Molecular Ecology (2006) **15**, 2905–2919 doi: 10.1111/j.1365-294X.2006.02990.x

The recent history and population structure of five *Mandarina* snail species from subtropical Ogasawara (Bonin Islands, Japan)

ANGUS DAVISON*†‡ and SATOSHI CHIBA†

Institute of Genetics, School of Biology, Queen's Medical Centre, University of Nottingham, Nottingham NG7 2UH, UK, †Division of Ecology and Evolutionary Biology, Graduate School of Life Sciences, Tohoku University, Aoba, Sendai 980-0875, Japan, ‡Institute of Evolutionary Biology, University of Edinburgh, Kings Buildings, West Mains Road, Edinburgh EH9 3JT, UK

Abstract

The effect of Pleistocene climate change on the organisms of tropical and subtropical regions is rather poorly understood. We therefore studied the land snail genus *Mandarina* (Bradybaenidae) of oceanic Ogasawara (Bonin Islands, Japan), with the aim of using population genetic data to understand their recent history. Our analysis of a mitochondrial 16S ribosomal RNA region from more than 600 snails in five ground-living species suggests that populations on the small islands of Mukoujima, Anejima, Imotojima and Meijima, as well as on the low-lying southern and central parts of Hahajima, have probably undergone recent bottlenecks followed by subsequent expansions. Except between the main island of Hahajima and Mukouijima, there is almost no evidence for gene flow among islands even though the islands were connected repeatedly by land bridges through the Pleistocene. Within islands the population structure is severe, suggestive of a long-term, low level of gene flow (F_{ST} is frequently greater than 0.5 among geographically close populations). Finally, there is a marked genetic patchiness, meaning that genetically close populations are sometimes separated by genetically distant populations. These patterns could be a consequence of expansion from bottlenecks, low active dispersal and founder effects caused by rare long-distance migrants. Unfortunately, the exact nature of the refugia and bottlenecks remains unknown because the palaeoclimate of this region is poorly understood. Dating the population size changes is also challenging because the molecular clock is uncertain. We suggest, however, that arid conditions or deforestation induced by decreased atmospheric CO_2 may have been the main factor in determining population size.

Keywords: 16S ribosomal RNA, bottleneck, land snail, mitochondrial phylogeography, oceanic islands, Pleistocene refugia

Received 8 February 2006; revision accepted 30 March 2006

Introduction

Increasingly numerous species have been investigated from a phylogeographic perspective (Emerson *et al*. 2001; Hewitt 2004; Hofreiter *et al*. 2004), yet most research has concentrated on species from temperate regions. So, while many studies have shown that temperate species were confined to one or a few refugia during much of the Pleistocene because the environment was inhospitable or the land was glaciated (Hewitt 2004; Hofreiter *et al*. 2004), the response of tropical and subtropical species to climate change is less well understood (Schneider & Moritz 1999; Hugall *et al*. 2002; Dutech *et al*. 2003; Lessa *et al*. 2003; Bell *et al*. 2004; Flanagan *et al*. 2004).

Several recent phylogeographic studies have been carried out on land snails and slugs (Davison 2002; Gittenberger *et al*. 2004; Pfenninger 2004; Pinceel *et al*. 2005), although few on tropical species (Holland & Hadfield 2002, 2004; Hugall *et al*. 2002; Rundell *et al*. 2004). One reason that land snails should be particularly useful in inferring the effect of Pleistocene climate change on

Correspondence: Angus Davison, Fax: +44 (0)115 8230313; E-mail: angus.davison@nottingham.ac.uk

population history is that they have high levels of population genetic structure, because of their limited active dispersal and high rate of mitochondrial DNA evolution (Chiba 1999a). In theory, the population structure can be used to enable a detailed reconstruction of past events.

As part of an ongoing project, we have studied the land snail genus *Mandarina* (Bradybaenidae) of subtropical, oceanic Ogasawara (Bonin Islands, Japan; Fig. 1), so as to understand its recent population history and, at the same time, augment the genetic data on subtropical species. In the past, the genus has been studied from the perspective of understanding how their exceptional morphological and species diversity arose (Chiba 1999a, 2004), since the sister genus *Euhadra* in Japan inhabits an area that is orders of magnitude larger (see inset to Fig. 1), yet has only a few more species (~22 compared with 15) and has less morphological variation within and between species (Davison *et al*. 2005). It has already been established using both mitochondrial and nuclear markers that one or a few individuals of an ancestral *Mandarina* species colonized Ogasawara between 0.9 and 1.8 million years ago, when the islands rose as a result of tectonic uplift (Chiba 1999a; Davison & Chiba 2006). Speciation on different islands was such that similar ecotypes evolved independently in different lineages and islands (Chiba 1999a). Also, the present-day species can take different, genetically determined

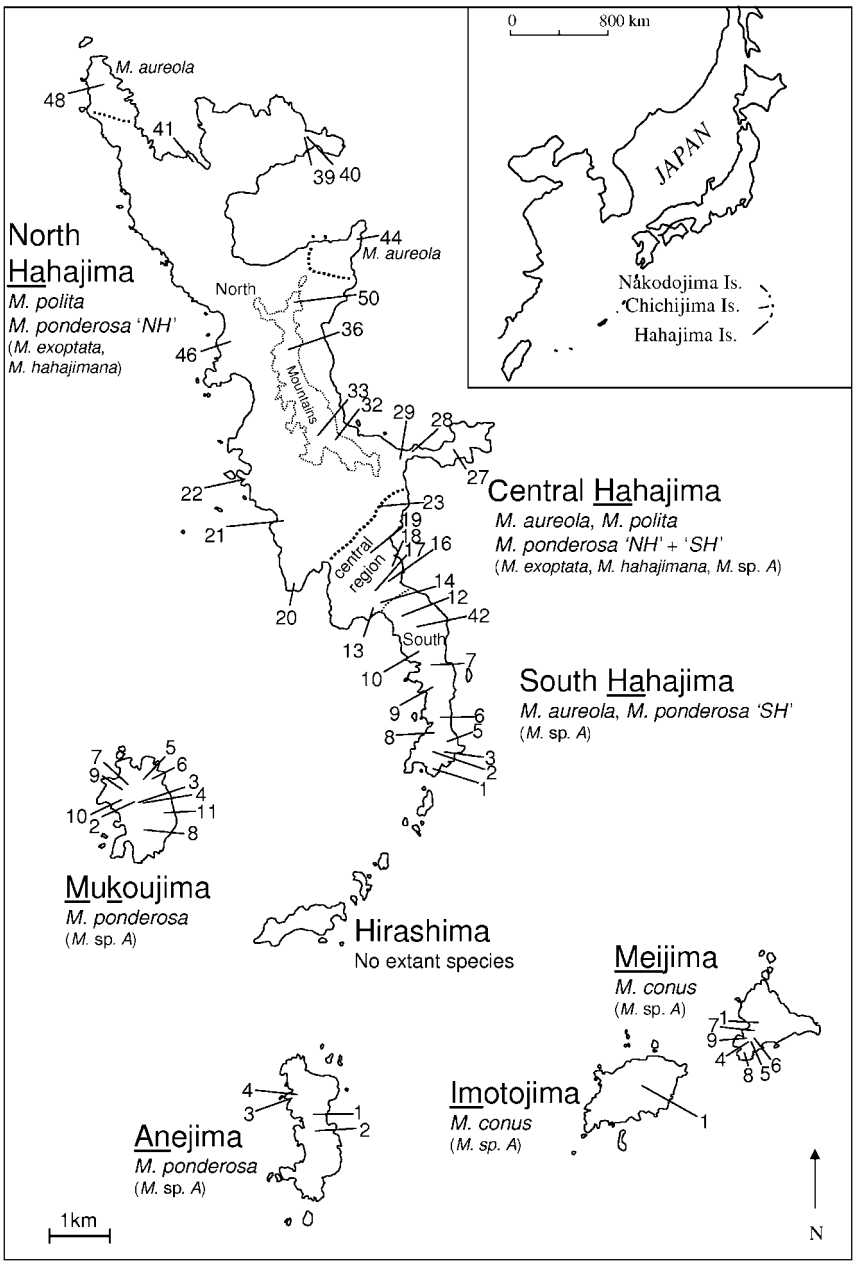

Fig. 1 The Hahajima archipelago showing the approximate distribution of species, and sample sites. Underlining indicates the site code name for that island. Note that while *Mandarina aureola* is mostly restricted to southern Hahajima, it is also found in two locations in the north and east, indicated by the dotted line (ha44 and ha48). Arboreal species (bracketed, small type) were not considered in this study. Inset: the location and size of the three Ogasawara archipelagos relative to the mainland of Japan. A variety of *Mandarina* species are present on each of the three archipelagos; the sister genus *Euhadra* is found throughout the four main islands of Japan.

ecotypes, depending on their geographic location and local competitors (Davison & Chiba 2006). The ability of species to switch ecotypes is a requirement for the burst of speciation that may have occurred shortly after the first colonization of the islands (Davison & Chiba 2006).

The specific aim of this study was to investigate in detail the phylogeographic structure and demographic history of the main ground-living species from one of the Ogasawara island groups — the Hahajima archipelago (Fig. 1). In temperate latitudes the main effect of Pleistocene climate change was to create large tracts of land covered by glaciers or permafrost so that most organisms were restricted to isolated refugia, before expanding again in the interglacial periods (Hewitt 2004). As sea level was at least 100 m lower during the glaciations, coastal regions expanded worldwide, and previously isolated islands became linked by land bridges. On the subtropical Hahajima archipelago, there were no glaciers at the height of the Pleistocene glaciations, the area covered by the islands was around two to three times greater compared with the present day, and each island was connected to another by a land bridge (Fig. 2). We therefore studied *Mandarina* to try to resolve the impact of climate change on their population genetic structure, specifically gene flow within and between islands. In line with expectations from prior palaeontologic and phylogeographic studies on snails, we found evidence for past population bottlenecks and limited gene flow between populations.

