

# Labile ecotypes accompany rapid cladogenesis in an adaptive radiation of *Mandarina* (Bradybaenidae) land snails

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The endemic land snail genus *Mandarina* of the oceanic Bonin Islands shows exceptionally diverse morphological and ecological traits. Previous studies have already provided evidence that speciation on different islands of the three main archipelagos was such that similar ecotypes evolved independently in different lineages and islands. Here we present data to show that the same species can have different ecotypes. As most of the characters involved are inherited, then variation between ecotypes must represent genetic differences between populations. We then show that the radiation on the Bonin Islands is derived from a single colonization event, and use a mitochondrial phylogeny to provide evidence for a burst of cladogenesis soon after colonization. As divergent selection has previously been implicated in causing differences between *Mandarina* species, and theory predicts that most of the speciation should have taken place early in their history, then the study adds to the evidence for an adaptive radiation by ecological speciation in *Mandarina*. However, while the diversity of ecotypes present at each site is dependent on the regime of natural selection and competition, geography still must have an important role. © 2006 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2006, 88, 269–282.

**ADDITIONAL KEYWORDS:** *Anolis* – character displacement – ecological speciation – ecomorph – Japan – Ogasawara – parallel evolution.

## INTRODUCTION

An adaptive radiation is the accumulation of both ecological and morphological diversity within a rapidly multiplying lineage, and is a process that can account for the origin of many of the world's species (Simpson, 1953; Schluter, 2000). An adaptive radiation can take place following the evolution of key morphological, physiological, or behavioural characters (Sanderson & Donoghue, 1994). Alternatively, the colonization of new and vacant habitats can release an organism from competition and predation, or simply provide new ecological niches, so that new lineages and species have the opportunity to rapidly evolve (Chiba, 1999a; Petren *et al.*, 1999; Schluter, 2000; Rüber, van Tassel

& Zardoya, 2003). Many of the classic studies of adaptive radiation concern this latter case, where speciation occurs following the colonization of isolated islands. The same studies have provided the best evidence for the role of ecology in speciation, in which reproductive isolation evolves from divergent selection on traits between environments (Schluter, 2000). In fact, perhaps the strongest evidence for the role of divergent natural selection in speciation comes from studies of parallel evolution, such as in sticklebacks (Rundle *et al.*, 2000), the adaptive radiation of *Anolis* lizards (Losos *et al.*, 1998, 2003), and cichlid fish (Allender *et al.*, 2003; Verheyen *et al.*, 2003).

Yet, despite the burgeoning interest, the connection between ecological speciation and adaptive radiation is far from established (Losos *et al.*, 2003). To date, much of the support for the theory of ecological speciation is sustained by only a few well studied cases, and

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the extent to which they can be generalized is uncertain (Schluter, 2000; Futuyma, 2003). There is also a potentially strong role for sexual selection in adaptive radiation, but there have been fewer case studies (Albert & Schluter, 2004; Knight & Turner, 2004; Seehausen & Schluter, 2004).

Nonetheless, great progress has been made in understanding adaptive radiations, especially as molecular phylogenies now enable performance of independent tests of ancestry (Sanderson & Donoghue, 1994; Schluter, 2000; Gillespie, 2004; Seehausen, 2004). An added benefit of using phylogenies is that they are able to provide a basis for investigating the tempo and mode of speciation. While theory predicts a burst of increasing lineage diversity (cladogenesis) and speciation during the early stages of an adaptive radiation, one problem has been to know the background rate against which to make comparisons. Another has been that genes rarely evolve in a clock-like manner. Recently, some of these difficulties have been overcome with the advent of methods that attempt to correct for between lineage rate variation, and assess the robustness of the conclusions (Kirkpatrick & Slatkin, 1993; Rambaut *et al.*, 1997; Sanderson, 1997; Pybus & Harvey, 2000; Pybus *et al.*, 2002).

Just as in *Anolis* lizards, cichlid fish and glacial lake sticklebacks, the endemic land snail genus *Mandarina* of the oceanic Bonin Islands of Japan shows exceptionally diverse morphological and ecological traits (Chiba, 1996, 2004), and there are several good reasons to believe that *Mandarina* has undergone an adaptive radiation (Chiba, 1999a; Chiba, 2004) (unlike some other snail species; see Gittenberger, 1991; Cameron *et al.*, 1996). Previous studies have already provided evidence that speciation on different islands of the three main archipelagos (Chichijima, Hahajima, and Nakodojima) was such that species with similar morphologies and life histories appeared repeatedly and independently in different lineages and islands at different times (Chiba, 1999a; Chiba, 1999b). As shell morphology is closely related to life history, it has been suggested that arboreal, semiarboreal and ground-living forms came to resemble snails on different islands from different lineages (Fig. 1; Chiba, 1999a).

