

Genetic evidence confirms the origin of the house mouse on sub-Antarctic Marion Island

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Abstract Biological invasions and climate change are two of the largest threats to biodiversity, and this is especially true for island ecosystems that have largely evolved in isolation. The house mouse is considered to have been introduced to sub-Antarctic Marion Island by sealers in the early 1800s. It is currently widespread across the island and has a large impact on the indigenous biota. To date, little information is available on genetic aspects of biological invasions in the sub-Antarctic. Ten specimens of the house mouse were collected from two geographically separated localities on Marion Island. Sequences of the mitochondrial DNA control region revealed only two haplotypes, separated by a single site change. More importantly, these haplotypes are shared between the eastern and western side of Marion Island. By comparing our sequences to data available on GenBank, we provide evidence that house mice on Marion Island is *Mus musculus domesticus* (Rutty 1772), and most closely related to haplotypes characterizing this species from Denmark, Sweden, Finland, and northern Germany.

Keywords *Mus musculus* · Marion Island · Mitochondrial DNA · Biological invasion

Introduction

The Southern Ocean contains only a few isolated groups of islands (Hall 2002), the majority of which are volcanic

in origin (Hall 1990). The sub-Antarctic Prince Edward Island group, situated approximately 2,300 km southeast of Cape Town, South Africa, comprises two islands namely Marion Island (46°54'S, 37°45'E) and Prince Edward Island (46°38'S, 37°57'E). Because of the inaccessibility of these islands, the direct impact of humans has, for the most part, been minimal, and their ecosystems are, in many ways, largely intact (Chown et al. 2001). However, although Prince Edward Island has remained virtually pristine, Marion Island has been significantly altered as a consequence of human activities, that include sealing, and the presence of a South African permanent scientific research station since 1947 (Hänel and Chown 1999). As a direct result of these human activities, a large number of alien species has been documented on Marion Island (Cooper and Condy 1988; Frenot et al. 2005). One of the most noticeable of these is the house mouse, *Mus musculus (sensu lato)*, which is thought to have been introduced by sealers in the early 1800s (Hänel and Chown 1999).

Following eradication of feral cats (*Felis catus*, Linnaeus) in the 1990s (Bester et al. 2000), the house mouse has become one of the most significant conservation concerns on the island (Chown and Smith 1993; Huyser et al. 2000). In recent years, it appears that populations of the house mouse have increased owing both to amelioration of climate and possibly to eradication of the feral cats (Van Aarde et al. 1996; Smith 2002). Consequently, the house mouse is having a significant impact on several indigenous species as well as on ecosystem functioning on the island. Crafford and Scholtz (1987) reported that the biomass of invertebrate decomposers was significantly higher on Prince Edward Island than on Marion Island and ascribed this to the absence of house mice on Prince Edward Island.

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Subsequent studies have shown that the biomass of invertebrates has indeed declined substantially (Chown et al. 2002), and that mice are affecting the body sizes of invertebrate species (Chown and Smith 1993). The decline in invertebrate biomass and densities, in turn, has direct effects on nutrient recycling in the terrestrial ecosystem (Smith 2002 and references therein), and indirect effects on populations of the endemic subspecies of the Lesser Sheathbill, *Chionis minor*, because this species relies on invertebrates for its overwinter survival (Huysen et al. 2000). Extensive damage is also caused to plant communities (specifically the cushion plant *Azorella selago*) through runways and extensive burrow systems (Avenant and Smith 2003). Finally, with the removal of feral cats, house mice are now the only terrestrial mammalian species, and circumstantial evidence suggests that they may be preying on albatross chicks and the chicks of other seabird species as has been found on Gough Island (Cuthbert and Hilton 2004; P.G. Ryan, personal communication).

Because of the profound effects of house mice, several discussions on the way forward for mouse control and possible eradication on Marion Island have been held (see, for example, Chown and Cooper 1995). Amongst the various recommendations that have emerged from these discussions has been the verification of the taxonomic status, origin, and the nature and extent of genetic variation in the house mouse population on Marion Island. To date, limited studies have been done on the genetics of the house mouse on Marion Island, with contrasting outcomes. Berry et al. (1978) proposed a Danish origin for the species on Marion Island based on the presence of a unique allele found only in Denmark and the Faroe Islands. In contrast, Robinson (1978) documented a Robertsonian translocation (the fusion of two chromosomes into one larger one) in the Marion Island house mouse population, a chromosomal rearrangement that had, at the time, only been reported from the Rhaetian Alps and Italy in southern Europe.

