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ORIGINAL ARTICLE

Phylogeography of the introduced species *Rattus rattus* in the western Indian Ocean, with special emphasis on the colonization history of Madagascar

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ABSTRACT

Aim To describe the phylogeographic patterns of the black rat, *Rattus rattus*, from islands in the western Indian Ocean where the species has been introduced (Madagascar and the neighbouring islands of Réunion, Mayotte and Grande Comore), in comparison with the postulated source area (India).

Location Western Indian Ocean: India, Arabian Peninsula, East Africa and the islands of Madagascar, Réunion, Grande Comore and Mayotte.

Methods Mitochondrial DNA (cytochrome *b*, tRNA and D-loop, 1762 bp) was sequenced for 71 individuals from 11 countries in the western Indian Ocean. A partial D-loop (419 bp) was also sequenced for eight populations from Madagascar (97 individuals), which were analysed in addition to six previously published populations from southern Madagascar.

Results Haplotypes from India and the Arabian Peninsula occupied a basal position in the phylogenetic tree, whereas those from islands were distributed in different monophyletic clusters: Madagascar grouped with Mayotte, while Réunion and Grand Comore were present in two other separate groups. The only exception was one individual from Madagascar (out of 190) carrying a haplotype that clustered with those from Réunion and South Africa. 'Isolation with migration' simulations favoured a model with no recurrent migration between Oman and Madagascar. Mismatch distribution analyses dated the expansion of Malagasy populations on a time-scale compatible with human colonization history. Higher haplotype diversity and older expansion times were found on the east coast of Madagascar compared with the central highlands.

Main conclusions Phylogeographic patterns supported the hypothesis of human-mediated colonization of *R. rattus* from source populations in either the native area (India) or anciently colonized regions (the Arabian Peninsula) to islands of the western Indian Ocean. Despite their proximity, each island has a distinct colonization history. Independent colonization events may have occurred simultaneously in Madagascar and Grande Comore, whereas Mayotte would have been colonized from Madagascar. Réunion was colonized independently, presumably from Europe. Malagasy populations may have originated from a single successful colonization event, followed by rapid expansion, first in coastal zones and then in the central highlands. The congruence of the observed phylogeographic pattern with human colonization events and pathways supports the potential relevance of the black rat in tracing human history.

Keywords

Commensal rodent, invasive species, island colonization, Madagascar, mitochondrial DNA, phylogeography, *Rattus rattus*.

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INTRODUCTION

Commensal small mammals, such as rats and mice, generally expand their distributions in association with humans, especially in the case of islands. For this reason, studying their colonization patterns can provide insight into human expansion history, as shown by the genetic analysis of *Rattus exulans* in Southeast Asia and the Pacific islands (reviewed in Matisoo-Smith & Robins, 2009) or of the house mouse in Europe (Gunduz *et al.*, 2001; Britton-Davidian *et al.*, 2007; Searle *et al.*, 2008). Colonization pathways, areas of origin and/or the time frame of introduction events can be inferred from genetic studies of invasive species through phylogeographic methods (Avise, 2000). New genetic methodologies applied to rats and mice can thus add to the archaeological toolbox and provide historical insights into human migrations (Searle, 2008).

Madagascar was probably first settled by Indonesian people and the Malagasy human population results from an admixture of African and Indonesian ancestors (Hurles *et al.*, 2005). Various pieces of evidence date the earliest human presence in Madagascar to about 2300 yr BP (Burney *et al.*, 2004), possibly in the south-west portion of the island; however, this early human occupation was probably sparse. Subsequent waves of immigration resulted in large human populations throughout the island about 1000 years ago (10th century AD; Burney *et al.*, 2004), with the establishment of settlements along several parts of the Malagasy coast (Wright & Rakotoarisoa, 2003). At that time, the entire Indian Ocean was a vast trading network, connecting societies between China and the Mediterranean. Arab traders (mainly from Oman) travelled from the Arabian Peninsula along the African coast towards the Comoros islands and Madagascar (Allibert, 1988; Liszkowski, 2000). This relatively well-known human history provides an opportunity to validate the approach of studying the colonization patterns of commensal small mammals as a proxy for human history.

In Madagascar, four commensal small mammals were introduced and now largely dominate small mammal communities in rural and urban regions (Goodman *et al.*, 2003): the black (*Rattus rattus*) and Norway (*Rattus norvegicus*) rats, the house mouse (*Mus musculus*) and the house shrew (*Suncus murinus*). A few studies have investigated the history of commensal small mammal colonization of Madagascar. Hutterer & Tranier (1990) suggested that the house shrew was carried by Arab traders from India to East Africa and Madagascar, and Duplantier *et al.* (2002) showed that house mice from Madagascar were genetically close to populations found in Yemen. In the Malagasy central highlands, the black rat (*R. rattus*) represents more than 95% of rodent captures in fields and inside houses (Duplantier & Rakotondravony, 1999). This species has overrun Madagascar (Goodman, 1995) and occurs in practically all habitats (Duplantier & Duchemin, 2003). The black rat thus appears to be a particularly relevant species for studying historical colonization processes in Madagascar.

Moreover, as in other parts of the world, especially islands (Towns *et al.*, 2006; Harris, 2009), the black rat is strongly implicated in ecosystem damage (Lever, 1994; Jones *et al.*, 2008) and serious agricultural and health problems (Gratz, 1997) in Madagascar (Duplantier & Rakotondravony, 1999). Several studies (Goodman, 1995; Ganzhorn *et al.*, 2003; but see Ramanamanjato & Ganzhorn, 2001; Ganzhorn, 2003) suggest an important impact of the black rat on Malagasy endemic rodents and small lemurs. It is the main reservoir of plague (Brygoo, 1966; Duplantier *et al.*, 2005), a disease which in Madagascar accounted for 41% of the world's reported cases in 2000–01 (World Health Organization, 2003). Improving genetic knowledge of this pest species may thus also have conservation and health implications. However, and despite its world-wide distribution, *R. rattus* invasions have been infrequently studied using genetic methods (but see Abdelkrim *et al.*, 2005; Hingston *et al.*, 2005).

The genus *Rattus* originated in Southeast Asia and the black rat, *Rattus rattus* (Linnaeus, 1758), is native to the Indian Peninsula and has since been introduced world-wide (Musser & Carleton, 2005). *Rattus rattus* appears to have many close relatives (Aplin *et al.*, 1996; Musser & Carleton, 2005). It can be distinguished from its sister species *Rattus tanezumi* (Temminck, 1844), restricted to the south and east of Asia, by cytological studies (*R. rattus* $2n = 38$, whereas *R. tanezumi* $2n = 42$; Baverstock *et al.*, 1983). An earlier investigation of the karyotype of Malagasy rats identified only the $2n = 38$ form (Duplantier *et al.*, 2003). Hingston *et al.* (2005) provided the first genetic investigation of the colonization of Madagascar by *R. rattus*. Their results were consistent with an Indian origin of southern Malagasy populations, but the absence of samples from East Africa, the Arabian Peninsula and northern Madagascar prevented them from confirming this hypothesis.