In this paper, we further test support for an adaptive radiation in *Mandarina* by establishing the monophyly of the radiation then using a phylogeny to investigate rates of cladogenesis. If there was an adaptive radiation, then we expect that most lineage diversification should have taken place early in the history of the genus. We then compare the phylogeny with the ecology of the present day species. In particular, we aim to understand the role of labile ecotypes in developing community structure and speciation. If single

species have variable ecotypes now, then it is consistent that selection for different ecological niches caused speciation in the past. Together the results should add to the weight of evidence that divergent natural selection is the ultimate cause of reproductive isolation in *Mandarina* snails.

## MATERIAL AND METHODS

### SAMPLES

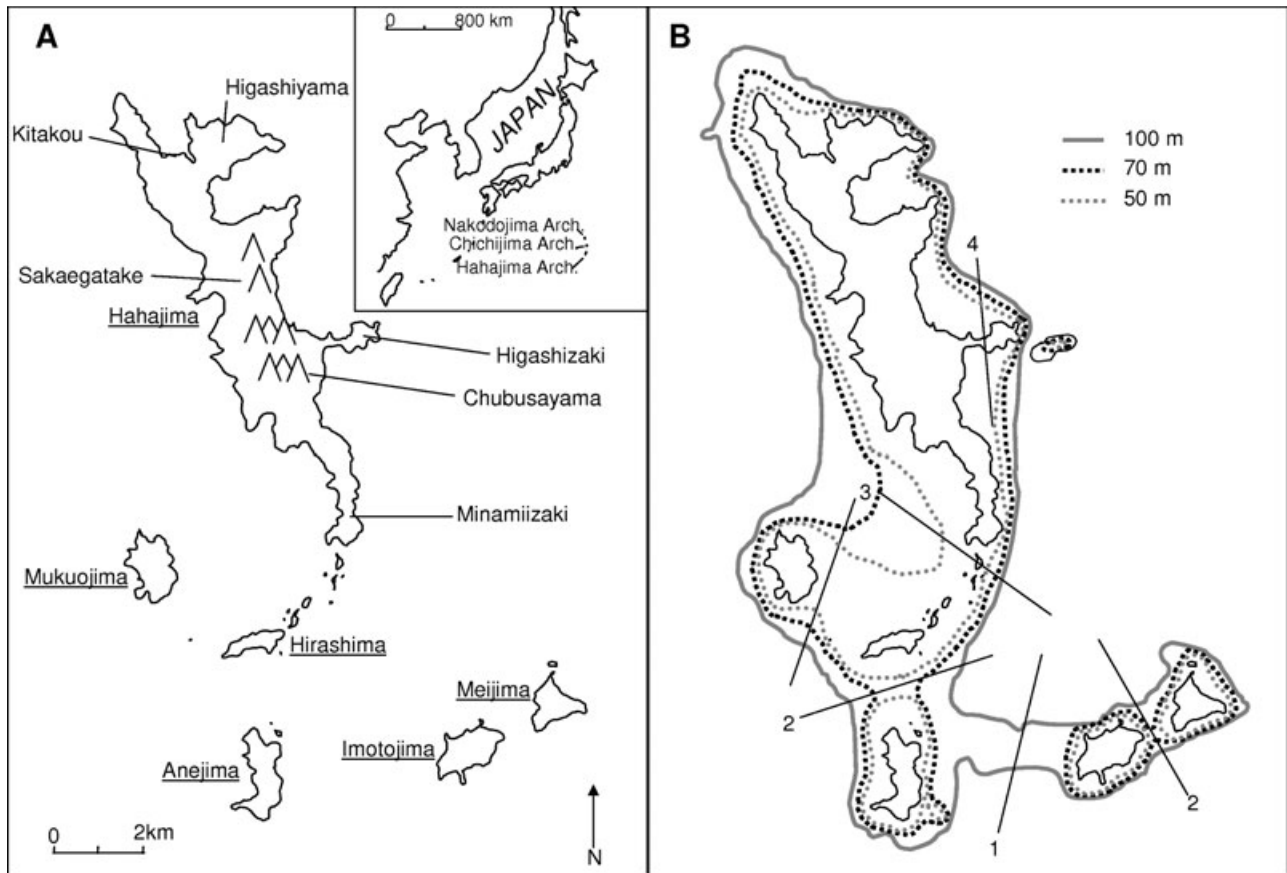
The Bonin (Ogasawara) Islands (inset to Fig. 2A) were formed during the Palaeogene, but their uplift started in the Pleistocene, and they appeared above sea level before the middle Pleistocene (Kaizuka, 1977; Imaizumi & Tamura, 1984). The three separate archipelagos of Nakodojima, Chichijima and Hahajima arose independently and have never been connected to each other or to the mainland by a land bridge. The progenitors of the endemic organisms in the islands must have reached them from elsewhere during the early to middle Pleistocene (*c.* 1.8–0.9 Mya).