Notwithstanding a plethora of data, taxonomic relationships within the genus *Mus* (Linnaeus, 1758) remain contentious. Several recent studies based on a wide array of molecular genetic markers (see, for example, Duplantier et al. 2002; Lundrigan et al. 2002; Ihle et al. 2006) have failed to reach consensus mostly because very few sister taxon relationships are robust. The genus is characterized by wild-living taxa with little or no human associations, taxa that remain wild-living but with human-mediated dispersal (such as *M. m. musculus* occurring throughout eastern Europe and northern Asia), and certain taxa that appear to be fully

commensal (including *Mus musculus domesticus* found throughout western Europe, northern Africa, and southwestern Asia) (Nowak 1999).

Molecular data have been useful in establishing colonization histories for species on other oceanic islands (see, for example, Gündüz et al. 2001). In this study, we therefore use sequences of the mitochondrial DNA (mtDNA) control region to investigate the origin of *M. musculus* (*sensu lato*) on Marion Island. We further provide preliminary information on the spread of genetic variation across the island.

Materials and methods

Sample collection

Mice were collected from two localities on Marion Island during the 2004 relief voyage. Eight house mice were caught on the eastern side of the island at the scientific station at Transvaal Cove (46°52'S, 37°51'E), and two mice were caught on the western side of the island at Mixed Pickle Cove field hut (46°52'S, 37°38'E). To determine the subspecies status of the house mice introduced to Marion Island, mtDNA sequences of nine *M. musculus* (*sensu lato*) were downloaded from GenBank representing five (putative) subspecies, namely *Mus musculus castaneus*, *Mus musculus gentilulus*, *M. m. musculus*, *Mus musculus molossinus*, and *M. m. domesticus*. The geographic origin and GenBank accession numbers of these putative subspecies are indicated in Table 1. *Mus spretus* (Table 1), believed to be a sister taxon to *M. musculus* and allocated to the same subgenus (see, for example, Nowak 1999; Duplantier et al. 2002; Lundrigan et al. 2002), was used to root the derived tree topology. In an attempt to pin-point the origin of Marion Island house mice, mtDNA sequences of 20 *M. m. domesticus* were downloaded from GenBank (Table 1). These mtDNA sequences were selected to provide a good geographic coverage of the distribution of this subspecies.

DNA amplification and sequencing

Total genomic DNA was extracted using standard procedures described in Maniatis et al. (1982). Standard polymerase chain reactions, employing control region primers N777 and DLH1, were set up following conditions described in Alpers et al. (2004). Amplicons were directly cycle sequenced using BigDye™ chemistry (version 3, Applied Biosystems, Foster City, CA, USA). Unincorporated dye label was removed by sephadex columns before the samples were run on an ABI 3100™

Table 1 *Mus musculus* subspecies designations, geographic origin and GenBank accession numbers to sequences used in the present study. The *M. spretus* was used as an outgroup

Species/subspecies	Geographic origin	Accession number
<i>M. spretus</i>	Spain	U47539 ^a
<i>M. m. castaneus</i>	Pakistan	AF074539 ^b
	Iran	AJ286324 ^c
<i>M. m. gentilulus</i>	Yemen	AF074541 ^b
	Madagascar	AY091524 ^d
<i>M. m. musculus</i>	Hungary	AY091526 ^d
	Georgia	AF506182 ^e
<i>M. m. molossinus</i>	Not reported	NC_006915 ^f
<i>M. m. domesticus</i>	Madeiran Archipelago	AJ313379 ^g
	Madeiran Archipelago	AJ313378 ^g
	Denmark, Sweden, Finland, northern Germany ^h	U47455 ^a
	Denmark	U47460 ^a
	Madeiran Archipelago, northern Germany, Denmark, Sweden, Finland ^h	AJ313361 ^g
	Greece	AY551961 ⁱ
	Greece	AY551960 ⁱ
	Italy	U47471 ^a
	Italy	U47477 ^a
	Italy	U47482 ^a
	Southern Germany	U47474 ^a
	Morocco	AJ313381 ^g
	England	U47430 ^a
	Mauritania	AJ313380 ^g
	Turkey	AJ843838 ^j
	Turkey	AJ843837 ^j
	France	AM182742 ^k
	France	AM182741 ^k
	Cameroon	AM182713 ^k
	Cameroon	AM182714 ^k

^a Prager et al. (1996)^b Prager et al. (1998)^c Gündüz et al. (2000)^d Duplantier et al. (2002)^e Orth et al. (2002)^f Akimoto et al. (2005)^g Gündüz et al. (2001)^h Haplotype found across several localitiesⁱ Tryfonopoulos et al. (2005)^j Gündüz et al. (2005)^k Ihle et al. (2006)

automated sequencer (Applied Biosystems, Foster City, CA, USA). Electropherograms were checked using Sequence Navigator (ABI™, version 1.01) and aligned by eye. Sequences (haplotypes) were submitted to GenBank under accession numbers DQ792846–DQ792847.