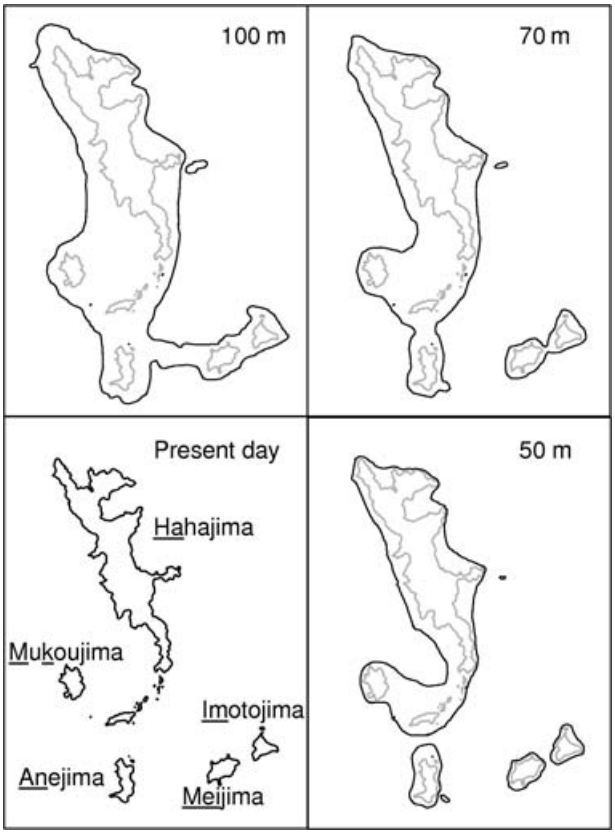

Fig. 2 Pleistocene sea level changes, showing extent of land mass with sea level 100, 70, and 50 m below present. As the sea level rose, Meijima and Imotojima were isolated first, followed by Anejima, then Mukoujima.

Materials and methods

Samples

Fifteen *Mandarina* (Bradybaenidae) species are recorded from Ogasawara. Each species is ultimately recognized by differences in the genitalia, but shell shape and banding pattern are useful in the first instance. For this study, we sampled all four ground-living species from Hahajima (the only human-inhabited island in the Hahajima archipelago) as well as the single ground-living species that live on each of the smaller islands of Mukuojima, Anejima, Imotojima and Meijima (Table 1; Fig. 1). In total, 56 sites were sampled on five islands (Table 1). Unfortunately, we were not able to find any living *Mandarina* on Hirashima; they are presumed extinct due to habitat degradation caused by introduced goats (now exterminated). The arboreal species of the Hahajima archipelago were not considered in this study; morphological and DNA evidence indicates that the arboreal and ground-living species are separate lineages (Davison & Chiba 2006, unpublished).

On the main island of Hahajima, two principal types of ground-living species (or 'ecotypes') usually coexist on a broad scale, although each is found in a different niche. One species tends to be associated with the litter of broad-leaved trees (*Mandarina polita* or *Mandarina aureola*) and the other is associated with palm litter (*Mandarina ponderosa*) (Chiba 1996a, b, 2004). *M. ponderosa* on Hahajima take one of two forms, which are distinguished by both shell and genital differences. As they are probably two separate species, they are referred to here as *M. ponderosa* '*SH*' or '*NH*', the name depending upon their mostly southern or northern distribution in Hahajima, respectively. *M. ponderosa* '*SH*' usually coexists with *M. aureola*, and *M. ponderosa* '*NH*' with *M. polita* (Fig. 1), but there is a central region where similar ecotypes may live together and hybridize (e.g. *M. polita* × *M. aureola* and *M. ponderosa* '*NH*' × '*SH*') (Chiba, unpublished). *M. aureola* is also found in two regions in the extreme north and northeast of Hahajima (sites ha44 and ha48 in our sample; Fig. 1).

On each of the small islands only one ground-living species is present (Fig. 1), either *M. ponderosa* or *M. conus*. On Mukoujima the genitalia of the ground-living species are similar to *M. ponderosa* '*SH*' from South Hahajima. On Anejima, the genitalia of the snails are approximately intermediate between both *M. ponderosa* '*NH*' and '*SH*' forms from Hahajima. On Imotojima and Meijima, the

Table 1 Species, sample sites, standard diversity indices and mitochondrial DNA haplotypes recovered from each population

Species	Distribution	Site	N 26°	E 142°	Sample size	Number of haplotypes	Nucleotide diversity	Haplotype diversity	Group*	Individual haplotypes
M. aureola	central and	ha1	36′15″41	10′53″36	10	3	0.0027	0.64	A5 (10)	au20 (4), au27 (5), au28
	southern Hahajima,	ha2	36′24″41	10′53″00	2	2			A5 (2)	au18, au20
	plus two outlying	ha3	36′23″76	10′59″55	17	4	0.0022	0.57	A5 (17)	au18 (2), au19 (3), au20 (11), au22
	northern populations	ha5	36′30″00	11′1″82	8	5	0.0017	0.79	A5 (8)	au14, au16 (4), au18, au19, au20
	(ha44 + ha48)	ha6	36′42″81	10′57″73	5	4	0.0030	0.90	A5 (5)	au17, au20 (2), au21, au23
		ha7	37′12″00	10′52″09	5	2	0.0020	0.40	A5 (4), A6	au2, au20 (4)
		ha8	36′34″05	10′53″64	15	3	0.0019	0.45	A5 (15)	au18, au19 (3), au20 (11)
		ha9	37′0″00	10′53″82	7	2	0.0007	0.29	A5 (7)	au15, au20 (6)
		ha10	37′18″97	10′45″45	10	3	0.0052	0.51	A5, A6 (7), A9 (2)	au2 (7), au20, au24 (2)
		ha12	37′8″11	10′33″91	2	2			A6, A9	au2, au26
		ha13	37′12″16	10′19″18	1	1			A9	au24
		ha16	37′21″81	10′18″55	5	3	0.0060	0.70	A5, A6 (4)	au1 (3), au8, au19
		ha17	37′25″78	10′25″45	4	1			A6 (4)	au2 (4)
		ha18	38′4″30	10′28″91	3	1			A6 (3)	au1 (3)
		ha19	38′12″32	10′15″55	10	6	0.0019	0.64	A6 (10)	au2 (4), au5, au6, au7, au11, au12 (2)
		ha23	38′36″49	10′23″55	4	2			A6 (4)	au2, au12 (3)
		ha42	37′0″41	10′43″91	5	3	0.0070	0.70	A6 (3), A9	au2 (3), au3, au25
		ha44	40′56″76	10′12″18	10	3	0.0064	0.38	A1 (8), A7 (2)	po45, po46, po61 (8)
		ha48	42′21″89	7′42″73	11	9	0.0150	0.96	A3 (9), A4 (2)	po44, po47, po48, po49, po52, po53 (2), po54, po55 (2), po59
		Total			134					
M. polita	Central and northern Hahajima	ha20	37′57″16	9′30″45	17	13	0.0115	0.96	A2 (16), A8	po7, po8, po9 (2), po10 (3), po11 (2), po12, po13, po14, po15, po16, po17, po18, po19
		ha21	38′31″22	9′25″27	2	2			A2 (2)	po22, po25
		ha22	38′53″03	9′3″91	9	4	0.0034	0.58	A2 (9)	po20 (6), po21, po23, po24
		ha23	38′36″49	10′23″55	15	7	0.0031	0.71	A6 (15)	au1 (5), au2 (3), au8 (2), au9, au10, au12, au13 (2)
		ha27	39′7″14	11′6″64	11	2	0.0008	0.33	A7 (11)	po3 (9), po43 (2)
		ha28	39′6″49	10′42″27	11	4	0.0018	0.67	A7 (11)	po2, po3 (3), po4 (6), po5
		ha29	39′2″51	10′36″27	10	2	0.0018	0.36	A6 (8), A7 (2)	au1 (8), po3 (2)
		ha32	39′13″05	9′56″82	11	4	0.0023	0.62	A7 (11)	po1 (6), po3 (4), po6
		ha33	39′15″81	9′46″82	2	1			A2 (2)	po23 (2)
		ha39	41′55″22	9′42″00	11	7	0.0084	0.87	A1 (11)	po26, po28, po31 (4), po32 (2), po33, po34, po35
		ha40	41′55″62	9′43″09	11	6	0.0079	0.89	A1 (11)	po26, po27 (2), po29, po30 (2), po31 (3), po32 (2)
		ha41	41′41″27	8′35″91	12	7	0.0092	0.83	A4 (10), A1 (2)	po36, po37, po38, po39 (2), po40 (5), po41, po42
		ha46	40′3″49	8′1″36	10	6	0.0041	0.91	A2 (10)	po50, po51 (2), po56 (2), po57, po58 (2), po60 (2)
		Total			132					
M. ponderosa 'SH'	Southern Hahajima	ha1	36′15″41	10′53″36	10	6	0.0053	0.87	P5 (10)	sp1 (3), sp5, sp26, sp28 (3), sp29, sp30
		ha2	36′24″41	10′53″00	9	5	0.0052	0.86	P5 (9)	sp1 (3), sp5 (2), sp13, sp26, sp28 (2)
		ha3	36″23′76	10′59″55	9	6	0.0034	0.89	P5 (9)	sp1 (3), sp5 (2), sp6, sp7, sp15, sp18
		ha8	36′34″05	10′53″64	11	4	0.0018	0.75	P5 (11)	sp1 (5), sp3 (3), sp5 (2), sp9
		Total			39					