Note: to avoid confusing islands and island groups with the same name, the latter are consistently referred to as archipelagos (e.g. the island of Hahajima compared with the Hahajima archipelago).

Fifteen or more *Mandarina* (Bradybaenidae) species are recorded from the Bonin Islands, each with a unique genital morphology, which is used for identification. Unlike more commonly studied snails such as *Cepaea* (Davison, 2002), shell characters of *Mandarina* are relatively invariant within populations, but tend to vary between populations. Three main ecotypes have been described previously: (1) terrestrial (ground-living) palm snails; (2) terrestrial, broad-leaved litter snails; and (3) arboreal snails (Chiba, 1996, 2004). Terrestrial palm snails tend to have tall-spined, thick shells that are dark brown or black (Fig. 1, middle left panel). Terrestrial broad-leaved litter snails tend to be somewhat flatter and have thinner shells, with a larger aperture and variable banding pattern against a pale background colour (Fig. 1, middle right). Finally, arboreal snails have flat, pale and delicate shells and rarely have bands (Fig. 1, top panel). This latter group can be subdivided again into strict arboreal and semiarboreal species (Fig. 1; Chiba, 1996, 1999a, 2004). Sometimes a single species covers a range of niches at the same site (e.g. palm and broad-leaved litter), so they are correspondingly more variable (Fig. 1, bottom panel). Importantly, both shell characters and body colour are inherited (A. Davison & S. Chiba, unpubl. data), though the exact dominance relations have not been worked out, unlike for other species of snail (Cain & Sheppard, 1957; Murray & Clarke, 1966). The inheritance of habitat use preference is more uncertain, but it is possible that it is a pleiotropic effect of selection



**Figure 1.** The three main *Mandarina* ecotypes. Arboreal snails (top panel) are generally pale with low-spired, thin shells and usually lack bands. Terrestrial palm snails (middle left panel) are dark with a high spire and thick shells. Bands are fused together. Terrestrial broad-leaved litter snails (middle right panel) are somewhat intermediate and polymorphic for banding. In some populations, a single species covers more than one niche, so shell characters and body colour are polymorphic, e.g. *M. ponderosa* from Mukoujima (bottom panel). The shells of these species are around 1–2 cm.



**Figure 2.** A, the Hahajima archipelago within (inset) the Bonin Islands. B, Pleistocene sea level changes, showing extent of land mass with sea-level 50 m, 70 m, and 100 m below present. Meijima and Imotojima were isolated first (1), followed by Anejima (2), then Mukuojima and Hirashima (3). Higashizaki (4) is a 'habitat' island. *Mandarina* on Hirashima are probably extinct.

for shell form, e.g. dark shelled snails are predated less and overheat less in cool, dark palm litter (compared with open, broad-leaved litter), which may lead to linkage between shell type and habitat preference.

We investigated habitat use and shell morphology for all the extant species from the three archipelagos, including an especially detailed study of the Hahajima archipelago. Similar methods were used compared with previous studies (Chiba, 1996, 2004), so less detail is given here. Briefly, we recorded the resting site and substrate of adult and juvenile snails, in particular noting whether snails were below the litter, on the surface, on the trunk, or in the leaves of the tree. The islands were visited on five separate occasions, covering 65 field work days, in May 2001, December 2001, April 2002, November 2002, and June 2003. We made the first detailed survey of Mukuojima, Anejima, parts of Meijima, and much of Northern Hahajima, including also Higashizaki, a peninsula off Hahajima which is a habitat island.

