restricted use of the name *castaneus* to populations in southern India, southern China and Indochina. Boursot *et al.* (1996) referred to the northern Indian and Pakistani populations as an 'oriental group', while Yonekawa *et al.* (1994) subsequently applied the existing name *bactrianus* to these populations.

The version of CAS phylogeography by Prager *et al.* (1998) is based primarily on interpretation of mtDNA phylogeny. Although confirming a high diversity of mtDNA types in northern India and Pakistan, they regarded these to be part of a monophyletic *castaneus* lineage distinct from each of *domesticus*, *musculus* and the newly recognized *gentilulus* lineage of the Arabian Peninsula. Prager *et al.* (1998) developed a model of 'sequential' derivation of the lineages to reflect their phylogenetic branching order—*domesticus* being the oldest branch, followed by *gentilulus*, *castaneus* and *musculus*. They preferred to locate the ancestral pre-*domesticus* stock in the Near East, within the current range of *domesticus*, and regarded the progressive derivation of other lineages as a consequence of sequential dispersal events that took house mice south onto the Arabian Penisula, then east onto the Indian subcontinent and, finally, north through the mountains of northwest India and Pakistan to occupy the great Eurasian steppe. Within CAS, Prager *et al.* (1998) suggest a relatively long phase of regional diversification on the Indian subcontinent, followed by a 'more recent' dispersal into the 'humid lowlands of Southeast Asia'.

Our sampling for CAS is relatively extensive and the results go far towards illuminating the historical phylogeography of this HG. In keeping with the findings of Prager *et al.* (1998) and contrary to the predictions of Boursot *et al.* (1993, 1996), we recovered reciprocal monophyly with good to excellent support among all of the major HGs, including CAS. Although Prager *et al.* (1998) considered the branching order among the major HGs to be resolved, our larger CR and *Cytb* data set fails to provide a robust phylogenetic structure at this level, although there is a suggestion of special affinity between CAS and MUS and between DOM and NEP. Like both groups of previous researchers, we found the highest mtDNA diversity and depth in CAS populations inhabiting the mountainous region of northwest India and Pakistan, with a loss of haplotype lineage diversity from north to south on the Indian subcontinent (Boursot *et al.*, 1996) and from west to east into Southeast Asia (Prager *et al.*, 1998). Despite this general agreement, Boursot *et al.* (1996) clearly regard *castaneus* in their restricted application of the name to be a long-term resident of Southeast Asia, while Prager *et al.* (1998) portray this as a relatively recent phase of dispersal of *castaneus*, though without specifying any time frame.

Low nucleotide diversity in the widely distributed CAS-1 subgroup is evident in this study. This is consistent with that observed by the recent work on the *castaneus* subspecies group done by Rajabi-Maham *et al.* (2012) (see also Bonhomme and Searle, 2012). Our

results are suggestive of a relatively recent range expansion of CAS-1 to a large geographic areas covering the south and east Indian subcontinent, Southeast Asia, Indonesia, South China, Northeast China and the Russian Far East (Figure 5). On the other hand, the presence of the locally restricted phyletic group, CAS-1a is suggestive of stepwise historical range expansion of CAS-1. Haplotype diversity within CAS-1a, the sub-group found in mice from South China (Kunming and Guilin), northern Honshu, Hokkaido and South Sakhalin, was most likely produced by subsequent dispersal and is suggestive of several thousands of years of *in situ* evolution. Furthermore, the location of the Japanese cluster at the far eastern periphery of the CAS distribution implies a significantly earlier onset for dispersal onto the Indian subcontinent and thence through to East Asia.

We suspect that the dispersal of CAS-1 mice occurred in response to ecological transformation of the landscape by early agriculturalists and the emergence of urban centers and trade networks. As has been postulated for the Middle East (Auffray *et al.*, 1990; Cucchi and Vigne, 2006), South Asian populations of *M. musculus* are likely to have benefited from the creation of new agricultural landscapes, and the common practice of storing harvested grain inside villages and even inside houses provided the context for development of commensalism. Long-distance dispersal is part and parcel of commensalism, with mice being carried as stowaways during transport of grain, building materials, clothing and bedding (Pocock *et al.*, 2005).