The specific objectives of this study are to describe the phylogeographic patterns of the black rat in the western Indian Ocean using mitochondrial sequence data. We predicted that the migration patterns would match what has been proposed for the house mouse and the house shrew (see above): colonization of East Africa and of the islands of the Indian Ocean (Madagascar, Mayotte and Grande Comore) from India through the Arabian Peninsula (Fig. 1). Thus, we expected India (source populations) and, to a lesser extent, the Arabian Peninsula (an old settlement) to present basal and diverse haplotypes, while recently (the past few thousand years) introduced populations (on islands and, to a lesser extent, in East Africa) would be genetically less diverse. In particular, we addressed the issue of the origin and timing of the arrival of *R. rattus* in Madagascar. To this end, we extended the sampling of Hingston *et al.* (2005) in southern Madagascar by adding new populations in the central and northern parts of the island, as well as samples from neighbouring countries and islands. Colonization of the central highlands of Madagascar was expected to be more recent than that of the coastal areas, as human settlement is thought to have first occurred on the coasts and subsequently in the highlands (Wright & Rakotoarisoa, 2003).

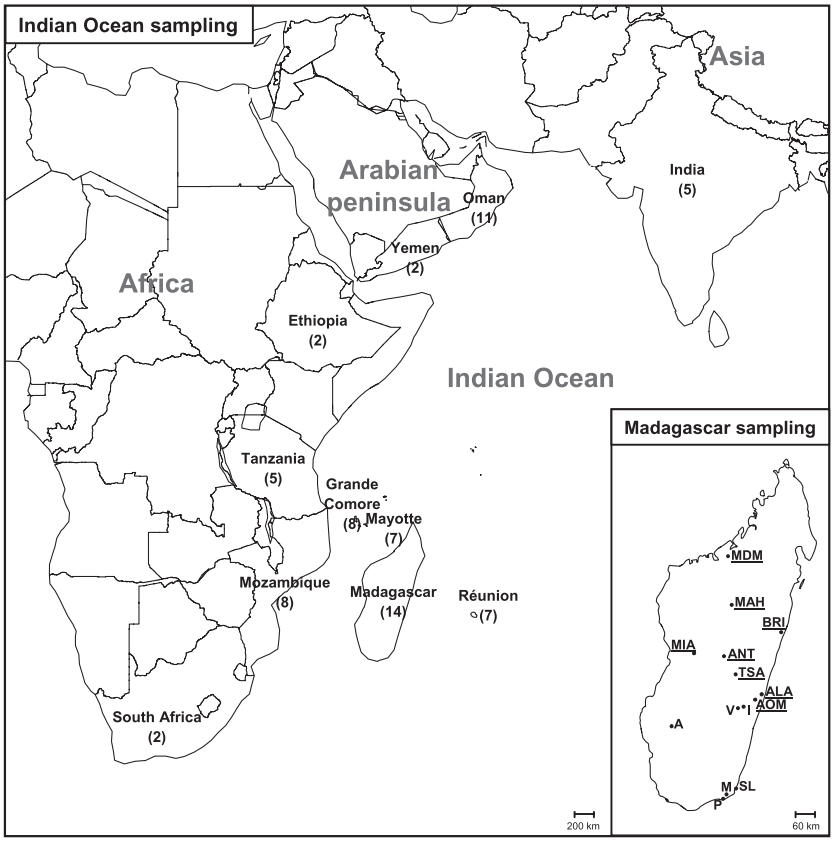

Figure 1 Map showing the countries and islands of the western Indian Ocean relevant to this study. Sample sizes of *Rattus rattus* analysed for the Indian Ocean scale and the mitochondrial DNA sequence (1762 bp) are indicated in brackets. For Madagascar (see inset), the localities of all populations sampled (D-loop sequence, 419 bp) are shown, including those studied by Hingston *et al.* (2005) (A, Anavelona; I, Ioranjatsy; M, Mandena; P, Petricky; SL, Ste Luce; V, Vinantelo) and those sampled in this study (underlined: ALA, Ambalatenona; ANT, Antahobe; AOM, Ambohimiariana; BRI, Brickaville; MAH, Mahatsinjo; MDM, Madiomiangana; MIA, Miandrivazo; TSA, Tsarasambo).

MATERIALS AND METHODS

Sample collection

Black rat samples were organized into two datasets in order to investigate colonization patterns at two geographic scales: Madagascar and the Indian Ocean. In Madagascar, 97 black rats were collected by live trapping in various localities (12–13 rats per locality) that were widely distributed across the island (Fig. 1). Data from Hingston *et al.* (2005) comprising 93 other individuals from six populations from the south of Madagascar were included in some of our analyses (190 individuals total) (Fig. 1). Samples from outside of Madagascar (2–11 individuals from each country or island) were collected through different collaborations (Fig. 1). They were added to 14 individuals of the 97 sampled in Madagascar to constitute the Indian Ocean scale dataset (71 individuals).

Laboratory procedures

DNA was isolated from ethanol-preserved tissues (ear or tail) using the DNeasy® Tissue Kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions; 100 μL of buffer was used for the final elution.

For the 71 individuals of the Indian Ocean analyses, complete cytochrome *b* (cyt *b*) was amplified using primers L14723 (5′-ACC AAT GAC ATG AAA AAT CAT CGT T-3′) and H15915 (5′-TCT CCA TTT CTG GTT TAC AAG AC-3′). Polymerase chain reactions (PCR) were performed in a 25 μL total volume containing: 2 μL of extracted DNA, 1 μM of each primer, 100 μM of deoxyribonucleotides (dNTPs), and 0.1 U of *Taq* polymerase in the appropriate 1× Buffer (Qiagen). Samples were subjected to an initial denaturation at 94 °C for 3 min, followed by 35 cycles of denaturation at 92 °C for 30 s, annealing at 58 °C for 45 s, and extension at 72 °C for 1 min, with a final extension phase at 72 °C for 10 min.

For all individuals used in this study, 758 bp of the 3′-adjoining region of the cyt *b* comprising two tRNA (tRNA-Thr and tRNA-Pro) and a partial D-loop region were amplified. This sequence contained the 419-bp sequence analysed by Hingston *et al.* (2005). The forward primer (5′-GGC CAA CTA GCA TCC ATC AG-3′), located 92 bp before the end of the cyt *b* gene was designed from a Malagasy *R. rattus* cyt *b* sequence. The reverse primer (5′-GAC GGC TAT GTT GAG GAA GG-3′) was designed from a GenBank *R. norvegicus* mitochondrial sequence (accession number AB211039). This region was amplified using the same PCR conditions as for the cyt *b*.

The PCR were purified using ExoSAP® Kit (GE Healthcare, Buckinghamshire, UK). Sequencing was performed by Genoscreen (Lille, France) or Macrogen (Seoul, Korea).