For the morphological analysis, the same methods were used as in previous studies. Adult shell measurements were taken in the laboratory (see Chiba, 2004: fig. 2 for exact details) and the differences examined quantitatively.

Several of the species descriptions are in need of revision. There is a widespread unnamed species recorded from South Hahajima, Higashizaki, Mukuojima, Anejima, Imotojima, and Meijima that we refer to as *Mandarina* sp. A (previously designated *M. hahajimana*). As different populations have some genetal differences (S. Chiba & A. Davison, unpubl. data), then it is possible that *Mandarina* sp. A is actually two species, one limited to Hahajima and Higashizaki and the other to Mukuojima, Anejima, Imotojima and Meijima. Two forms of *M. ponderosa* are present in northern and southern Hahajima, which may be a parapatric species pair, as they have different genetal and shell morphology. We refer to them throughout as *M. ponderosa* 'SH' or 'NH'. *M. ponderosa* is also

present on Anejima and Mukoujima, but the relationship to *M. ponderosa* from Hahajima is uncertain.

#### DNA EXTRACTION AND PCR AMPLIFICATION

Genomic DNA was isolated, amplified and sequenced using methods described in Teshima *et al.* (2003). Primers for polymerase chain reaction (PCR) amplification of an approximately 900 base pair (bp) 16S ribosomal RNA (16S rRNA) and 700 bp 12S rRNA mitochondrial DNA (mtDNA) gene fragment are described in Chiba (1999a). An approximately 900 bp region of the nuclear rRNA, including the whole internal transcribed spacer 2 (ITS-2), was amplified by PCR using the primers of Wade & Mordan (2000). The 16S rRNA fragment was sequenced for all the samples, then the main divergent lineages identified, and the 12S rRNA gene fragment was sequenced. From the Chichijima archipelago, we sampled *M. mandarina* (Chichijima,  $N = 8$ ; Anijima,  $N = 11$ ), *M. chichijimana* (Chichijima,  $N = 22$ ), *M. anijimana* (Anijima,  $N = 10$ ), *M. hirasei* (South-west Chichijima,  $N = 9$ ; North Chichijima,  $N = 9$ ), and *M. suenoae* (Anijima,  $N = 6$ ). From the Nakodojima archipelago, we sampled *M. trifasciata* (Mukojima,  $N = 5$ ). On the Hahajima archipelago, we took multiple samples from across the main island, as well as each of the smaller islands of Mukuojima, Anejima, Imotojima and Meijima (Table 1; Fig. 2). *Euhadra*, the probable mainland sister taxa to *Mandarina*, and *Nesiohelix bipyramidalis* (outgroup) were also collected from Japan.

#### ANALYSES

Sequences were aligned using the CLUSTALX software, and then checked manually (Thompson *et al.*, 1997). All insertion and deletion sites (indels), as well as several regions that were difficult to align, were then removed before phylogenetic analysis. Phylogenetic relationships between lineages were analysed using maximum likelihood (PHYML v2.4, Guindon & Gascuel, 2003), neighbour-joining (PAUP\*4.0b10, Swofford, 2002) and Bayesian inference methods (MrBayes v3, Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003). Using PHYML, the HKY model of DNA substitution was used to estimate the maximum likelihood phylogeny, beginning with an initial neighbour-joining tree. At the same time, the transition/transversion ratio, proportion of invariant sites (I) and gamma ( $\Gamma$ ) distribution shape parameter were also estimated. Similarly, for the neighbour-joining phylogeny, multiple hits were corrected using the GTR + I + G model in PAUP. The rate matrix, base frequencies and shape parameter of the gamma distribution were estimated using likelihood, by iteration from an initial neighbour-joining tree. Parameters esti-

mated from the initial tree were used to make a new neighbour-joining tree. The parameters were then re-estimated, and the process was repeated until there was no further improvement in likelihood. Support for the resulting maximum likelihood and neighbour-joining trees was tested by bootstrap re-sampling, using routines within the same packages to produce majority rule consensus trees (values above 70% only shown in figures). Finally, a Bayesian inference of phylogeny was performed with MrBayes by Markov chain Monte Carlo sampling for a million generations (four chains), using the GTR + I +  $\Gamma$  model. Prior to estimating support for the topology (consensus above 95% shown in figures), we checked that chains had converged and that the log likelihood was stationary.