Although the archaeological record of agriculture is less comprehensive for Asia than for the Middle East and Europe, there is good evidence for domestication of cereal crops, including rice and millet, by about 9000 years ago in several parts of South and East Asia (Khush 1997; Londo *et al.*, 2006; Zheng *et al.*, 2009; Molina *et al.*, 2011) and even earlier evidence for long-distance overland and maritime trade (Oka and Kusimba, 2008). Assuming that populations experienced a sudden or exponential growth, we calculated τ values from the *Cytb* sequences and estimated times since the onset of population expansions. Higher rates of mutation (for example, 10% or 20% per million years per lineage) rather than lower rates (for example, 2.5%) are considered to be realistic for assessing rather recent diversifying events (Ho *et al.*, 2005). We obtained a τ value of 1.7 for CAS-1b ($n = 17$), which under mutation rates (per million years per lineage) of 10 and 20% gives expansion times of 7600 and 3800 years, respectively (Table 3). A τ value was not calculated for CAS-1a, but it too is likely to have commenced its dispersal and diversification in China, the Russian Far East and Japan in prehistoric times. In this regard, it is of interest to note archaeological evidence for rice cultivation along the upper Yangtze river (for example, Yunnan province, here represented by mice from Kunming) at 4500 years ago (Fuller *et al.*, 2010) and recent genetic evidence from an intensive genome survey on wild and cultivated rice, suggesting the Pearl River (Guangxi province, here represented by mice from Guilin) in southern China is the place of the first development of cultivated rice (Bonhomme and Searle, 2012; Huang *et al.*, 2012).

Comparatively recent long-distance dispersal of CAS mice most likely explains the detection of CAS-2 haplotypes at isolated localities in Taiwan and Vietnam, though we could not exclude out the possibility that these are relictual haplotypes, either rare survivors of an earlier dispersal of CAS-2 mice out of India that was swamped by a later CAS-1 dispersal or the last remnants of incomplete lineage sorting of an immigrant population with a mixture of CAS-1 and CAS-2 haplotypes. In the case of the individual from Taiwan, the fact that its nuclear genetic profile is fully consistent with other East Asian populations of CAS and differ from mice from India and Pakistan with the CAS-2 mtDNA haplotypes (Nunome *et al.*, 2010a; Kodama *et al.*, unpublished) suggests that we are not dealing with a novel invader but perhaps with a product of mtDNA introgression.

Phylogeography of the MUS lineage

Previous phylogeographic interpretations of the house mouse group do not vary much with regard to the geographic origin of the MUS HG. Boursot *et al.* (1993) speculated that 'the cradle of *M. m. musculus* could be in Transcaucasia or east of the Caspian Sea', while Prager *et al.* (1993, 1996) saw the origin of MUS as the product of northward dispersal from a proto-CAS population occupying the region east of the Caspian Sea, followed by range expansion. Both groups of researchers also agree that MUS populations subsequently dispersed west into central Europe and east into China and Japan, and this scenario has been adopted as paradigmatic by Japanese researchers interested in the origin of the indigenous *molossinus* population (Yonekawa *et al.*, 1988; Terashima *et al.*, 2006; Nunome *et al.*, 2010a). Yonekawa *et al.* (1988) postulated that MUS populations relatively recently expanded into China where CAS populations had already colonized, but few other workers have expressed an opinion on the earlier timing of the remarkable eastward expansion of MUS. Nunome *et al.* (2010a) suggested a latitudinal division within MUS between the northern (MUS-I) and southern (MUS-II) groups, based on phylogeographic analyses of nuclear gene sequences, and posited that range expansion of the MUS HG from west to east across continental Eurasia followed separate northern and southern dispersal routes, with separate expansion again into eastern Europe.