Table 1 *Continued*

Species	Distribution	Site	N 26°	E 142°	Sample size	Number of haplotypes	Nucleotide diversity	Haplotype diversity	Group*	Individual haplotypes
M. ponderosa	Central and northern	ha14	37′15″41	10′21″64	1	1			P6 (1)	np17
'NH'	Hahajima	ha16	37′21″81	10′18″55	10	2	0.0009	0.36	P6 (10)	np17 (8), np18 (2)
		ha18	38′4″30	10′28″91	11	5	0.0035	0.82	P6 (11)	np16, np17 (4), np19 (3), np20 (2), np22
		ha36	40′1″62	9′30″91	4	2			P6 (4)	np17, np21 (3)
		ha50	40′22″30	9′32″27	8	3	0.0048	0.61	P6 (8)	np21 (5), np17 (2), np23
		Total			34					
M. ponderosa	Mukoujima	mk2	36′0″00	7′56″00	6	4	0.0167	0.87	P5 (5), P6	np2, sp10, sp16 (2), sp19 (2)
		mk3	35′59″00	7′57″00	16	4	0.0103	0.44	P5 (14), P6 (2)	np6 (2), sp5 (12), sp16, sp19
		mk4	35′59″00	7′59″00	18	7	0.0021	0.69	P5 (18)	sp5 (10), sp12, sp14, sp16, sp17, sp23 (2), sp24 (2)
		mk5	36′12″16	8′0″45	20	5	0.0187	0.57	P5 (7), P6 (13)	np2 (13), sp5 (3), sp19 (2), sp21, sp24
		mk6	36′11″35	8′5″45	28	6	0.0182	0.56	P5 (9), P6 (19)	np2 (18), np4, sp5 (6), sp22, sp24, sp27
		mk7	36′8″11	7′51″36	17	4	0.0160	0.70	P5 (4), P6 (13)	np2 (8), np8 (5), sp5 (3), sp25
		mk8	35′45″00	8′0″91	19	9	0.0218	0.85	P5 (12), P6 (7)	np2 (2), np5 (2), np9, np14, np15, sp2, sp5 (7), sp8, sp20 (3)
		mk9	36′5″68	7′47″73	21	8	0.0087	0.76	P5, P6 (20)	np1 (2), np2 (3), np7, np8, np10, np11 (10), np12 (2), sp11
		mk10	36′0″00	7′47″00	13	7	0.0194	0.83	P5 (4), P6 (9)	np2 (5), np3, np6, np7, np13, sp5 (3), sp19
		mk11	35′53″92	8′13″18	8	1	0.0000	0.00	P5 (8)	sp5 (8)
		Total			166					
M. ponderosa	Anejima	an1	33′6″49	9′35″91	5	2	0.0010	0.40	P3 (5)	an2 (4), an5
		an2	32′57″81	9′36″82	9	4	0.0027	0.69	P3 (9)	an2 (5), an3, an6, an9 (2)
		an3	33′16″22	9′23″45	10	5	0.0054	0.84	P3 (10)	an2, an3 (3), an4, an7 (3), an8 (2)
		an4	33′17″43	9′26″45	13	6	0.0052	0.82	P3 (13)	an1, an2 (2), an4, an7 (5), an8 (3), an9
		Total			37					
M. conus	Meijima	mei1	33′54″57	14′0″45	3	2			P2 (3)	c2 (2), c5
		mei4	33′43″95	13′53″45	7	4	0.0030	0.81	P2 (7)	c1 (3), c2, c5, c14 (2)
		mei5	33′43″78	13′55″82	4	3			P2 (4)	c1 (2), c2, c14
		mei6	33′46″14	13′57″91	8	5	0.0030	0.81	P2 (8)	c1 (2), c3, c5 (4), c6, c12
		mei7	33′50″43	13′56″36	11	10	0.0038	0.98	P2 (11)	c1, c2, c3, c4, c5, c7, c9, c10, c11, c14 (2)
		mei8	33′38″11	13′51″82	12	5	0.0044	0.74	P1, P2 (11)	c1 (2), c3 (6), c8, c10 (2), c16
		mei9	33′46″22	13′53″18	10	6	0.0032	0.78	P2 (10)	c1 (5), c2, c5, c7, c13, c14
		Total			55					
M. conus	Imotojima	im1	33′20″51	12′50″91	9	3	0.0042	0.56	P1 (9)	c15 (2), c17, c18 (6)

*For *Mandarina ponderosa*/*Mandarina conus*, the 'group' corresponds to a well-supported lineage; for *Mandarina aureola*/*Mandarina polita*, the groupings are much more arbitrary.

genitalia and shell morphology of the ground-living species are sufficiently distinct that they are considered a separate species — *M. conus*.

DNA methods

For the present study, we were interested in the recent evolution of *Mandarina*, so efforts concentrated on a short (~410 bp), but highly variable region of the 16S ribosomal RNA (16S rRNA) gene.

Genomic DNA was isolated using methods described by Teshima *et al*. (2003). Primers for polymerase chain reaction (PCR) amplification of an approximately 900-bp 16S rRNA gene fragment have been described by Chiba (1999a). All PCRs used Takara rTaq™ (Takara Biomedicals) and buffers, with annealing temperatures of 50 °C. Cycle sequencing was carried out with the forward primer only, using about 80–100 ng of PCR product in the reaction and the BigDye™ Terminator version 3.0 Cycle Sequencing Ready Reaction Kit (Applied Biosystems). DNA sequences were electrophoresed on an Applied Biosystems 310 Genetic Analyser.

Sequences were aligned using the CLUSTAL_X software, and then checked manually. The number of unique haplotypes was then counted. For most analyses, all insertion and deletion sites (indels) were removed, meaning that some pairs of haplotypes that differed by only one indel were pooled. This was necessary because of potential alignment problems, and also because several of the analytical methods are not able to use gaps as characters, or are not able to use them effectively. Given that snail populations are highly structured, ignoring gaps should have a relatively small effect (Pearce 2006).

Phylogenetic and phylogeographic analyses

To assess the variation present within each population of five or more sampled individuals, nucleotide diversity and haplotype diversity were calculated using ARLEQUIN 2.001 (Schneider *et al*. 2000). Fu's F_S test of neutrality was used, also in ARLEQUIN, to test for evidence of selection under an assumption of neutral evolution (Fu 1997).

Prior phylogenetic analyses with longer sequences (~1600 bp, Davison & Chiba 2006), as well as analyses with the shorter sequences (unpublished), showed that the mitochondrial DNA sequences of *M. aureola* and *M. polita* always cluster together, as do sequences of *M. ponderosa* and *M. conus* (supported by bootstrapping). The sequences from these two groups were therefore treated separately in the population genetic analyses.

The relationships *within* the two groups were therefore investigated by constructing unrooted phylogenies using neighbour-joining (NJ) and maximum likelihood (ML), both with PAUP* 4.0b10 (Swofford 1999). Multiple hits were corrected using the general time reversible model. The rate matrix, base frequencies and shape parameter (α) of the gamma distribution (based on 16 rate categories) were estimated using likelihood, by iteration from an initial NJ tree. Parameters estimated from the initial tree were used to make a new NJ tree. The parameters were then re-estimated, and the process was repeated until there was no further improvement in likelihood. The proportion of invariant sites was not used because the extra parameter did not improve the model significantly, as judged by a likelihood-ratio test. Bootstrap values were calculated using 1000 replicates for the NJ method. ML methods used a heuristic procedure with tree-bisection–reconnection and 100 bootstrap replicates. There was no useful outgroup because DNA sequences from other ground-living species are too distantly related.

The partitioning of genetic variation among pairs of populations was estimated using F_{ST} (minimum five sampled individuals per population), based on the maximum-likelihood estimate of distance between haplotypes, with significance determined by a permutation analysis in ARLEQUIN 2.001 (Schneider *et al*. 2000). Evidence for an association of geographical distance with genetic distance was tested by plotting a regression of ln (geographical distance) against F_{ST}, with significance determined by a randomization (Mantel) test. This was also implemented in ARLEQUIN.

The relationship between unique haplotypes was described using a median-joining (MJ) network, with the sequences as nodes of a network instead of the terminal tips of a tree. First, sites with more than one substitution were removed from the alignment. Then, MJ networks were estimated using the reduced median algorithm of NETWORK 4.1.0.9 (www.fluxus-engineering.com) (Bandelt *et al*. 1999). For *M. ponderosa* and *M. conus*, the analyses were carried out on defined haplotype lineages (i.e. five distinct groups, each with high bootstrap support), as identified in the prior phylogenetic analysis. As there are no well-defined lineages in *M. polita* or *M. aureola*, the analysis was carried out on the full data set.

Population bottlenecks followed by growth, or selective sweeps, may both lead to a Poisson distribution of substitutional differences between pairs of haplotypes (Slatkin & Hudson 1991; Rogers & Harpending 1992). The hypothesis of recent demographic expansion was therefore tested using mismatch analyses, carried out in ARLEQUIN. Expected distributions were fitted to the observed mismatch distributions using a generalized least squares method to estimate the demographic expansion parameters τ, θ_0 and θ_1. Parametric bootstrapping with 1000 pseudo-replicates was used to obtain confidence intervals around the parameter estimates and to test whether the observed mismatch distributions fitted the sudden expansion model.

Results

Around 410 bp of the 16S rRNA gene fragment were sequenced from 606 individuals, the exact length varying according to the number of insertion deletions (166 unique sequences; GenBank reference numbers DQ084905 to DQ085071). The levels of within-species mitochondrial diversity were relatively low compared with other snails that have been studied (e.g. Thomaz *et al*. 1996). Subsequent analysis showed that the populations with the highest nucleotide diversity (> 1%) are those that contain more than one distinct divergent lineage, such as *Mandarina ponderosa* populations on Mukoujima.

Unrooted phylogenies were used to describe the relationship between the mitochondrial DNA lineages (Fig. 3). The *Mandarina ponderosa*/*Mandarina conus* group contains five distinct groups or clades (P1 to P5), each recovered using both NJ and ML methods, and supported by bootstrapping (Fig. 3a). Haplotype groups P1 to P3 were found exclusively on the outlying islands of Imotojima, Meijima and Anejima, respectively (Fig. 4a), with a single exception: a snail from Meijima had a haplotype (c16) that fell with the P1 lineage sequences from Imotojima (c15, c17, c18). The two remaining haplotype lineages, P4 and P5, were both found on Mukoujima and Hahajima, although they have discrete distributions on the latter island (Fig. 4a): P4 was found only in *M. ponderosa* '*NH*' populations and P5 in *M. ponderosa* '*SH*'.