A likelihood ratio test was performed in PAUP using the neighbour-joining phylogeny, with and without a molecular clock constraint. To test lineage diversification rates, the maximum likelihood trees were first converted to ultrametric trees using the nonparametric rate smoothing method of Sanderson (Sanderson, 1997), with rate differences weighted by the mean at all nodes, as implemented in TreeEdit v1 (Rambaut & Charleston, 2001). Then, a semilogarithmic lineage through time plot was generated from the ultrametric trees using GENIE v3.0 (Pybus & Rambaut, 2002). The expectation of lineage through time plots is that convex plots indicate a burst of lineage production early in the history of the genus (Nee *et al.*, 1994, 1995; Rüber *et al.*, 2003). To roughly estimate a minimum and maximum divergence, we assumed that base of the phylogeny derives from a single colonization event 1.8 Mya, though it is possible the colonization was more recent.

To test the robustness of the inferences for lineage diversification, we used the constant rate test in GENIE (Pybus & Harvey, 2000; Pybus & Rambaut, 2002). One limitation to this method is that it assumes equal diversification between lineages, so is sensitive to both incomplete taxon sampling and the effects of both topology and branch length in the phylogeny. Therefore, the  $B1$  index of Shao & Sokal (1990) and the Colless Index  $I_C$  (Colless, 1982) were used to assess phylogeny imbalance, using the simulations of Kirkpatrick & Slatkin (1993) to assess confidence limits. Finally, the relative cladogenesis statistic was calculated using End-Epi, to characterize the lineages that show greater than expected rates of lineage diversification (Nee *et al.*, 1994; Nee *et al.*, 1995; Rambaut *et al.*, 1997). All of the above calculations were carried out on the *Mandarina* mtDNA sequences, using the rate-smoothed maximum likelihood tree.

As the base of the *Mandarina* phylogeny was poorly resolved, and rate smoothing methods assume the correct topology, then we also tested the robustness of the conclusions by assessing the effect of

**Table 1.** Habitat use and shell characters in *Mandarina* from the Hahajima Islands. Some populations have wide-ranging habitat use, so are shown in two rows

Island	Region	Species	Sample size (mtDNA)	Adult snails		Juveniles		Shell shape	Shell colour‡	Body colour‡	
				Habitat*	Niche†	Habitat*	Habitat*‡				
Hahajima	South	<i>M. ponderosa</i> 'SH'	12	T	P	T	T	Tall	Dark	Dark	
		<i>M. aureola</i>	17	T	B	T	T + A	Flat	Light	Light	
	Central	<i>M. sp. A</i>	16	A	P	A	A	Flat	Light	Light	
		<i>M. ponderosa</i> 'NH'	11	T	P	T	T	Tall	Dark	Dark	
		<i>M. polita</i>	15	T	B	T	T + A	Flat	Light	Light	
		<i>M. sp. A</i>	17	A	P	A	A	Flat	Light	Light	
		<i>M. exoptata</i>	10	A	P	A	A	Flat	Light	Light	
		<i>M. hahajimana</i>	10	A	P + B	A	A	Flat	Light	Light	
	North	Higashizaki	<i>M. polita</i>	11	T	P	T	T	Polymorphic	Dark	Grey
			<i>M. sp. A</i>	15	T	P + B	A	A	Polymorphic	Polymorphic	Polymorphic
Kitakou Higashiyama		<i>M. polita</i>	12	T	P	T	T	Polymorphic	Dark	Polymorphic	
		<i>M. polita</i>	22	T	B	T	T + A	Polymorphic	Light	Light	
		<i>M. hahajimana</i> §	13	T	B	A	A	Polymorphic	Polymorphic	Light	
Mukoujima	<i>M. ponderosa</i>	17	T	P + B	T	T + A	Polymorphic	Polymorphic	Polymorphic		
	<i>M. sp. A</i>	10	A	P	A	A	Flat	Light	Light		
Anejima	<i>M. ponderosa</i>	13	T	P + B	T	T + A	Polymorphic	Polymorphic	Polymorphic		
	<i>M. sp. A</i> ¶	17	T	P + B	A	A	Flat	Light	Light		
Imotojima	<i>M. conus</i>	9	T	P	T	T	Tall	Dark	Dark		
	<i>M. sp. A</i>	4	T	P	T	T + A	Polymorphic	Polymorphic	Light		
Meijima	<i>M. conus</i>	12	T	P	T	T	Tall	Dark	Dark		
	<i>M. sp. A</i>	12	T	P	T	T + A	Polymorphic	Polymorphic	Light		