Much of the interest in the geographic distribution of MUS has focused on its genetic interaction with mice of other HGs. In the European context, numerous studies have examined the evolutionary dynamics of a narrow hybrid zone with DOM that runs from Norway through Denmark, Germany and Austria to eastern Bulgaria (Hunt and Selander, 1973; Sage *et al.*, 1993; Boursot *et al.*, 1993; Jones *et al.*, 2010). There are grounds to believe that initial contact may have occurred further west in Europe with the current position stabilizing after a period of eastward retreat of *musculus* (Gyllensten and Wilson, 1987). Whatever the case, the age of the contact zone is constrained by the timing of the DOM migrations along the shores of the Mediterranean, an event that is thought to date to within the past 2–3000 years (Cucchi *et al.*, 2005).

In Transcaucasia, gene flow between complexly parapatric populations of MUS and DOM is thought to explain a 300-km wide zone of genetic admixture (Mezhzherin *et al.*, 1998; Frisman *et al.*, 1990; Milishnikov *et al.*, 1990); however, an alternative interpretation attributes the genetic diversity to a high level of ancestral polymorphism in the regional MUS population (Milishnikov *et al.*, 2004), equivalent to that observed among the 'oriental group' of mice in northern India and Pakistan (Boursot *et al.*, 1993, 1996; Din *et al.*, 1996). This would be consistent with long residency of the MUS population in this area. MUS and CAS populations also come into secondary contact in China (Moriwaki *et al.*, 1994); however, both the geography and the genetic outcome of these interactions remain poorly documented.

Our expanded sampling among eastern house mouse populations sheds significant new light on the evolutionary history of the MUS HG. We identify two major sub-groups within MUS—MUS-1 and MUS-2—and a total of three phylogeographic components within MUS-1: MUS-1a in Moldova, Ukraine, N Caspian Sea and Russian Siberia; MUS-1b in East Europe, Kazakhstan and China; and MUS-1c in Korea and Japan. The origin of the MUS-1 and MUS-2 sub-groups is ancient, with a divergence estimate from BEAST of

150 000 ± 13 000 years (Figure 6, Table 2). Both sub-groups are represented in the area around the Caspian Sea, and it seems likely that both matrilines originated within this ancestral geographic area.

Rapid population expansion was inferred for each of MUS-1b and MUS-1c (Table 3). Estimates of expansion times for these lineages (Table 3) suggest an early expansion of MUS-1b in northern China ($\tau = 4.9$ CI: 2.9–6.5; for example, 21 000 and 10 800 years ago, with an assumption of the mutation rate of 10% and 20% per million years per lineage, respectively), followed by a later expansion of MUS-1c in northeastern Russia, Korea and Japan at ($\tau = 1.5$ CI: 0.7–2.5; for example, 6600 and 3300 years ago).

The notion of ancient population expansions in eastern Eurasia is clearly at odds with the conventional notion of a recent west-to-east dispersal of the MUS HG. However, other lines of genetic evidence similarly point to a long residency of the MUS HG in central Russia and the Far East. For example, the beta-hemoglobin gene (*Hbb*) shows contrasting predominant alleles in the lower Yellow River basin and in the remaining western portion of northern China (Hbb^{p} and Hbb^{w1}, respectively; Miyashita *et al.*, 1994; see also Moriwaki, 1994); and mice from the eastern part of China are known to have relatively longer tails (tail ratio: ~93%) than those from the rest of MUS territory in China (81%; Tsuchiya *et al.*, 1994).