The *M. aureola*/*M. polita* group was poorly resolved compared with the *M. ponderosa*/*M. conus* analysis (Fig. 3b), with none of the main branches supported by bootstrapping. However, there was still evidence for genetic structure, with some haplotype groups confined to specific sample sites and species (Table 1; see below for further details). The level of variation within *M. aureola*/*M. polita* on Hahajima is about the same as in *M. ponderosa* from Hahajima (Fig. 3).

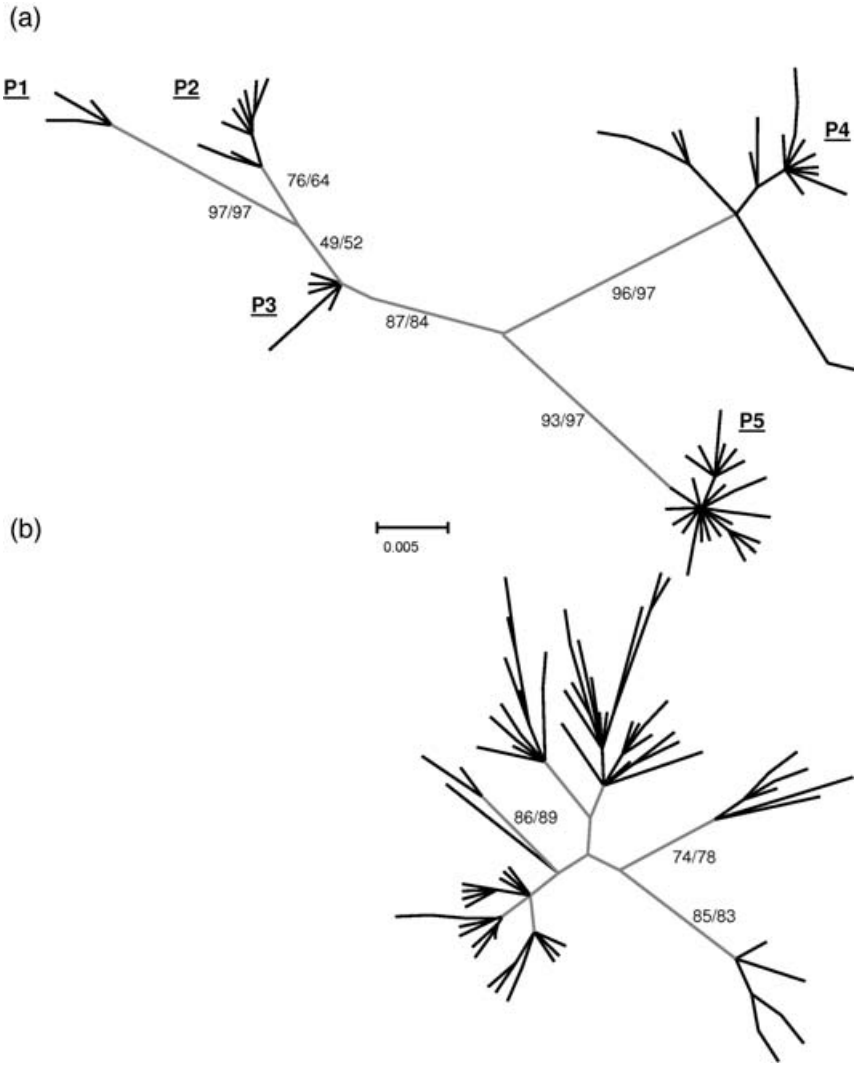

Fig. 3 Unrooted rate-corrected maximum-likelihood phylogenies showing the relationship between mitochondrial DNA lineages. The scale is the same for both phylogenies; bootstrap support is shown for the main branches of the maximum-likelihood and neighbour-joining topologies, respectively. (a) In the palm litter species, *Mandarina ponderosa* and *Mandarina conus*, there are several relatively well-supported lineages. Lineages P4 and P5 were found on Hahajima, whereas P1 to P3 were found on the other islands. (b) In the broad-leaved litter species *Mandarina aureola*/*Mandarina polita* (only found on Hahajima), the topology is not supported by bootstrapping. The scale of DNA variation within *M. aureola*/*M. polita* is about the same as that within *M. ponderosa* from Hahajima (P4 and P5).

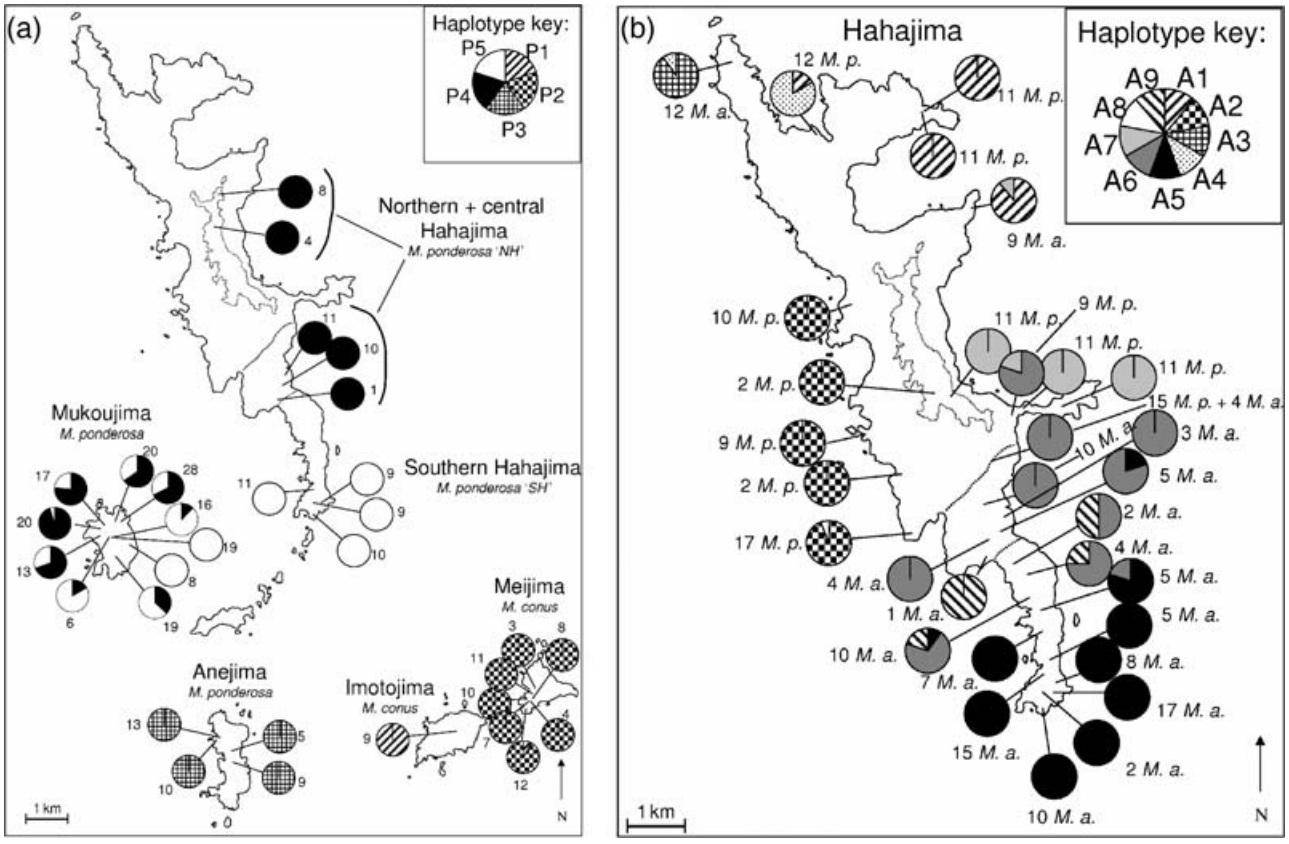

Fig. 4 The distribution of (a) the *Mandarina ponderosa* and *Mandarina conus* haplotype groups on the Hahajima archipelago and (b) the *Mandarina polita* and *Mandarina aureola* haplotype groups on Hahajima. Sample size is shown next to each pie graph.

In both phylogenies, the starlike structure of the tips might be a result of past bottlenecks. As phylogenies are also not ideal to describe the relationship between closely related haplotypes, the MJ method was used to construct a haplotype network. For *M. aureola*/*M. polita*, all of the sequences were used in a single analysis. This showed that there are several clusters, some of which have a common central haplotype, from which multiple, closely related haplotypes radiate out (Fig. 5). The starlike pattern is commonly taken to be a signature of a recent bottleneck and demographic expansion (Slatkin *et al.* 1991; Rogers *et al.* 1992).

In most cases, *M. aureola*/*M. polita* have different haplotypes (Fig. 5a). The exceptions were haplotype au1 (found at site ha16, ha18 in *M. aureola*; ha23, ha29 in *M. polita*), au2 (site ha7, ha10, ha12, ha17, ha19, ha23, ha42 in *M. aureola*; ha23 in *M. polita*), au8 (site ha16 in *M. aureola*; ha23 in *M. polita*) and au12 (site ha19, ha23 in *M. aureola*; ha23 in *M. polita*) (Table 1). Snails in the central part of Hahajima, corresponding to the contact zone between *M. aureola* and *M. polita*, were therefore much more likely to share haplotypes between species, with site ha23 making by far the greatest contribution to the sharing of haplotypes. As both species have similar or identical mitochondrial haplotypes in the central region, then this suggests recent or historic gene flow between them; shell morphology also suggests hybridization (Davison & Chiba, unpublished).