\*T, terrestrial (leaf litter); A, arboreal

†B, broad-leaved, deciduous, such as *Pouteria obovata* and *Wikstroemia pseudoretusa*; P, mostly palm *Livistonia chinensis* or pandanus *Pandanus boninensis*

‡Dark = black to dark brown, light = white to yellow

§Wide-ranging habitat use: adults live either on the ground in broad-leaved litter or else in palm trees

¶Wide-ranging habitat use: adults live either on the ground and in trees

changed topology and branch length. Twenty resampled bootstrap data matrices were generated using the SEQBOOT program in PHYLIP v3.62. Each data matrix was imported into PHYML, and the maximum likelihood tree calculated using the same parameters as above. Then, an ultrametric tree was created and lineage through time plots calculated as before.

## RESULTS

### DIVERSE *MANDARINA* ECOTYPES

One of the remarkable aspects of the *Mandarina* radiation is that species with the same ecotype are found on different islands, and that species with different ecotypes are found in broadly the same place but have different niches (Chiba, 1996, 2004). We found multiple terrestrial and arboreal snail species present at many sites (Table 1). On parts of the main island of Hahajima such as Chibusayama (Fig. 2) two arboreal and two terrestrial species are present (Table 1), and at some sites this may rise to three arboreal species, including *M. hahajimana*, and three terrestrial species, including *M. aureola*. There is a gradient in species richness and habitat occupancy from the small, relatively isolated islands of Meijima and Imotojima (two terrestrial species), to Anejima and Mukoujima (one or two terrestrial species, depending upon location; one arboreal species) and finally Hahajima (two terrestrial species; one or two arboreal species). Similar patterns are present on the Chichijima and Nakodojima archipelagos, though these archipelagos have not been surveyed so thoroughly (Chiba, 1996; Chiba, 2004).

We also found that the same species can have different ecotypes in different locations, while at the same time clustering together by mtDNA sequence. Thus, the monophyletic mtDNA lineages that characterize *M. sp. A* are found on a variety of islands of the Hahajima archipelago (Figs 3, 4). Lineages in this group have long branches (Fig. 3) and show little if any variation in their genitalia, yet are characterized by a massive diversity in their ecotype between different islands of the Hahajima archipelago (Table 1; Fig. 5). This species can have either the palm ecotype (Meijima, Imotojima), be a ground living generalist (Higashizaki), or be found both on the ground and in trees (Anejima), or else is arboreal (Hahajima, Mukoujima) (Fig. 5). Similarly, the terrestrial '*M. ponderosa*' group (Table 1; Fig. 5) are sometimes ground living generalists (Mukoujima, Anejima), or else palm specialists (Hahajima, Meijima, Imotojima). *M. exoptata*, which may have evolved from an ancestor of *M. ponderosa* (Fig. 6), has the most extreme form of any snails (Fig. 1) and is strictly arboreal.