Finally, we note that the area in which MUS-1c is predominant—the Korean Peninsula and nearby continental area—harbors unique genetic components in both Y-specific gene sequences and nuclear gene sequences (for example, Nagamine *et al.*, 1994; Terashima *et al.*, 2006; Nunome *et al.*, 2010a). Under the existing paradigm of west-to-east dispersal, these phylogeographic patterns might be attributed either to genetic drift following migration of ancestral populations with diverse genetic components or to multiple migration events by mice carrying different genetic components, perhaps by different routes. However, neither of these scenarios can readily account for the evidence of ancient population expansions within geographically restricted matrilines. Accordingly, we favor the alternative model of regional differentiation within a long-term resident population.

The fossil record should be able to arbitrate this issue, and it is of great interest to note that paleontologists have long recognized *M. musculus* as a component of the Chinese mammal fauna since the middle part of the Middle Pleistocene (that is, ca. 500 000 years ago); for example, Zheng *et al.* (1997) and references cited therein. Although the taxonomic identification of the fossils might be challenged, the determination is at least plausible given the molecular evidence for early diversification among Chinese *musculus* populations. However, there is a risk of circularity in such arguments and an urgent need for critical appraisal of the relevant fossils.

The European sub-group MUS-1a contains substantial haplotype diversity, including persistent ancestral haplotypes and two deeply divided haplotype series, each of which contains relatively shallow stellar clusters derived from populations near the western limit of the MUS geographic range. This pattern is suggestive of a broad westward expansion of a MUS population into eastern Europe, with limited filtering of haplotype diversity. As summarized by Auffray *et al.* (1990), the long history of *M. musculus* in Europe is dominated by large expansions and contractions of range driven by glacial cycles. At the height of the last glaciation *M. musculus* was rare or absent across most of eastern Europe, which supported a mosaic of periglacial forest-steppe, steppe and semi-desert habitats (Markova *et al.*, 2009). Refugial forest habitats were restricted to small patches in the Crimea, in the Transcarpathian region and in the Caucasus (Markova *et al.*, 2009), and it is of interest to note fossil occurrences of *M. musculus* in the Carpathian–Balkan region during the warm interval (33–24 000 years ago) immediately before the last glacial maximum (Markova *et al.*, 2010). However, in view of the high level of genetic diversity within MUS-1a and the lack of a strong signal of recent population expansion, it seems likely that mice persisted in multiple localities, perhaps including both forest and semi-desert habitats. This issue warrants further consideration.

MUS-1a contains a discrete lineage characterized by a 75-bp duplication, first detected by Prager *et al.* (1998) in a mouse from Kishinev in Moldova. We found closely related haplotypes at low frequency in mice from eastern Europe (for example, Donetsk, Ukraine) and also from Khasan in Primorye, Russia (Figure 5b). Given the other evidence of regional differentiation of mtDNA within MUS, we are inclined to view MUS-1a as originally restricted to eastern Europe (Ukraine), with its more easterly occurrences being a product of long-range transport by modern means. The locality of Novosibirsk, for example, is situated on the Turkestan–Siberia Railway that was built in the early twentieth century (in 1930) and connects the Caspian Sea to localities in Central Asia. The link to the Primorye region of the Russia Far East is less readily accounted for by overland transportation but might be explained by the activities of the Russian government to introduce kazak and peasants to the Russian Far East in the late nineteenth century; upwards of 90 000 people (and perhaps a few mice) from Odessa in the Ukraine settled in the Ussuri Region of Primorye (http://www.fegi.ru/prim/geografy/etap.htm).

Phylogeography of the DOM lineage

Our small number of new DOM sequences contributes only a few insights into the history of this well-studied lineage (Gabriel *et al.*, 2011; Bonhomme *et al.*, 2011; Jones *et al.*, 2010). The onset of the expansion of DOM is estimated to be 12 000 years ago as the youngest timing, assuming the mutation rate of 20% per million years per lineage (Table 3), which is harmonious with the recent arguments based on zooarcheological records (Cucchi *et al.*, 2005; Rajabi-Maham *et al.*, 2008; Bonhomme and Searle, 2012).