Sequence alignment

Sequences were aligned using the multiple alignment algorithm implemented in ClustalW and further checked by eye.

At the Indian Ocean scale, both amplified loci were combined, resulting in a mitochondrial (mtDNA) sequence 1762 bp long, with 1103 bp corresponding to the cyt *b*, 138 bp to the tRNAs and 521 bp to the D-loop. At the Madagascar scale, the same 419-bp D-loop region (D-loop) as in Hingston *et al.* (2005) was analysed.

As outgroups, we used a sequence of *R. norvegicus* from GenBank (accession number X14848) and sequences from *R. tanezumi*, *Rattus losea* and *R. exulans* from Thailand (M. Pagès *et al.*, INRA-CBGP, France, in review).

We compared our mtDNA haplotypes (1762 bp) with previously published sequences: (1) homologous sequences of the whole mitochondrial sequence (16,305 bp, GenBank accession number EU273707) of one *R. rattus* sample from New Zealand (Robins *et al.*, 2008); (2) homologous sequences (1762 bp GenBank FJ897498–FJ897501) from three black rats sampled in Guadeloupe and two in Senegal; (3) 713 bp of the cyt *b* sequences and 550 bp of the D-loop sequences from five *R. rattus* samples from the islands of Oceania (New Zealand, Society, Samoa, Papua New Guinea) (GenBank EF186354–EF186360 and EF186469–EF186375) published by Robins *et al.* (2007); and (4) the 419-bp D-loop haplotype (GenBank DQ009794) named HaMI (Hingston *et al.*, 2005) found in samples originating from France (Lavezzi Islands and Ouessant Island), Great Britain (Lundy Island) and French Polynesia (Raïatéa and Tahiti). Hingston *et al.* (2005) noted that the HaMI haplotype has within it a 288-bp region identical to one sequence (GenBank U13754) from New York (Usdin *et al.*, 1995).

Indian Ocean scale analyses

All the analyses were performed on the mtDNA dataset (1762 bp). We estimated nucleotide diversity (π, Nei, 1987) and its standard deviation (Tajima, 1993) for each of three partitions using the DnaSP 4.0 program (Rozas *et al.*, 2003). No polymorphism was found within *R. rattus* samples in the tRNAs. Partitioned analyses (Bayesian phylogenetic reconstruction) were thus performed on the cyt *b* and the D-loop only (1624 bp) whereas global analyses [network construction and 'isolation with migration' (IM) simulations] were carried out on the 1762-bp mtDNA dataset. We performed Bayesian analyses to estimate phylogenetic relationships between haplotypes. First, we used MrAIC version 1.4.3 (Nylander, 2004) to determine the most suitable model of DNA substitution among 24 possible models. To this end we applied the corrected Akaike information criterion to each of the two partitions (cyt *b* and D-loop). The selected models of DNA evolution were the GTR model (Rodriguez *et al.*, 1990) with a proportion of invariant sites for the cyt *b*, and the HKY model (Hasegawa *et al.*, 1985) with a gamma distribution for the D-loop. The D-loop region alignment contained 13 gaps (among which six were polymorphic within *R. rattus* samples), and we added this information to our dataset by coding gaps as binary data, as recommended by the MrBayes manual. MrBayes version 3.1.2 (Ronquist & Huelsenbeck, 2003) was then run partitioning the dataset into three: cyt *b*, D-loop and gaps. For the cyt *b* and the D-loop, we applied the models selected by MrAIC. Each partition had its own set of parameters and we allowed partitions to evolve under different rates. We computed 10 million iterations (generations), four chains, and a burn-in of one million (10%) generated trees.

A haplotypes network (an appropriate method for intraspecific data; Posada & Crandall, 2001) was constructed using the median-joining method available in the network version 4.1.1.2 software (http://www.fluxus-engineering.com/, Bandelt *et al.*, 1999).

An IM program (Hey & Nielsen, 2004) was used to infer divergence time and migration rates between population pairs. Because *R. rattus* from Madagascar and Grande Comore are thought to originate from Oman (see above), we performed these analyses between Madagascar and Oman and between Grande Comore and Oman. We introduced the splitting parameter (*s*; Hey, 2005), which is the proportion of the ancestral population that contributed to Madagascar's or Grande Comore's population, respectively. The inheritance scalar was set to 0.25, and the model of evolution was set to HKY, as recommended for mitochondrial DNA (IM manual). To assess convergence, we checked effective sample sizes throughout the run and compared results between three independent runs. The burn-in period was set to 100,000 iterations. The first run of the IM program used parameter values recommended by Hey & Nielsen (2004) for priors of upper bounds of divergence time (t), migration rates (m_1 and m_2), and population sizes (θ_a, θ_1 and θ_2) parameters. In the final runs of divergence between Oman and Madagascar, priors were set to 6 for t, 2 for m, 300 for θ_a and 1000 for θ_1 and θ_2. For the divergence between Oman and Grande Comore, they were the same except for t, the upper bound of which was set to 12. Because development to sexual maturity takes about 4 months and reproduction stops (outdoors) or decreases (in houses) during the winter (J.-M. Duplantier, unpublished observations), the generation time of the black rat was estimated at 6 months. The mutation rate (μ_1) was inferred from the genetic distance (ML-dist: maximum-likelihood distance estimated considering the HKY model using paup* software; Swofford, 2000) between *R. rattus* and *R. tanezumi*, assuming a divergence time (T) of 450,000 years (Robins *et al.*, 2008) and using the formula $\mu_1 = (\text{ML-dist} \times \text{sequence length})/2T$. This mutation rate was used to convert IM parameters into demographic estimates following Hey & Nielsen (2004). The effective size of the founder population of each island population (n_F) was then estimated from the effective size of the ancestral population (N_a) and the splitting parameter using the formula $n_F = (1 - s)N_a$ (Hey, 2005).

Madagascar scale analyses

For Madagascar scale analyses, we aligned our dataset with six populations from the south of the island that were published by Hingston *et al.* (2005). Analyses were thus performed on the 419-bp D-loop sequence (corresponding to the region used

in Hingston *et al.*, 2005). A network was computed for these Malagasy D-loop haplotypes (using the same method as for the Indian Ocean scale analyses).

Haplotype (h) and nucleotide (π) diversities (Nei, 1987) and their standard deviations (Tajima, 1993) were estimated for each population using the DnaSP 4.0 program (Rozas *et al.*, 2003). We tested the hypothesis that *R. rattus* diversity was higher in the coastal areas than in the highlands by computing correlations (Spearman's rank) between haplotype diversity and minimum distance to the sea (calculated as the straight line distance to the coast line using Spatial Analyst in ArcMap 8.2, http://www.esri.com/).