To help visualize the distribution of the haplotypes, we grouped them into nine clusters (Fig. 5b). When their distribution was overlaid onto a map of Hahajima, a strong phylogeographic structure became apparent (Figs 4b and 5b). Populations from the low-lying southern and central parts of Hahajima show the greatest evidence for having undergone a bottleneck (A5, A6, A7 in Fig. 5b). In contrast, populations from other parts have a more diffuse structure (e.g. A1, A2, A3, A4 from central and northern parts of Hahajima; Fig. 5b). Note that because the grouping process was done by eye and was therefore relatively arbitrary, the geographic clusters do not necessarily correspond to real biogeographic units and hence the precise location of possible refugia cannot be inferred.

Finally, one of the isolated northern populations of *M. aureola* (site ha48) is mostly made up of haplotypes that are not found elsewhere (the northwest group in Fig. 5b). The other isolated population of *M. aureola* (site ha44) has haplotypes that are the same or similar to *M. polita* from nearby sites (ha39 and ha40; the northeast group in Fig. 5b).

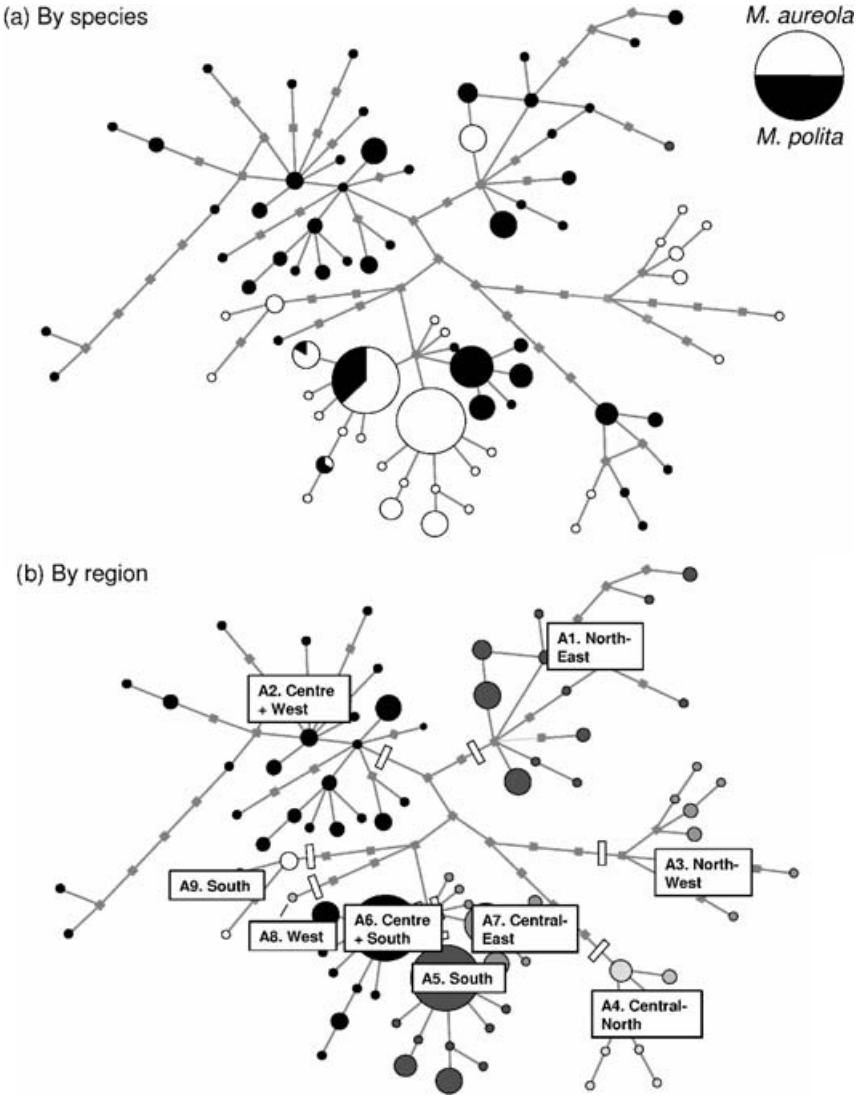

Fig. 5 The median-joining network for *Mandarina polita* and *Mandarina aureola* haplotypes. The size of the circle is proportional to sampling frequency. Unsampled or missing nodes/haplotypes are shown by squares. (a) By species. (b) By region; the white bars in the latter separate haplotypes from different regions.

We also carried out median-joining network analyses on the *M. ponderosa*/*M. conus* haplotypes (Fig. 6), with the added advantage that this time it was possible to divide the haplotypes into groups according to their placement in the phylogeny (Fig. 3a). Groups P2 to P5 all have one or two haplotypes at the centre of a star (Fig. 6), which is consistent with a bottleneck followed by a recent population expansion. As mentioned above, two types (P4 and P5) are present on both Hahajima and Mukoujima, but their distribution is only discrete on the former island (Fig. 4a). Their distribution on Hahajima corresponds to the distribution of the two putative species, *M. ponderosa 'SH'* and *'NH'* (Figs 1 and 4a).

Given the above visual representations, it was therefore not surprising to find that the level of population structuring (as defined by F_{ST}) is strong in *Mandarina* (Table 2). The other main characteristic is that the partitioning of genetic variation is patchy — geographically distant populations are sometimes genetically similar; in other circumstances they are genetically distant (Fig. 7a,c,d,f). Thus, F_{ST} was high and significantly greater than zero for most pairwise comparisons involving Mukoujima and Hahajima (Table 2; mean $F_{ST} = 0.46$). Within Mukoujima, some populations are genetically distant whereas others are genetically similar ($b = 0.11$; $P = 0.063$, not significant; Fig. 7a). On Hahajima, *M. ponderosa 'NH'* populations are genetically and geographically distant from one another, whereas *M. ponderosa 'SH'* populations are genetically and geographically similar to one another (Fig. 7b). There was no evidence for recent gene flow between *M. ponderosa 'SH'* and *'NH'* on Hahajima (Fig. 4a), since the lineages have separate distributions on that island; on Mukoujima, both lineages were found at most sites. When all the populations of *M. ponderosa* from Mukoujima and Hahajima were combined, a highly significant association of F_{ST} with geographic distance was found ($b = 0.10$; $P < 0.000$; Fig. 7c).

Table 2 F_{ST} values within and between *Mandarina aureola / Mandarina polita* (lower diagonal) and *Mandarina ponderosa* (upper diagonal) populations. Values that did not differ significantly from zero are shown in italics

	mk2	mk3	mk4	mk5	mk6	mk7	mk8	mk9	mk10	mk11	ha1	ha2	ha3	ha8	ha16	ha18	ha50							
ha1		*–0.070*	0.197	0.294	0.326	0.440	*–0.013*	0.717	0.314	0.116	0.152	0.127	0.181	0.217	0.809	0.786	0.774	mk2						
ha3	0.412		*0.062*	0.410	0.425	0.542	*0.076*	0.757	0.444	*0.007*	*0.112*	*0.079*	*0.132*	*0.118*	0.818	0.807	0.810	mk3						
ha5	0.394	*–0.089*		0.606	0.597	0.725	0.272	0.874	0.665	*–0.033*	0.261	0.206	0.297	0.260	0.963	0.944	0.945	mk4						
ha6	0.337	*0.003*	*–0.053*		*–0.042*	*–0.015*	*0.116*	0.263	*–0.062*	0.541	0.538	0.528	0.557	0.575	0.341	0.364	0.396	mk5						
ha7	0.353	*0.098*	*0.055*	*0.001*		*–0.018*	0.144	0.238	*–0.054*	0.544	0.542	0.534	0.561	0.573	0.312	0.339	0.375	mk6						
ha8	0.424	*–0.060*	*–0.096*	*0.011*	*0.105*		0.233	0.193	*–0.039*	0.671	0.655	0.648	0.675	0.695	0.326	0.353	0.379	mk7						
ha9	0.413	*0.090*	*0.061*	*0.042*	*0.033*	*0.098*		0.509	*0.127*	*0.196*	0.224	0.208	0.245	0.255	0.531	0.539	0.555	mk8						
ha10	0.549	0.532	0.483	0.420	*0.275*	0.535	0.489		0.246	0.858	0.838	0.836	0.850	0.863	0.490	0.505	0.506	mk9						
ha42	0.558	0.597	0.543	0.445	0.326	0.602	0.563	*–0.121*		0.591	0.577	0.567	0.599	0.626	0.354	0.374	0.394	mk10						
ha16	0.554	0.513	0.472	0.386	0.259	0.522	0.529	*0.008*	*–0.029*		0.239	0.184	0.353	0.378	0.989	0.956	0.954	mk11						
ha19	0.740	0.722	0.743	0.696	0.617	0.738	0.782	0.114	*0.090*	*0.082*		*–0.085*	*0.086*	*0.099*	0.933	0.910	0.907	ha1						
ha23	0.698	0.684	0.673	0.636	0.533	0.691	0.688	0.158	*0.129*	*0.008*	*0.045*		*0.056*	*0.056*	0.937	0.912	0.909	ha2						
ha22	0.839	0.850	0.847	0.815	0.818	0.856	0.864	0.730	0.725	0.741	0.846	0.806		*–0.032*	0.957	0.931	0.927	ha3						
ha27	0.792	0.764	0.816	0.778	0.782	0.786	0.868	0.573	0.626	0.654	0.792	0.713	0.879		0.971	0.945	0.944	ha8						
ha28	0.764	0.748	0.769	0.731	0.717	0.763	0.803	0.572	0.602	0.623	0.758	0.702	0.856	0.357		0.305	0.676	ha16						
ha29	0.713	0.685	0.710	0.663	0.559	0.703	0.754	*0.055*	*0.054*	*0.042*	*0.084*	0.128	0.839	0.714	0.680		0.612	ha18						
ha32	0.731	0.716	0.726	0.684	0.659	0.729	0.757	0.527	0.552	0.572	0.718	0.668	0.839	0.319	0.403	0.628		ha50						
ha39	0.751	0.778	0.735	0.685	0.683	0.776	0.736	0.657	0.607	0.652	0.741	0.749	0.600	0.766	0.762	0.734	0.747							
ha40	0.750	0.776	0.734	0.686	0.682	0.774	0.737	0.652	0.601	0.649	0.742	0.747	0.614	0.766	0.761	0.732	0.745	*–0.061*						
ha41	0.755	0.784	0.738	0.698	0.690	0.780	0.737	0.677	0.626	0.663	0.747	0.755	0.694	0.771	0.765	0.739	0.751	0.574	0.574					
ha20	0.649	0.676	0.617	0.575	0.562	0.669	0.611	0.569	0.535	0.536	0.639	0.645	0.281	0.657	0.661	0.625	0.648	0.500	0.508	0.614				
ha44	0.750	0.769	0.737	0.691	0.681	0.773	0.743	0.635	0.587	0.638	0.745	0.740	0.632	0.776	0.763	0.731	0.744	0.219	0.181	0.577	0.501			
ha46	0.839	0.852	0.842	0.811	0.813	0.856	0.854	0.746	0.737	0.750	0.843	0.814	0.521	0.871	0.853	0.837	0.839	0.638	0.650	0.709	0.377	0.666		
ha48	0.664	0.710	0.637	0.570	0.570	0.701	0.629	0.584	0.523	0.552	0.648	0.681	0.607	0.667	0.676	0.642	0.658	0.558	0.569	0.463	0.561	0.576	0.635	
	ha1	ha3	ha5	ha6	ha7	ha8	ha9	ha10	ha42	ha16	ha19	ha23	ha22	ha27	ha28	ha29	ha32	ha39	ha40	ha41	ha20	ha44	ha46	ha48