We recovered the expected 'Clade F' haplotypes from mice collected in North America, Australia and Africa (Senegal, Somalia) but also detected them in mice from several localities in Asia, namely Lanzhou and Xining in China, and Bogor on Java in Indonesia. At Bogor, CAS and DOM mtDNA haplotypes were found to co-occur in one population.

A high frequency of DOM haplotypes was also detected in the Russian Far East, thereby supporting previous claims of DOM–MUS–CAS interactions in this area based on studies of chromosomes, allozymes and random-amplified-polymorphic DNA (RAPD) markers (Frisman *et al.*, 2011; Spiridonova *et al.*, 2011). Interestingly though, the DOM haplotypes recovered at Primorye (HS1466) and Sakhalin (HS3606, HS3607) are not 'Clade F' but are related specifically to haplotypes from Cameroon (for example, AFWCMR41; Bonhomme *et al.*, 2011). This connection is very likely explained by long-distance dispersals associated with human activities in modern times.

The detection of DOM haplotypes in numerous corners of the world is testimony to the ongoing dispersal of *M. musculus* and encourages further study of the impact of occasional arrival of 'exotic' mice on the genetic constitution of pre-established mouse populations (Rajabi-Maham *et al.*, 2008; Searle *et al.*, 2009a, b; Gabriel *et al.*, 2010; Bonhomme *et al.*, 2011). To further illustrate this point, mice with both DOM and CAS mtDNA haplotypes have been captured in Japanese international ports (Tsuda *et al.*, 2007) and Nunome *et al.* (2010a) provided robust evidence from their nuclear haplotype analysis of genetic introgression by DOM components of Japanese

house mice. The extent to which genetic introgression may now be shaping the future evolution of the house mouse is an interesting topic—one that has bearing on other commensal mammals, including the black rat *Rattus rattus* which also displays comparable signals of former geographic sub-division and recent intermingling as a consequence of commensalism and human-assisted dispersal (Chinen *et al.*, 2005; Aplin *et al.*, 2011; Bastos *et al.*, 2011; Lack *et al.*, 2012).

Concluding remarks

The expanded mtDNA data set raises a number of important new issues regarding the prehistory of the house mouse. Most significantly, it has identified one particular CAS sub-group (CAS-1) that has expanded into southern India, Southeast and East Asia and raised the possibility that this expansion is linked to the emergence of agricultural lifestyles and of Asian civilizations. Also of significance is our suggestion that MUS populations have a long history of residency in eastern Russia and China, contrary to the existing paradigm of recent expansion from west to east. Finally, our results emphasize the role of long-distance dispersal in shaping contemporary pattern of distribution and opportunities for interaction between each of the major lineages within *M. musculus*.

Our study also demonstrates the value of continuing efforts to fill gaps in geographic coverage of *M. musculus* mtDNA. Moreover, it highlights the need for ongoing field collection to increase local sampling and the need for more comprehensive assessments of population genetic history using nuclear markers. From our preliminary work with nuclear genes on this group, it is clear that much deeper divergence between subspecies groups is observed in some regions of the genome than in others (for example, Suzuki *et al.*, 2004; Nunome *et al.*, 2010a) and is also evident that different markers can yield strongly contrasting phylogeographic structure, such as in southern China where CAS mtDNA dominates but both CAS and MUS components are detected in nuclear genes (for example, Nunome *et al.*, 2010a). Finally, it is worth mentioning the as yet unexplored potential for detailed study of Central and East Asian house mouse populations to reveal important new aspects of human history, including the emergence of agricultural lifestyles and of regional trade networks.

DATA ARCHIVING

The nucleotide sequences reported in this paper appear in the DDBJ, EMBL and GenBank nucleotide sequence databases under the following accession numbers AB649455–AB649770, AB819902–AB819920 and AB820897–AB820942, respectively (Table S1). Sequence data files in the nexus file format, together with Supplementary Information files are stored at Dryad repository: doi:10.5061/dryad.rf161.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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