Demographic analyses were performed using DnaSP to test for recent (on an evolutionary time-scale) expansion in each population and in the complete Malagasy dataset. F_S (Fu, 1997) and R_2 (Ramos-Onsins & Rozas, 2002) statistics were estimated. For populations having experienced a recent demographic change (at least one of these two tests were significant), we performed pairwise mismatch distribution analysis (Rogers & Harpending, 1992) to estimate the 'growth–decline' model parameter *Tau* (mode of the curve, Rogers & Harpending, 1992). This parameter allows the estimation of the expansion time using the formula: T (in years) = ($Tau \times$ generation time $\times$ 1,000,000)/($2\mu_2 \times$ sequence length). The generation time of black rats was estimated to be 6 months (see above). The parameter μ_2 is the mutation rate per million years and was inferred from the genetic distance (uncorrected p estimated using PAUP* software) between *R. rattus* and *R. tanezumi*.

RESULTS

Indian Ocean scale analyses

Overall, 74 variable sites were found in the *R. rattus* samples (71 individuals, Table 1), resulting in a total of 40 mtDNA haplotypes within the 1762-bp sequence (GenBank accession numbers GQ891569–GQ891608). Haplotype composition for each country is reported in Table 1 (for details of individuals analysed see Appendix S1 in Supporting Information). Nucleotide diversities were 0.0056 ± 0.003 for the cyt *b* (40 variable sites), and 0.0098 ± 0.0006 for the D-loop (34 variable sites).

Samples from outside the Indian Ocean were all similar to the mtDNA haplotype Hap 40 we found in South Africa. The

Table 1 Geographic location, *Rattus rattus* sample size and haplotypes found for each locality considered. Further details (individual data and corresponding GenBank accession numbers) are provided in Appendix S1.

Country or island	Site	*N*	Haplotypes
India	Attur	1	Hap 5
	Avallanchi	1	Hap 4
	Mudumalai	3	Hap 1, Hap 2, Hap 3
Oman	Arazat	3	Hap 6
	Sahanout	4	Hap 9, Hap 10, Hap 11, Hap 12
	Tibraq	4	Hap 7, Hap 8
Yemen		2	Hap 13, Hap 14
Ethiopia		2	Hap 15, Hap 16
Tanzania	Lushoto	3	Hap 17, Hap 18, Hap 19
	Morogoro	2	Hap 17
Mozambique	Maputo	2	Hap 18, Hap 22
	Tete	3	Hap 18, Hap 20
	Zambezi	3	Hap 18, Hap 20, Hap 21
Grande Comore	Moroni	8	Hap 18, Hap 23, Hap 24, Hap 25, Hap 26, Hap 27, Hap 28
Mayotte	Site 1	4	Hap 20
	Site 2	2	Hap 29, Hap 30
	Site 3	1	Hap 20
Madagascar*	Ambalatenona (ALA)	3	Hap 20 (H1), Hap 34 (H1), Hap 35 (H17)
	Ambohimiariana (AOM)	3	Hap 36 (H23), Hap 37 (H9), Hap 38 (H3)
	Brickaville (BRI)	2	Hap 32 (H14), Hap 33 (H3)
	Madiomiangana (MDM)	4	Hap 20 (H1), Hap 31 (H16)
	Miandrivazo (MIA)	2	Hap 20 (H1)
Réunion	Site 1	3	Hap 39
	Site 2	3	Hap 39
	Site 3	1	Hap 39
South Africa	Cape Town	2	Hap 40
Total		71	

N, sample size for *R. rattus* individuals analysed for the mtDNA sequence (1762 bp).

*For Malagasy haplotypes, correspondence between haplotypes for the mtDNA sequence (1762 bp, named Hap *X*) and those for the D-loop sequence only (419 bp, named H*X*) are mentioned.

haplotype found in New Zealand (1762 bp) was identical to Hap 40. Within the four mtDNA haplotypes observed in Guadeloupe and Senegal (1762 bp), one was identical to Hap 40 and the others differed only by one or two substitutions (0.06–0.11% difference). Among the five *R. rattus* samples from the islands of Oceania (Robins *et al.*, 2007), 682 bp of the cyt *b* were identical to Hap 40, while only three positions differed out of 585 bp of the D-loop (0.51% divergence). The HaMI haplotype (Hingston *et al.*, 2005) was identical (D-loop, 419 bp) to Hap 40.

The phylogenetic tree in Fig. 2 estimates the coalescence for the 40 mtDNA haplotypes, rooted by *R. tanezumi*, *R. losea*, *R. exulans* and *R. norvegicus*. Haplotypes from India (Hap 1–5) and Oman (Hap 6–12) were all found in basal positions and in different branches of the tree. Mayotte and Madagascar shared their most common haplotype (Hap 20, Table 1). Haplotypes found in these two islands were all (except for Hap 31 from Madagascar) in a monophyletic group [Group B, posterior probability (PP) = 1.00], which also contained some individuals from Mozambique and Ethiopia. The haplotype closest to this group (Hap 11, PP = 1.00) was found in Oman. The only Malagasy haplotype found outside of Group B, Hap 31, originated from the locality Madiomiangana (MDM, north-west Madagascar, Fig. 1). It clustered in a monophyletic group (Group A, PP = 1.00), which also contained haplotypes from South Africa and from Réunion (where all seven individuals shared the same haplotype, Table 1). All the haplotypes found in Grande Comore were in one group (Group C; PP = 0.63) together with some haplotypes from Mozambique and Tanzania. The comparison of the phylogenetic tree (Fig. 2) with the haplotype network (Fig. 3) revealed that both representations were quite similar, and the three groups identified in the phylogenetic tree were also found within the network. Groups B and C formed star-like topologies, which are characteristic of recent (on an evolutionary time-scale) demographic expansion events.

Simulations under the IM model were computed using all individuals available for mtDNA sequences, except for the Malagasy individual carrying Hap 31. Removal of this individual was justified by the fact that this divergent haplotype represents 7.1% of the Malagasy sample (1/14 individuals), and as such may highly influence results although it is only one out of 190 in the total sample (0.5%, see below). We obtained reliable estimations for parameters of divergence time (t), splitting (s), migration rates (m_1 and m_2) and ancestral population size (θ_a). In contrast, posterior probabil-