Data analyses

Sequences generated from the house mice collected from Marion Island were aligned to those deposited in

GenBank. Phylogenetic relationships among taxa were estimated using parsimony and neighbour-joining methods carried out by PAUP version 4.0b10 (Swofford 2000). Parsimony analyses were based on heuristic searches with 100 random additions of taxa and TBR branch swapping. For neighbour-joining analyses, trees were constructed from uncorrected *p*-distances as well as under a general time-reversible model with gamma shape parameter of 0.5. Robustness of these analyses was assessed using 1,000 bootstrap replications. For a general description of laboratory techniques and methods of analyses see Hillis et al. (1996).

Results and discussion

To determine the taxonomic status of house mice from Marion Island, we generated 535 bp of the control region sequence for ten individuals collected from the two localities. Two haplotypes (Marion haplotypes 1 and 2) characterized the eight specimens sampled at the meteorological station. Marion haplotype 1 characterized six specimens with the remaining two individuals sharing haplotype 2. The two individuals caught at Mixed Pickle Cove field hut, situated on the western side of the island, shared the most common haplotype detected at the meteorological station. Previous investigations, based on RFLP analyses, similarly detected a single haplotype for house mice collected from across the island (C.A. Matthee, personal communication). Although a detailed study documenting the spatial distribution of genetic variation is needed, preliminary indications are that the variability is quite low. Although nuclear genes may indeed reflect specific adaptations, this finding is nonetheless of concern since it would imply that the low level of variation present in this invasive commensal species has not impeded its ability to spread and colonize new habitats across the island.

Within *M. musculus (sensu lato)*, between three and nine subspecies have historically been recognized (see, for example, Nowak 1999; Duplantier et al. 2002; Lundrigan et al. 2002; Ihle et al. 2006) although it is now widely accepted that most are of hybrid origin (for example *M. m. molossinus*). To determine the taxonomic status of the house mice on Marion Island, Marion haplotypes 1 and 2 were aligned to nine sequences representing five subspecies, namely *M. m. castaneus*, *M. m. gentilulus*, *M. m. musculus*, *M. m. molossinus*, and *M. m. domesticus* as well as to *M. spretus* (see Table 1) which is believed to be a sister taxon to *M. musculus* and belongs to the same subgenus, *Mus* (Linnaeus, 1758). Parsimony analyses retrieved five equally most parsimonious trees of 83 steps, with a

consistency (CI) of 0.830. Neighbour-joining analyses (irrespective of whether uncorrected p -distances or the parameter rich GTR model of evolution was assumed) resulted in an identical tree topology. The neighbour-joining topology was similar to the 50% majority rule consensus tree constructed under parsimony analyses. The Marion haplotypes clustered with *M. m. domesticus* with high bootstrap support (100%) (see Fig. 1a).

It is well-known that the colonization histories of commensal species closely follow the movement of humans. Specifically relating to Marion Island, humans have frequented the island since the early 1800s (Hänel and Chown 1999). For almost 60 years, the island formed the hub of large-scale sealing activity by several European (including the United Kingdom and Scandinavia) and North American companies (Hänel and Chown 1999). In addition, several shipwrecks also occurred around the island (see Hänel and Chown 1999 for an account of the island's history). In an attempt to identify the country of origin, we aligned our sequences to 20 *M. m. domesticus* sequences from GenBank (see

Table 1) covering much of the geographic distribution of this subspecies throughout Europe and the Middle East. We also included sequences from Africa and the Madeiran Archipelago (Table 1).

Parsimony analyses retrieved 298 equally parsimonious trees of 54 steps (CI = 0.815). A consistent finding, irrespective of the method of analyses (parsimony or neighbour-joining) used, is that the Marion Island haplotypes clustered within a northern European clade comprising sequences derived from Denmark, Sweden, Finland, and northern Germany (Fig. 1b). The Marion Island haplotypes were most similar to haplotypes derived from house mice sampled on Porto Santo (Madeiran Archipelago). Although the history of islands in the Madeiran Archipelago has been closely linked to Portugal, Gündüz et al. (2001) argued that northern Europeans must have frequented these islands long before Portuguese settlers first arrived. It has indeed been documented that Danish Vikings made several raiding expeditions along the Iberian coast and into the western Mediterranean (Gündüz et al. 2001).