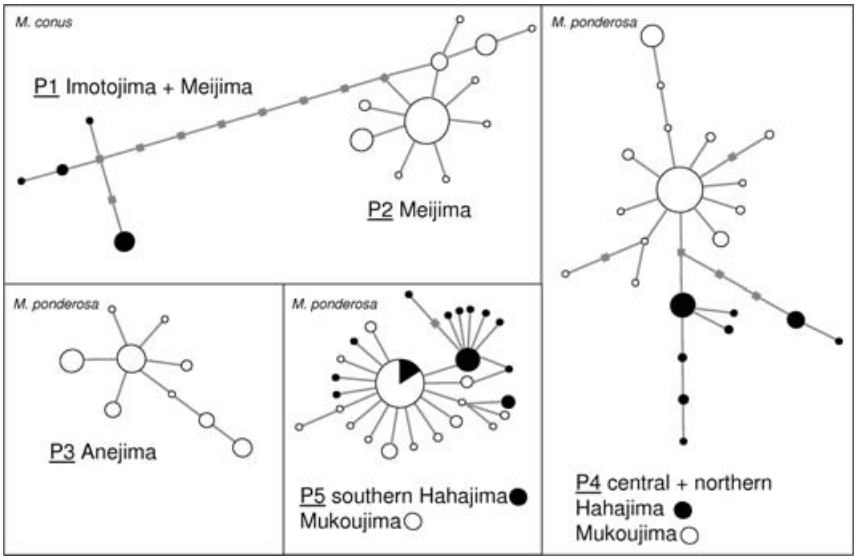

Fig. 6 The median-joining networks for *Mandarina ponderosa* and *Mandarina conus*. The size of the circle is proportional to sampling frequency. Unsampled or missing nodes/haplotypes are shown by a square.

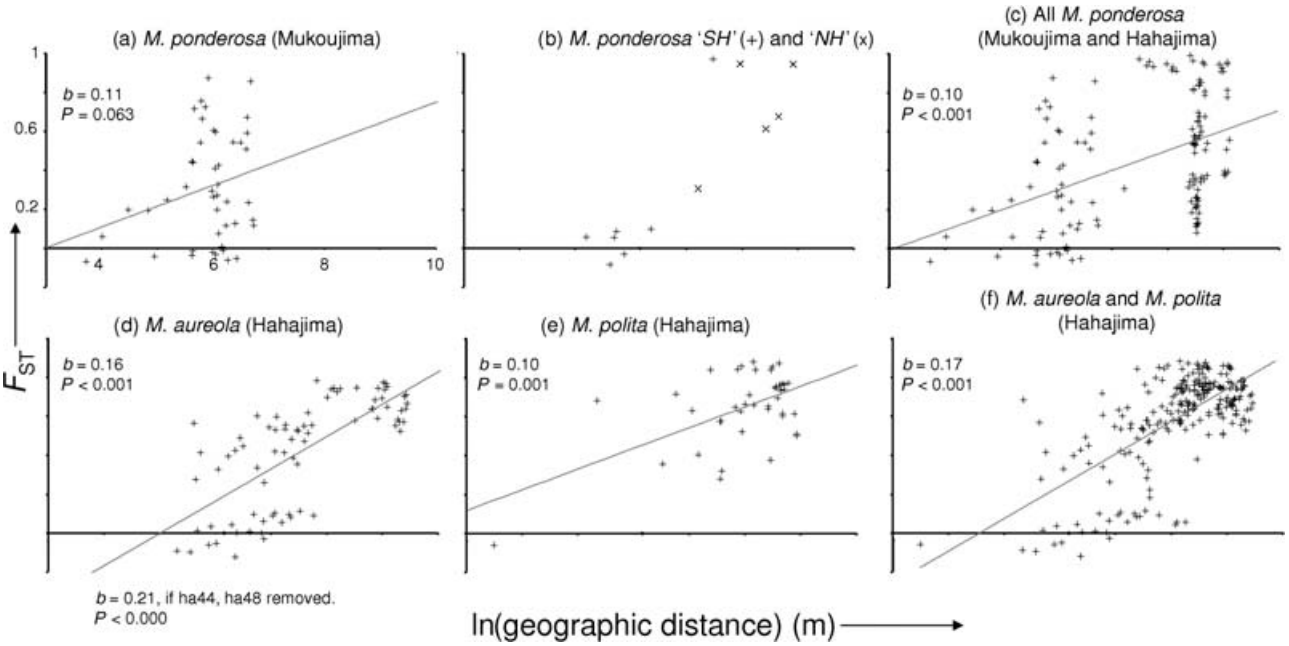

Fig. 7 Isolation-by-distance plots for the *Mandarina ponderosa* group (top line) and *Mandarina aureola*/*Mandarina polita* group (bottom line). All the axes have the same scale. As there are few data, the linear regression for (b) was not calculated.

The geographically distant populations (> ~3 km apart) that are genetically similar (F_{ST} ~0.1; Fig. 7c) are a result of pairwise comparisons between populations on Mukoujima and north Hahajima.

Similarly, in *M. aureola* and *M. polita*, there was a high level of structuring (Table 2; mean $F_{ST} = 0.59$; Fig. 7f), only part of which is explained by the differential distribution of haplotypes between the two species. Thus, there was a highly significant correlation of F_{ST} with geographic distance for *M. aureola* ($b = 0.16$, $P < 0.001$; Fig. 7d), even when the populations from the far north of Hahajima were excluded from the analysis ($b = 0.21$, $P < 0.001$; not shown). There was also a highly significant correlation of F_{ST} with geographic distance for *M. polita* ($b = 0.10$, $P = 0.001$; Fig. 7e).

We tested the hypothesis that the star-like patterns in *M. ponderosa* and *M. conus* have arisen because of a bottleneck followed by a recent demographic expansion. Unfortunately, a constructive analysis of the *M. aureola*/*M. polita* data could not be carried out, because of uncertainties regarding the unit of analysis. Fu's F_S was significantly less than zero in all analyses, except group P3 (Table 3). As the strong deviation from neutrality could be explained by a recent increase in population size, we examined the mismatch distribution of differences between haplotypes

Table 3 Sample size of each of the haplotype groups, basic statistics and results from mismatch distribution analysis of *Mandarina*

Haplotype group	Sample size	Number of haplotypes	Nuclectide diversity	P (F_S)	P (SSD)	P (H_{rag})	τ	θ_0	θ_1
P2	55	14	0.003	0.002**	0.75 NS	0.57 NS	1.19 (0.35–1.60)	0.00 (0.00–0.96)	778 (3–5383)
P3	37	9	0.004	0.097 NS	0.25 NS	0.43 NS	1.82 (0.77–2.95)	0.00 (0.00–1.03)	31 (3–6971)
P4	118	25	0.006	0.001**	0.79 NS	0.95 NS	3.70 (1.03–7.58)	0.00 (0.00–1.39)	4 (2–2774)
P5	121	29	0.003	0.000**	0.42 NS	0.40 NS	1.28 (0.55–1.57)	0.00 (0.00–0.79)	1654 (6–5562)

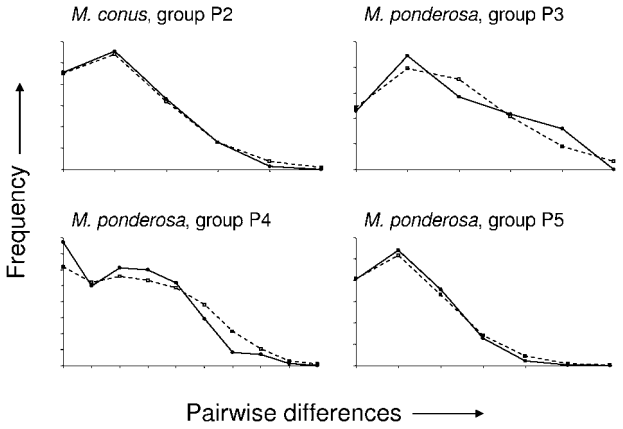

Fig. 8 Mismatch distributions (solid line) of four haplotype groups from *Mandarina* sp. with expected, simulated distributions (dotted lines) under the sudden demographic expansion model. None of the observed distributions differed significantly from the simulated distribution.