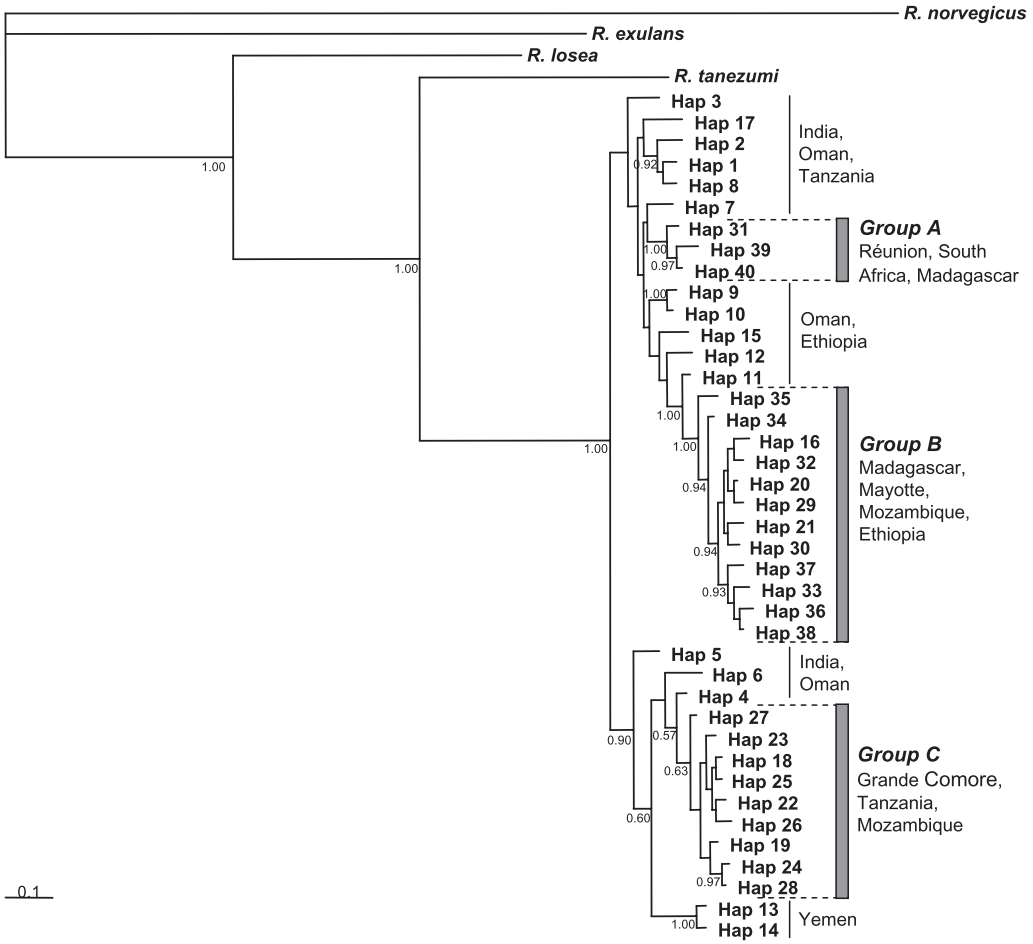

Figure 2 Bayesian tree of the 40 *Rattus rattus* mitochondrial DNA haplotypes and four closely related species: *Rattus tanezumi*, *Rattus losea*, *Rattus exulans* and *Rattus norvegicus*. Posterior probabilities are shown when higher than 0.5.

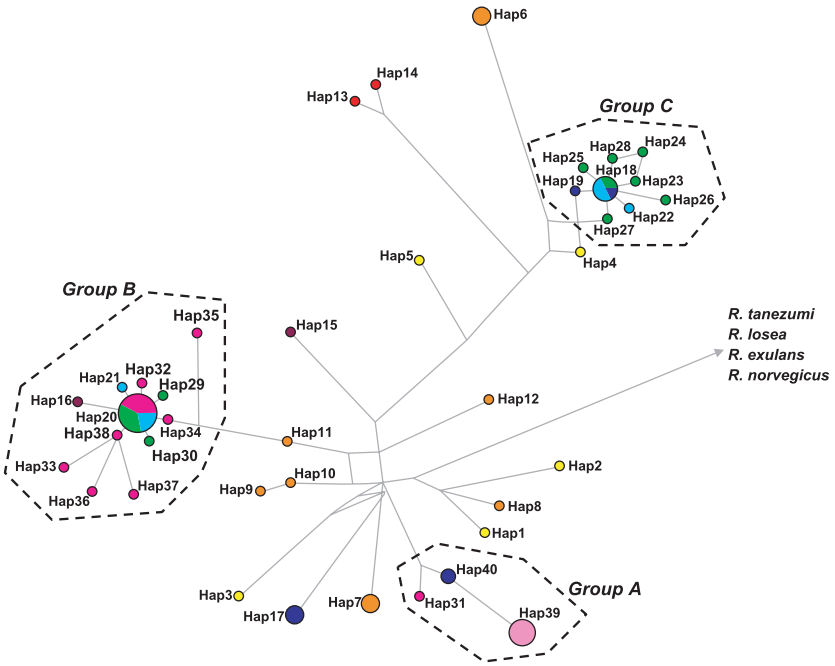

Figure 3 Median-joining network of the 40 *Rattus rattus* mitochondrial DNA haplotypes (1762-bp sequence) found in the western Indian Ocean (and four closely related species). Node sizes are proportional to haplotype frequencies. Groups mentioned in the text are circled and named as in Fig. 2. Each country is symbolized by a different colour: India, yellow; Oman, orange; Yemen, red; Ethiopia, brown; Tanzania, blue; Mozambique, bright blue; Grande Comore, dark green; Mayotte, light green; Madagascar, pink; Réunion, light pink; South Africa, dark blue.

ity distribution never gave clear results for actual population sizes θ_1 and θ_2. The divergence time parameter was estimated to be 0.91 (95% confidence interval: 0.36–2.25) for Madagascar and 0.82 (0.23–5.41) for Grande Comore (Fig. 4a). Both migration parameters had peaks at the lower limit of resolution: the migration parameter from Oman to Madagascar or Grande Comore was estimated at 0.007 (0.001–1.309) and 0.001 (0.001–1.101), respectively (Fig. 4b). The splitting parameter (s) was estimated at 0.9985 (0.9505–0.9995) for Madagascar and 0.9995 (0.8775–0.9995) for Grande Comore. The ancestral population size parameters (θ_a) were estimated at 46.05 (19.65–102.45) for Madagascar and 33.45 (0.75–87.15) for Grande Comore. To convert parameter estimates into time-scale units, we used a mutation rate (μ_1) of 8.9×10^{-5} mutation events/locus/year (calculated from the ML distance between *R. rattus* and *R. tanezumi* of 0.0455). The estimated time of divergence of Oman with Madagascar and Grande Comore was 10,215 yr BP (4012–25,252) and 9238 yr BP (2630–60,752), respectively. Estimations of the splitting parameter (s) and of the ancestral population size (N_a) resulted in an effective number of founders (n_F) of about 388 for Madagascar and 94 for Grande Comore (as it results from a product of estimators, the 95% confidence interval of n_F was not worked out but would certainly be very large).