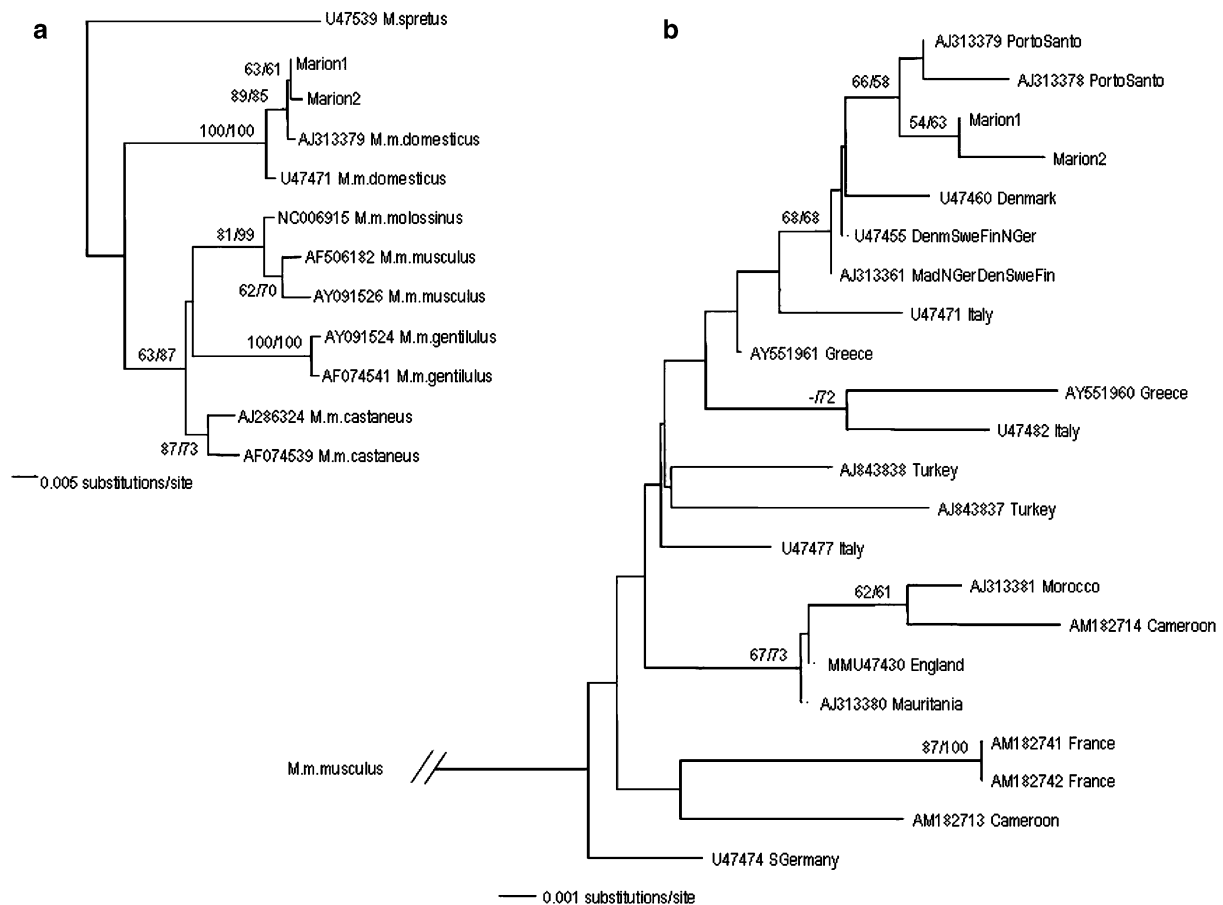


Fig. 1 Neighbour-joining trees indicating the taxonomic affinity (a) and origin (b) of house mice on Marion Island. Parsimony and neighbour-joining analyses retrieved identical or near identical

topologies. Bootstrap support (1,000 iterations) is indicated for nodes (parsimony/neighbour-joining)

The close association of the Marion Island haplotypes to those sampled on Porto Santo and northern Europe would similarly indicate a Scandinavian origin for house mice on Marion Island, or the transport of mice from the Madeiran Archipelago to Marion Island.

The presence of a Robertsonian translocation in house mice collected from Marion Island (Robinson 1978) may not necessarily be contradictory to a northern European origin. It should be noted that at the time that Robinson (1978) described the translocation, limited chromosomal data were available, with most of the published records being from southern European populations (mostly the work done by Capanna et al. 1976). Since then, more than 100 Robertsonian fusions have been described of which none are specific to southern European populations (Piálek et al. 2005). Unfortunately, difficulties with bone-marrow preparations did not allow Robinson (1978) to document the exact chromosomes involved in the Marion Island house mouse population which, in turn, does not allow us to make a comparison to chromosomal data currently available, specifically those unique to Denmark. All available data, when taken in concert, therefore support a northern European origin for house mice on Marion Island.

Whether such an origin will be found for *M. musculus* (*sensu lato*) populations established on the other Southern Ocean islands is difficult to forecast at this point. However, given that the species is widely distributed across these islands (Jones et al. 2003; Frenot et al. 2005), and is having substantial impacts on many of them (Le Roux et al. 2002; Smith et al. 2002; Jones et al. 2003; Cuthbert and Hilton 2004; Frenot et al. 2005), ascertaining the origins of, and relationships among, these populations is clearly important, especially in light of the increasing interest in rodent eradication on islands throughout the region.

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