(Fig. 8). The distribution of the differences in all cases was similar to that obtained from simulations that assume a recent demographic expansion (Table 3). Both test statistics, Harpending's (1994) raggedness index and the sum of the squares deviation of Rogers (1995), were consistent with the explanation of a recent population bottleneck for all four cases. The genetic patterns could be as a result of either a demographic expansion in an isolated and un-subdivided founder population, or an expansion in a subdivided population. Unfortunately, our sample was inadequate to enable the use of new methods that are able to distinguish one from the other (Ray *et al*. 2003; Excoffier 2004; Hamilton *et al*. 2005).

Discussion

Refugia, demographic expansion and extreme population structure

The ground-living *Mandarina* snails of the Hahajima archipelago, especially those of the small islands and the low-lying southern part of Hahajima, have undergone recent population bottlenecks, surviving in multiple refugia. The bottlenecks were followed by a large and rapid population expansion out of the refugia. Present-day populations are highly structured, with little gene flow among them. As there is also only limited evidence of gene flow among the islands (except between Mukoujima and Hahajima), even though the islands must have been connected repeatedly by land bridges, the best interpretation of the data is that the climate was sometimes extreme in the Pleistocene, so that snails were restricted to refugia. Even when two populations on the same island are connected by suitable habitat, or two islands are connected by land bridges, snails must only rarely move between them.

As Ogasawara was not glaciated, the most likely explanation for the bottlenecks may be arid conditions, or reduced forest cover (Asahara 1999; Shen *et al*. 2005) that could have been initiated by decreased atmospheric CO_2 (Levis *et al*. 1999). Unfortunately, it is difficult to be more definitive, because the palaeoclimate of Ogasawara (as in other subtropical islands) is not well understood. Elsewhere, dry conditions may have forced wet-dependent species into refugia. In South America in particular, it has been hypothesized that dry conditions caused the rainforest to retreat to similar refugial 'islands', and that much of the Amazonian diversity arose during this period (Haffer 1969; Dutech *et al*. 2003; Flanagan *et al*. 2004). In Ogasawara, the only evidence is correlative, the best of which comes from subfossil material from the Chichijima archipelago (Chiba 1996a, 1998). There, it has been shown that there was a rapid and synchronized morphological change in several *Mandarina* species, between 18 000 and 25 000 radiocarbon years before the present (corresponding to slightly older true dates), roughly coincident with the last glacial maximum. At more or less the same time several other species became extinct. It is therefore reasonable to suppose that the bottlenecks in *Mandarina* from the Hahajima archipelago may have occurred around that time, or else in the periods leading up to earlier glacial maxima.

Some of the data interpretation also requires caution. In general, the results give rather strong evidence that most, if not all, populations of *Mandarina ponderosa* and *Mandarina conus* have recently expanded from a severe population bottleneck: lineages from different islands are reciprocally monophyletic, and have long internodes that are supported by bootstrapping (Fig. 3); there is a central, common haplotype surrounded by rarer haplotypes (Fig. 6);

and nucleotide differences are Poisson-distributed, almost perfectly matching the pattern expected assuming a demographic expansion after a bottleneck (Fig. 8). In contrast, the evidence is somewhat weaker that *Mandarina aureola*/*Mandarina polita* underwent population bottlenecks. None of the groups were supported by bootstrapping, making some of the analyses difficult, and only parts of the network show the characteristic signature of a bottleneck (Fig. 5).

More generally, the nature of the refugia and population size changes is open to question, whether disjunct and severe, or diffuse and moderate. There could have been a demographic expansion from an un-subdivided population (founder effect), because the refugia were small and isolated populations. Alternatively, the population density of snails might have decreased, then expanded again more or less equally across large areas. The genetic patterns could therefore be a consequence of a subsequent expansion from within a subdivided population. The genetic methods that we used are unable to distinguish which is correct.

The results may have a parallel in a recent paper on Hawaiian damselflies. Jordan *et al.* (2005) found two classes of population, one from the island of Hawaii that maintained genetic diversity through range shifts induced by climate change, and another from a smaller island that suffered a loss of genetic diversity (Jordan *et al.* 2005). It is therefore striking that for *M. aureola* and *M. polita*, the greatest evidence for a bottleneck and subsequent expansion is in snails from the southern and central southern part of Hahajima (groups A5, A6 and A7 in Fig. 5b). As the southern parts of Hahajima as well as Mukoujima, Anejima, Imotojima and Meijima are all low-lying compared with the mountainous northern part of Hahajima, snails in the latter could have remained at relatively high densities in the more moist parts of the mountain tops. In the future, it will be interesting to see if many other tropical-island species demonstrate similar kinds of patterns.

In the Introduction, it was implied that the populations of some tropical species may have increased in size as the area of land above sea level also increased in the period leading up to each glacial maximum of the Pleistocene. Arguably therefore, the population genetic signature of a demographic expansion following a population bottleneck in *Mandarina* could have been caused by an increased area of available habitat (land above sea level) during one of the last glaciations. However, several factors point towards a recent expansion; conversely, the data are not consistent with the bottlenecks being caused by a rising sea level. First, if snails were living on land bridges when the sea level was low, then we might have expected to find greater evidence for gene flow between islands. Second, if snails were living on land bridges and the sea flooded in, this would have not caused a bottleneck effect (or an imperceptible one) on the genetic structure of snails living on the main islands, unless the climate was unfavourable at the same time. Third, at the greatest extent when the sea level was lowest, the area of land above sea-level doubled or tripled, so the population could only have doubled or tripled. Compared with the rather large bottlenecks and subsequent expansions, which theory suggests are necessary to leave a genetic signature of a bottleneck (Slatkin *et al.* 1991; Rogers *et al.* 1992), as well as the empirical evidence in northern latitude species (Hewitt 2004), it is extremely doubtful that a two- to threefold expansion would leave the genetic signature that was detected in the small island and southern/central Hahajima populations.

Long-distance migration and genetic patches

We found some evidence for long distance gene flow. Presently, on Mukoujima *M. ponderosa* is the only ground-living species, with a shell form and genitalia most similar to *M. ponderosa* '*SH*'. As two divergent *M. ponderosa* mitochondrial lineages (P4 and P5) are present on Mukoujima, it is likely that *M. ponderosa* '*NH*' (or possibly '*SH*') recently colonized Mukoujima from Hahajima. This must have been followed by extensive introgression, since both lineages are now found across the whole island. However, long-distance migration does not seem to have had an effect on the other islands, even though they were all repeatedly connected by land bridges through the Pleistocene. The only exception is in *M. conus*, where a related lineage was found in a snail from Meijima and several in Imotojima. Long-distance migration could also be used to explain the disjunct distribution of *M. aureola* on Hahajima (there are two isolated populations in the north, at least some of which have a distinct haplotype group; site ha48). However, given the fragmented distribution, and the fact that *M. aureola* in the north of Hahajima are genetically quite distant from other *M. aureola* and arguably more similar to *M. polita*, then it is perhaps more likely that the current distribution of the former species is because it is a remnant of a once more widespread species that has become fragmented, and perhaps hybridized with *M. polita*.

The genetic structure of populations is also characterized by a marked patchiness. Some populations that are separated by 2 to 3 km are sometimes genetically very similar, whereas geographically close populations are sometimes genetically divergent. Similar effects have been observed in another snail, *Cepaea nemoralis* (Davison & Clarke, 2000). In the case of *Mandarina*, the patchiness could be attributed to a variety of factors, such as the effect of bottlenecks, low active dispersal, and founder effects occasioned by rare long distance migrants (leptokurtic dispersal) (Ibrahim *et al.* 1996).

Divergence in allopatry

The ground-living species of *Mandarina* on each outlying island of the Hahajima archipelago differ from each other in terms of both their genital and shell morphology (Chiba 2004), to the extent that some have been designated as different species (hence *M. ponderosa* and *M. conus*). The genetic divergence within and between various groups may be due to combined action of drift and selection during the Pleistocene and the Holocene, as the genetic evidence suggests that they were more or less isolated during both periods. The most interesting comparisons are between *M. aureola*/*M. polita*, the broad-leaved litter species, and *M. ponderosa* '*SH*' and '*NH*', the palm litter species, which are both found on Hahajima. The largely allopatric or parapatric distribution of species with the same ecotype contrasts with the largely sympatric pattern of species pairs that have distinct ecotypes (Chiba 1999b, 2004; Davison & Chiba 2006). *M. aureola* and *M. polita* are rarely found together because they have mostly southern or northern distributions on Hahajima. When they are found together, they may hybridize (e.g. site ha23; Fig. 1; Table 1) because they inhabit the same broad-leaved litter niche. Similarly, *M. ponderosa* '*SH*' and '*NH*' usually live in palm litter, but in largely separate southern and northern regions of Hahajima. Ultimately, these contrasting patterns could be the result of two different modes of divergence, ecological speciation and allopatric divergence in refugia (Schluter 2000; Coyne & Orr 2004). Thus, the initial divergence between ground-living forms could have come about because of divergent selection for the relatively dry litter of broad-leaved trees or the wet litter of palm trees. Subsequent divergence between *M. aureola* and *M. polita* or *M. ponderosa* '*SH*' and '*NH*' may have arisen due to drift in refugia. Alternative explanations are, however, possible, since theory suggests that phylogeographic breaks can sometimes form in a continuously distributed species (Irwin 2002).

Concluding remarks

Our long-term aim is to try to understand how *Mandarina* speciated, by combining genetic and ecological methods. Together with other studies on the relatively ancient history of *Mandarina* (Chiba 1999a; Davison & Chiba 2006), as well as a growing understanding of how the sister genus *Euhadra* has evolved on the mainland of Japan (Ueshima & Asami 2003; Davison *et al*. 2005), we hope that this study will contribute to a greater understanding, as well as more generally inform the debate on how species respond to climate change.