Madagascar scale analyses

Within the 419-bp D-loop region analysed for the 190 Malagasy samples of *R. rattus* (including those of Hingston *et al.*, 2005), 26 polymorphic sites were found (6.2%), corresponding to 27 mutations (no indels were found). A total of 29 D-loop haplotypes were identified, including 13 previously described by Hingston *et al.* (2005) (GenBank accession numbers DQ009781–DQ009793 and GQ891553–GQ891568). The D-loop haplotype network (Fig. 5) revealed a star-like topology as observed for the mtDNA analysis (Fig. 3). D-loop haplotype frequencies found in each population are reported in Fig. 6. The same main haplotype (H1, corresponding to Hap 20 in the mtDNA dataset) was found in each population except for one located in the highlands (Antahobe, ANT), where H28 was the main D-loop haplotype. The haplotype and nucleotide diversity indices are indicated for each population in Table 2. Higher haplotype diversity was found in Ambalatenona (ALA), where high nucleotide diversity was also found. However, the highest nucleotide diversity was found in Madiomiangana (MDM), because of a very distinct D-loop haplotype (H16, see Fig. 5, corresponding to Hap 31, see Fig. 3). The correlation between haplotype diversity and distance to the coast was non-significant (Spearman's rank, $S = 594.7$, $P = 0.285$), probably due to the population Antahobe (ANT) which is the most distant from the sea (about 212 km) and presents high haplotype diversity (Table 2). However, a tendency for the expected pattern (higher diversity near the sea, $\rho = -0.307$) was observed.

Six populations experienced a population demographic change (Table 2): two from the east coast (AOM and BRI), two from the west coast (A and MIA) and two from the central highlands (MAH and TSA). The uncorrected genetic distance between *R. rattus* and *R. tanezumi* was 0.68, resulting in a mutation rate (μ_2) of 15.1% Myr^{-1}. Time since expansion was estimated using this mutation rate for all populations having experienced a demographic change (Table 2). Expansion times were the largest for populations on the east coast (populations AOM and BRI), followed by the west coast (populations A and MIA) and finally the central highlands (populations MAH and TSA). The pool of all Malagasy individuals also revealed significant patterns of recent population growth, estimated at about 3000 yr BP (Table 2).

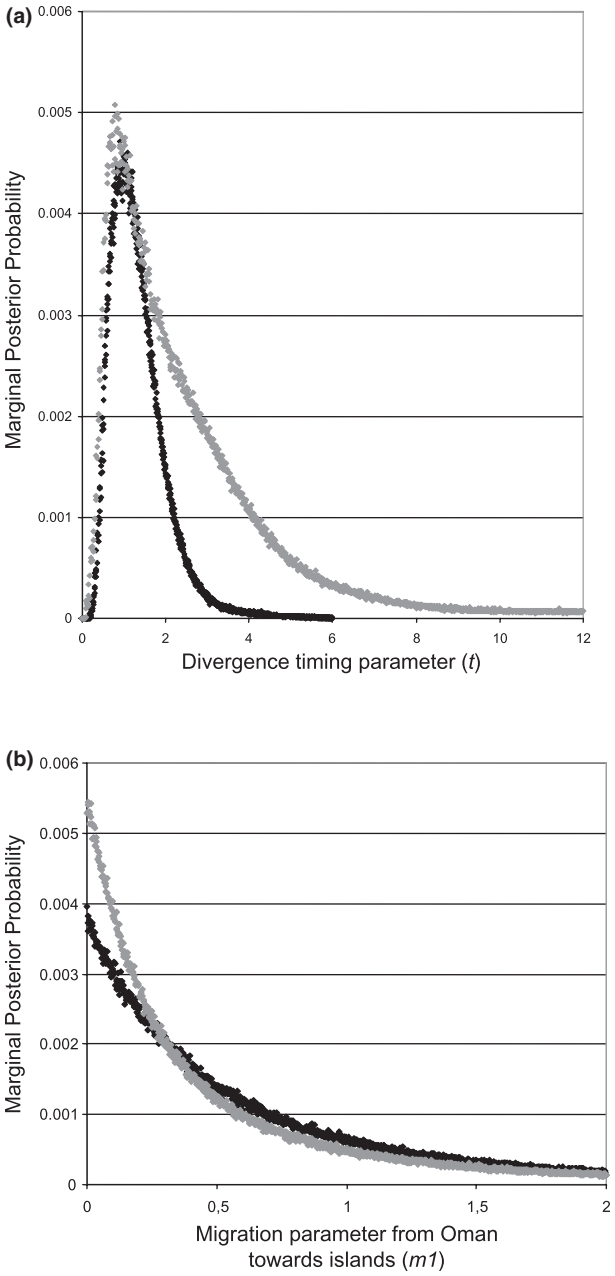

Figure 4 Results of simulations under the 'Isolation with migration' (IM) model for *Rattus rattus* population divergence between Oman and Madagascar (in black) and between Oman and Grande Comore (in grey): marginal posterior probability distribution for the time since divergence parameter (t, a) and the recurrent migration towards islands parameter (m_1, b). For IM simulations, the dataset included 11 individuals from Oman, 13 from Madagascar and 8 for Grande Comore.

DISCUSSION

Colonization routes in the Indian Ocean

The colonization history of the black rat, *R. rattus*, towards western Europe and Africa is hypothesized to be strongly connected to human migration and trade routes. This species was first found in North Africa (Egypt, Libya) in 2000 BC (de Graaf, 1981; Pascal *et al.*, 2006). In East Africa, no fossils or archaeological remains have been found, preventing any dating of the arrival of *R. rattus*. However, it may have been present since the beginning of the Christian era, as commercial links between the Arabian Peninsula, the Middle East and the East African coast were already important during this period (Hutterer & Tranier, 1990). In accordance with this relatively recent history, our results indicate little geographic structure, with shared haplotypes occurring across large geographic areas (for example, Hap 20 was found in Mozambique, Mayotte and Madagascar) and many countries (India, Oman, Tanzania and Mozambique) containing very distant haplotypes. Haplotypes from India were always found in basal positions, as expected for the native area of the species. Haplotypes from Oman were also found in basal positions of the phylogenetic tree and were not grouped in the network. Each of three sampled localities in Oman presents a different haplotype composition. The high genetic diversity found in the Oman *R. rattus* populations could originate from a relatively old introduction (compared to the other localities) with a high number of founders or through multiple colonization events.

Samples found in GenBank, originating from European Islands, Pacific Islands, Senegal, Guadeloupe and New York, are all identical or similar to the mtDNA haplotype found in our South African samples. Although this result requires confirmation with world-wide sampling and longer sequences, it suggests that rats bearing this haplotype were recently (about a few centuries ago) disseminated around the world, probably from western European populations. *Rattus rattus* arrived into southern Europe by the second to fourth centuries BC but would have remained confined to main commercial roads until a period of rapid urban growth; consequently, the rat population expanded around the 11th to 13th centuries AD (Audouin-Rouzeau & Vigne, 1994; Pascal *et al.*, 2006). However, north-western Mediterranean islands such as Lavezzi were probably colonized at an early stage of European colonization, probably by a few centuries BC (Ruffino *et al.*, 2009). Guadeloupe is thought to have been colonized by *R. rattus* during the 17th century AD (Abdelkrim *et al.*, 2005) and Senegal between the 17th and 19th centuries (Konecny, 2009). Pacific islands (including New Zealand) were probably colonized by the black rat during European exploration from the 16th to 18th centuries AD (Atkinson, 1985). Indeed, rats are reported to have heavily infested the ships of Cook's expeditions in AD 1785 (de Graaf, 1981). Finally, *R. rattus* is thought to have been introduced in South Africa only within the last 100 years and spread along with European settlements (de Graaf, 1981).