Acknowledgements

We thank the owners of the Pension Ryotoko for general assistance, as well as boat hire and use. *Mandarina* were collected under permit, from the Agency for Cultural Affairs (No. 4-519) and by the South Kanto branch, Ministry of the Environment (No. 709). AD was funded by a Royal Society/Japanese Society for the Promotion of Science 2+2 fellowship; the project was supported by a grant to SC, funded by the Japanese government under the 'Global environmental research fund F051'. We thank both B. Holland and M. Pfenninger for useful comments on the manuscript prior to submission, as well as detailed suggestions and criticisms from three anonymous referees, once of whom also helped improve the style and grammar.

References

Asahara Y (1999) Sr-87/Sr-86 variation in north Pacific sediments: a record of the Milankovitch cycle in the past 3 million years. *Earth and Planetary Science Letters*, **171**, 453–464.

Bandelt HJ, Forster P, Rohl A (1999) Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution*, **16**, 37–48.

Bell KL, Yeates DK, Moritz C, Monteith GB (2004) Molecular phylogeny and biogeography of the dung beetle genus Temnoplectron Westwood (Scarabaeidae: Scarabaeinae) from Australia's wet tropics. *Molecular Phylogenetics and Evolution*, **31**, 741–753.

Chiba S (1996a) A 40 000-year record of discontinuous evolution of island snails. *Paleobiology*, **22**, 177–188.

Chiba S (1996b) Ecological and morphological diversification within single species and character displacement in *Mandarina*, endemic land snails of the Bonin Islands. *Journal of Evolutionary Biology*, **9**, 277–291.

Chiba S (1998) Synchronized evolution in lineages of land snails in oceanic islands. *Paleobiology*, **24**, 99–108.

Chiba S (1999a) Accelerated evolution of land snails *Mandarina* in the oceanic Bonin Islands: Evidence from mitochondrial DNA sequences. *Evolution*, **53**, 460–471.

Chiba S (1999b) Character displacement, frequency-dependent selection, and divergence of shell colour in land snails *Mandarina* (Pulmonata). *Biological Journal of the Linnean Society*, **66**, 465–479.

Chiba S (2004) Ecological and morphological patterns in communities of land snails of the genus *Mandarina* from the Bonin Islands. *Journal of Evolutionary Biology*, **17**, 131–143.

Coyne JA, Orr HA (2004) *Speciation*. Sinauer Associates, Sunderland, Massachusetts.

Davison A (2002) Land snails as a model to understand the role of history and selection in the origins of biodiversity. *Population Ecology*, **44**, 129–136.

Davison A, Chiba S (2006) Labile ecotypes accompany rapid cladogenesis in an adaptive radiation of *Mandarina* (Bradybaenidae) land snails. *Biological Journal of the Linnean Society*, in press.

Davison A, Clarke B (2000) History or current selection? A molecular analysis of 'area effects' in the land snail *Cepaea nemoralis*. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **267**, 1399–1405.

Davison A, Chiba S, Barton NH, Clarke BC (2005) Speciation and gene flow between snails of opposite chirality. *Public Library of Science Biology*, **3**, e282.

Dutech C, Maggia L, Tardy C, Joly HI, Jarne P (2003) Tracking a genetic signal of extinction — recolonization events in a neotropical tree species: *Vouacapoua americana aublet* in French Guiana. *Evolution*, **57**, 2753–2764.

Emerson BC, Paradis E, Thebaud C (2001) Revealing the demographic histories of species using DNA sequences. *Trends in Ecology & Evolution*, **16**, 707–716.

Excoffier L (2004) Patterns of DNA sequence diversity and genetic structure after a range expansion: lessons from the infinite-island model. *Molecular Ecology*, **13**, 853–864.

Flanagan NS, Tobler A, Davison A *et al.* (2004) Historical demography of Mullerian mimicry in the neotropical *Heliconius* butterflies. *Proceedings of the National Academy of Sciences, USA*, **101**, 9704–9709.

Fu YX (1997) Statistical test of neutrality of mutations against population growth, hitch-hiking and background selection. *Genetics*, **147**, 915–925.

Gittenberger E, Piel WH, Groenenberg DSJ (2004) The Pleistocene glaciations and the evolutionary history of the polytypic snail species *Arianta arbustorum* (Gastropoda, Pulmonata, Helicidae). *Molecular Phylogenetics and Evolution*, **30**, 64–73.

Haffer J (1969) Speciation in Amazonian forest birds. *Science*, **165**, 131–137.

Hamilton G, Currat M, Ray N *et al.* (2005) Bayesian estimation of recent migration rates after a spatial expansion. *Genetics*, **170**, 409–417.

Harpending HC (1994) Signature of ancient population growth in a low-resolution mitochondrial DNA mismatch distribution. *Human Biology*, **66**, 591.

Hewitt GM (2004) Genetic consequences of climatic oscillations in the Quaternary. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, **359**, 183–195.

Hofreiter M, Serre D, Rohland N *et al.* (2004) Lack of phylogeography in European mammals before the last glaciation. *Proceedings of the National Academy of Sciences, USA*, **101**, 12963–12968.

Holland BS, Hadfield MG (2002) Islands within an island: phylogeography and conservation genetics of the endangered Hawaiian tree snail *Achatinella mustelina*. *Molecular Ecology*, **11**, 365–375.

Holland BS, Hadfield MG (2004) Origin and diversification of the endemic Hawaiian tree snails (Achatinellidae: Achatinellinae) based on molecular evidence. *Molecular Phylogenetics and Evolution*, **32**, 588–600.

Hugall A, Moritz C, Moussalli A, Stanisic J (2002) Reconciling paleodistribution models and comparative phylogeography in the wet tropics rainforest land snail *Gnarosophia bellendenkerensis* (Brazier 1875). *Proceedings of the National Academy of Sciences, USA*, **99**, 6112–6117.

Ibrahim KM, Nichols RA, Hewitt GM (1996) Spatial patterns of genetic variation generated by different forms of dispersal during range expansion. *Heredity*, **77**, 282.

Irwin DE (2002) Phylogeographic breaks without geographic barriers to gene flow. *Evolution*, **56**, 2383.

Jordan S, Simon C, Foote D, Englund RA (2005) Phylogeographic patterns of Hawaiian *Megalagrion* damselflies (Odonata: Coenagrionidae) correlate with Pleistocene island boundaries. *Molecular Ecology*, **14**, 3457.

Lessa EP, Cook JA, Patton JL (2003) Genetic footprints of demographic expansion in North America, but not Amazonia, during the late Quaternary. *Proceedings of the National Academy of Sciences, USA*, **100**, 10331–10334.

Levis S, Foley JA, Pollard D (1999) CO_2, climate, and vegetation feedbacks at the last glacial maximum. *Journal of Geophysical Research-Atmospheres*, **104**, 31191–31198.

Pearce JM (2006) Minding the gap: frequency of indels in mtDNA control region sequence data and influence on population genetic analyses. *Molecular Ecology*, **15**, 333–341.

Pfenninger M (2004) Comparative analysis of range sizes in Helicidae s.1. (Pulmonata, Gastropoda). *Evolutionary Ecology Research*, **6**, 359–376.

Pinceel J, Jordaens K, Pfenninger M, Backeljau T (2005) Rangewide phylogeography of a terrestrial slug in Europe: evidence for Alpine refugia and rapid colonization after the Pleistocene glaciations. *Molecular Ecology*, **14**, 1133–1150.

Ray N, Currat M, Excoffier L (2003) Intra-deme molecular diversity in spatially expanding populations. *Molecular Biology and Evolution*, **20**, 76–86.

Rogers AR (1995) Genetic evidence for a Pleistocene population explosion. *Evolution*, **49**, 608.

Rogers AR, Harpending H (1992) Population growth makes waves in the distribution of pairwise genetic differences. *Molecular Biology and Evolution*, **9**, 552–569.

Rundell RJ, Holland BS, Cowie RH (2004) Molecular phylogeny and biogeography of the endemic Hawaiian Succineidae (Gastropoda: Pulmonata). *Molecular Phylogenetics and Evolution*, **31**, 246–255.

Schluter D (2000) *The Ecology of Adaptive Radiation*. Oxford University Press, Oxford.

Schneider C, Moritz C (1999) Rainforest refugia and evolution in Australia's wet tropics. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **266**, 191–196.

Schneider S, Roessli D, Excoffier L (2000) *ARLEQUIN Version 2001: A Software for Population Genetic Data Analysis*. Genetics and Biometry Laboratory, Department of Anthropology, University of Geneva, Switzerland.

Shen J, Liu XQ, Wang SM, Matsumoto R (2005) Palaeoclimatic changes in the Qinghai Lake area during the last 18 000 years. *Quaternary International*, **136**, 131–140.

Slatkin M, Hudson RR (1991) Pairwise comparisons of mitochondrial DNA sequences in stable and exponentially growing populations. *Genetics*, **129**, 555–562.

Swofford DL (1999) *PAUP* 4.B10*. Sinauer Associates, Sunderland, Massachusetts.

Teshima H, Davison A, Kuwahara Y *et al.* (2003) The evolution of extreme shell shape variation in the land snail *Ainohelix editha*: a phylogeny and hybrid zone analysis. *Molecular Ecology*, **12**, 1869–1878.

Thomaz D, Guiller A, Clarke B (1996) Extreme divergence of mitochondrial DNA within species of pulmonate land snails. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **263**, 363–368.

Ueshima R, Asami T (2003) Single-gene speciation by left-right reversal — a land-snail species of polyphyletic origin results from chirality constraints on mating. *Nature*, **425**, 679–679.

This work was begun while Angus Davison was a research fellow at Tohoku University, Sendai, Japan, continued at Edinburgh University and completed at Nottingham University. It forms part of a wider collaboration with Satoshi Chiba on the land snails of Japan, with a particular focus on their speciation. Both Angus Davison and Satoshi Chiba have complementary interests in the ecology, evolution, population genetics and chirality of snails.