Colonization of some western Indian Ocean small islands

Despite their geographic proximity, each Indian Ocean island has a distinct *R. rattus* genetic composition, probably revealing different colonization histories.

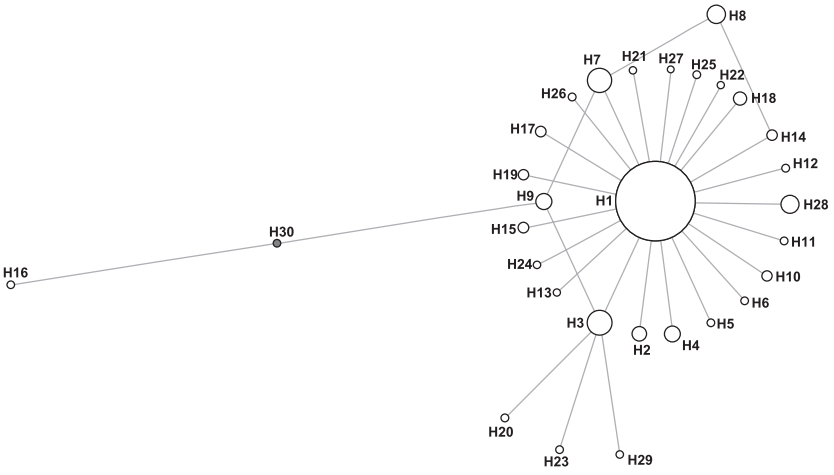

Figure 5 Median-joining network of Malagasy *Rattus rattus* D-loop haplotypes (419-bp region, 29 haplotypes H1–29) and one Oman individual (H30, in grey). Circle sizes represent haplotype frequency in the whole dataset (190 individuals from Madagascar).

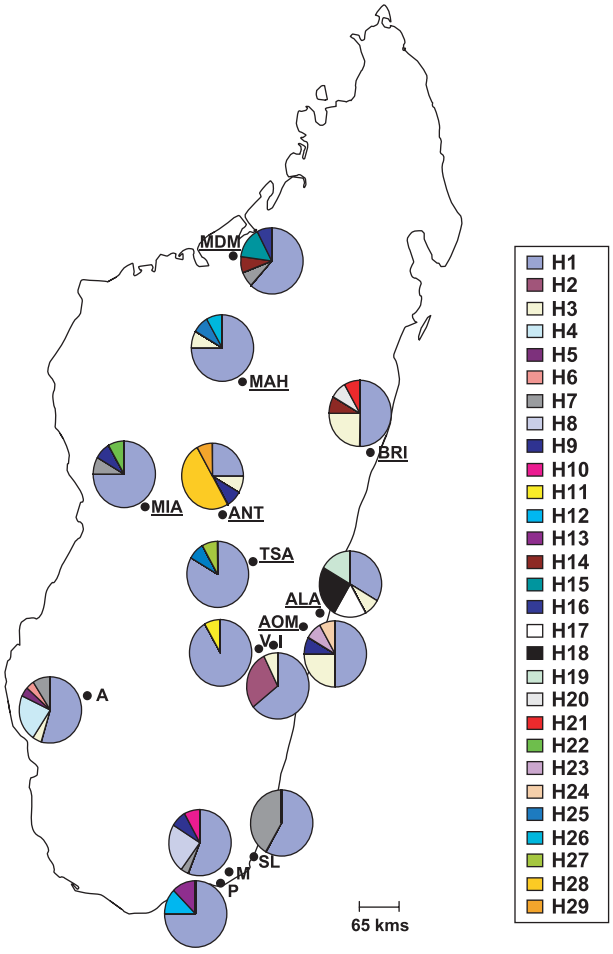

Figure 6 Location and D-loop haplotype composition (419-bp sequence) of each population of *Rattus rattus* from Madagascar. Population codes are the same as in Fig. 1. Underlined populations are those sampled in this study, whereas others were studied by Hingston *et al.* (2005).

In Grande Comore, the mtDNA haplotypes all belonged to a well-supported group, which also contained sequences from East Africa (Mozambique and Tanzania). This group was closely related to Indian and Arabian (Oman and Yemen) sequences but was very distinct from Malagasy sequences. Therefore, independent colonization events may have occurred in Madagascar and Grande Comore. However, simulations under IM estimated a similar divergence time with Oman for both islands. Colonization events may thus have been simultaneous, but the genetic composition of the founder population in Grande Comore would have been highly different from that of Madagascar. However, our sampling in Grande Comore only covered the town and the vicinity of the Moroni harbour, and different haplotypes may be present in other parts of the archipelago.

Mayotte's mtDNA haplotypes were very similar to those of Madagascar, and they shared their most common haplotype (Hap 20), which may be indicative of a two-step colonization pattern. Mayotte was first colonized by East Africans and Arabians, but important migrations from Madagascar occurred later on. In this way, the Malagasy black rat could have been the first to settle in Mayotte, or, alternatively, it could have driven out earlier occupants.

Only one mtDNA haplotype was found in Réunion Island, although three distant (20–60 km apart) localities, widely distributed over the island, were sampled. This haplotype was similar to the one found in South Africa and to those reported in the literature from various continents (Europe, America, West Africa and Oceania). This result is in agreement with a colonization of Réunion independently of that of Madagascar, Mayotte and Grande Comore, presumably directly from Europe. The settlement of Europeans and the colonization by the black rat is thought to date from AD 1680 in Réunion Island (Moutou, 1983; Atkinson, 1985).

Insights into the colonization of Madagascar

Rattus rattus sampled from Madagascar formed a monophyletic group (except for one individual, see below). This observation favours the hypothesis of a single colonization of Madagascar, followed by *in situ* diversification (which is plausible considering the timing and the high mutation rate of the D-loop region). The haplotype most closely related to the Malagasy haplotype group (Hap 11) was found in Oman,

Table 2 Genetic diversities and results of population expansion tests for each population of *Rattus rattus* in Madagascar and the whole Malagasy dataset.

Population (distance to the sea, km)	*N*	$h \pm$ SD	$\pi \pm$ SD	F_S	R_2	*Tau* (time, yr BP)
Anavelona (A) (71.4)	22	0.67 ± 0.09	0.0020 ± 0.0020	−2.75*	0.087	0.814 (3217)
Ioranjatsy (I) (68.6)	14	0.54 ± 0.11	0.0014 ± 0.0001	−1.12	0.169	
Vinantelo (V) (88.7)	12	0.17 ± 0.13	0.0004 ± 0.0010	−0.48	0.276	
Ste Luce (SL) (0.3)	12	0.53 ± 0.08	0.0013 ± 0.0010	0.72	0.265	
Mandena (M) (10.7)	25	0.64 ± 0.08	0.0029 ± 0.0020	−0.29	0.146	
Petricky (P) (3.6)	8	0.46 ± 0.20	0.0012 ± 0.0010	−1.00	0.216	
Ambalatenona (ALA) (10.6)	12	0.83 ± 0.07	0.0028 ± 0.0005	−1.48	0.144	
Antahobe (ANT) (211.6)	12	0.73 ± 0.11	0.0031 ± 0.0007	−1.27	0.151	
Ambohimiariana (AOM) (31.9)	12	0.73 ± 0.11	0.0025 ± 0.0006	−1.82*	0.127*	1.030 (4070)
Brickaville (BRI) (11.6)	12	0.73 ± 0.11	0.0024 ± 0.0006	−1.94*	0.124*	0.985 (3892)
Mahatsinjo (MAH) (185.2)	12	0.46 ± 0.17	0.0012 ± 0.0005	−2.12**	0.144*	0.500 (1976)
Miandrivazo (MIA) (101.3)	12	0.46 ± 0.17	0.0015 ± 0.0007	−1.59*	0.134*	0.636 (2513)
Madiomiangana (MDM) (23.5)	13	0.63 ± 0.14	0.0036 ± 0.0017	−0.78	0.171	
Tsarasambo (TSA) (140.9)	12	0.32 ± 0.16	0.0008 ± 0.0004	−1.32*	0.186*	0.333 (1316)
All Madagascar	190	0.63 ± 0.04	0.0023 ± 0.0002	−35.95***	0.019*	0.787 (3110)

Haplotype (h) and nucleotide (π) diversities and their standard deviations (SD). Results for F_S- and R_2-tests. In populations having gone through a recent expansion (at least one of these tests was significant), mismatch distribution analysis was performed, and time since population expansion was estimated from *Tau*, using a mutation rate estimated at 15.1% Myr^{-1} from the *Rattus rattus*/*Rattus tanezumi* divergence. All these analyses were performed on the 419-bp D-loop region. Underlined populations are those sampled in this study, while others were published by Hingston *et al.* (2005).

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

suggesting that the Arabian Peninsula may be the origin of the black rat colonization of Madagascar, as was argued for the house mouse (Duplantier *et al.*, 2002). Simulations under the IM model (divergence between the populations of Oman and Madagascar) favour the model without recurrent migration and estimate the number of founders on Madagascar (and Grande Comore) as a few hundred. Our results thus agree with one main colonization event in Madagascar, that is to say the arrival of *R. rattus* in a few boats coming from approximately the same region of Oman at the same time. These founder rats would thus present low genetic diversity and subsequent drift would have led to the retention of only the major haplotype.

Finally, it is worth noting that the only exception to this general trend, i.e. one individual (carrying Hap 31) out of the 190 (0.5%) analysed, belonged to another group, which included the haplotypes from South Africa, Réunion and probably many other localities outside the western Indian Ocean. This individual was found in the Madiomiangana (MDM) population, a site located along a large river, about 25 km from the large harbour of Majunga. This variant haplotype is probably the result of a post-colonization immigration event. The successful integration of such migrants is thus still possible in Madagascar, but is expected to be extremely rare owing to the advantage of resident rats over migrants during competitive interactions (Granjon & Cheylan, 1989; Russell & Clout, 2004).

IM simulations estimated the split of the ancestral populations of Oman and Madagascar at about 10,000 years ago. However, this does not mean that the Malagasy population was established at that time, but that the gene pools had started to diverge (Nichols, 2001). The Malagasy black rat populations have experienced recent population expansion, and population growth was estimated to have occurred about 3000 yr BP. As expected, this value is more recent than the estimated divergence time and is compatible with the date of human arrival in Madagascar, about 2300 yr BP (Burney *et al.*, 2004). Moreover, the substitution rate inferred from the inter-specific genetic distance may underestimate the intra-specific mutation rate (Ho & Larson, 2006); in this case, our population expansion would have occurred more recently than 3000 years ago.

One population located on the east coast (Ambohimiariana, AOM) presents the highest haplotype diversity (and nucleotide diversity if we remove the MDM population, for which the high nucleotide diversity is due to a single individual), the haplotype most closely related to that of Oman and the oldest expansion time. Other east coast populations also have high haplotype diversities (ALA and BRI) and old expansion times (BRI). These data suggest that the first arrival of *R. rattus* occurred on the east coast of Madagascar, rather than in the Tolagnaro region (south) as argued by Hingston *et al.* (2005). The oldest Malagasy black rat fossils were found in Mahilaka (north-west of the island), an Islamic port dating back to the 11th to 14th centuries AD (Rakotozafy, 1996; Radimilahy, 1997). However, the arrival of the rat probably pre-dates this period because human settlements were present very early in the east coast (about the 9th century AD; Wright & Rakotoarisoa, 2003).

Population growth dating suggests that the central highlands (at least for the two populations TSA and MAH; no signal of population expansion was found for ANT) were colonized later than the coasts. This is in accordance with historical data that describe the first human colonization to be restricted to the coastal zones, with later settlement of the central highlands by the 12th to 13th centuries AD (Wright & Rakotoarisoa, 2003).

In the central highlands, two populations (TSA and MAH) out of three also have low haplotype and nucleotide diversities, which supports the hypothesis of a more recent colonization. However, higher diversity was found in one population in the centre of the highlands (ANT), which also had a non-typical haplotype composition (the most common haplotype is H28 instead of H1). This high diversity could result from a bottleneck (a rare haplotype being retained by chance), followed by immigration (recovery of genetic diversity by the addition of new haplotypes). Such demographic crashes may be common in rat populations in the central highlands due to the occurrence of plague (Brygoo, 1966; Duplantier *et al.*, 2005).

CONCLUSION

Due to their high mutation rate, mitochondrial sequences are useful for inferring species history, especially as regards the colonization patterns of introduced commensal rodents (Gunduz *et al.*, 2001; Searle *et al.*, 2008). In addition, the phylogeographic histories of rats and mice can be valuable for tracing human history, as for example the use of *R. exulans* as a proxy for the movement of prehistoric people in the Pacific (Matisoo-Smith & Robins, 2009). We show here that mitochondrial sequences provide useful information for inferring the history of *R. rattus* and tracking human movement in the Indian Ocean, even for recent evolutionary time-scales. Nevertheless, our results require confirmation because they rely only on mitochondrial DNA, the use of which for phylogeography is under debate (Ballard & Whitlock, 2004; Zink & Barrowclough, 2008). Future research should also include nuclear markers such as microsatellites, which are currently being developed for *R. rattus* (Loiseau *et al.*, 2008).

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Detailed sampling at the Indian Ocean scale.

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