### PHYLOGENY OF *MANDARINA*

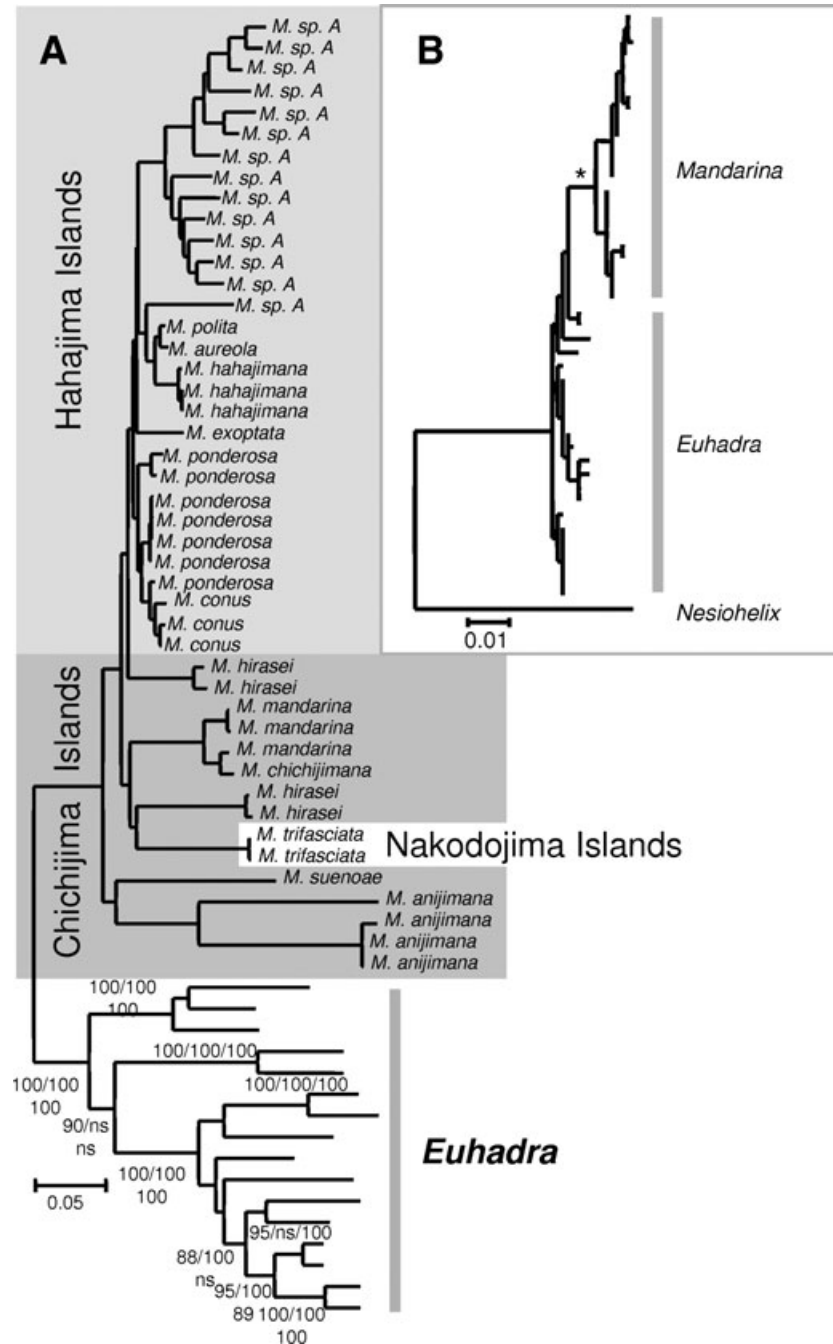
The ITS-2 phylogeny (Fig. 3B) confirms the earlier suggestion that *Mandarina* are monophyletic (Chiba, 1999a), having arisen from a single progenitor up to 1.8 Mya (GenBank AY251820–AY251837 and AY829576–AY829597), but the degree of variation within *Mandarina* is insufficient to resolve the relationship between species.

The two mtDNA rRNA fragments are highly variable, as in other snails (Thomaz *et al.*, 1996) (GenBank AY829598–AY829639 and AY829533–AY829575). The final alignment was 908 bp (16S rRNA) and 700 bp (12S rRNA), of which 678 bp and 496 bp were used in the phylogenies, respectively. As expected, *Mandarina* and *Euhadra* group separately in the combined phylogeny, irrespective of the method used (Fig. 3; supported by 100% of Bayesian consensus trees and 100% of neighbour-joining/maximum likelihood bootstraps).

In contrast to the structure shown between *Euhadra* lineages (Fig. 3A), the relationship between *Mandarina* lineages is poorly resolved (Figs 3A, 4). The phylogeny is characterized by large groups of well supported 'clouds' of sequences, with an uncertain relationship between them (Figs 3, 4). The likelihood (*L*) ratio test on unconstrained ( $-\ln L = 12\,744.1$ ) and clock-enforced trees ( $-\ln L = 12\,541.5$ ) confirmed rate variation between lineages ( $X^2 = 405.2$ ; d.f. = 58;  $P < 0.01$ ), with similar highly significant results obtained using subtrees from the phylogeny (e.g. the *Mandarina* group alone). In the absence of rate constancy, ultrametric trees were used for subsequent analyses, rooted on *Euhadra*. The monophyletic *Mandarina* group of Figure 3 is shown in Figure 4 as an ultrametric tree, including bootstrap support for all three methods.

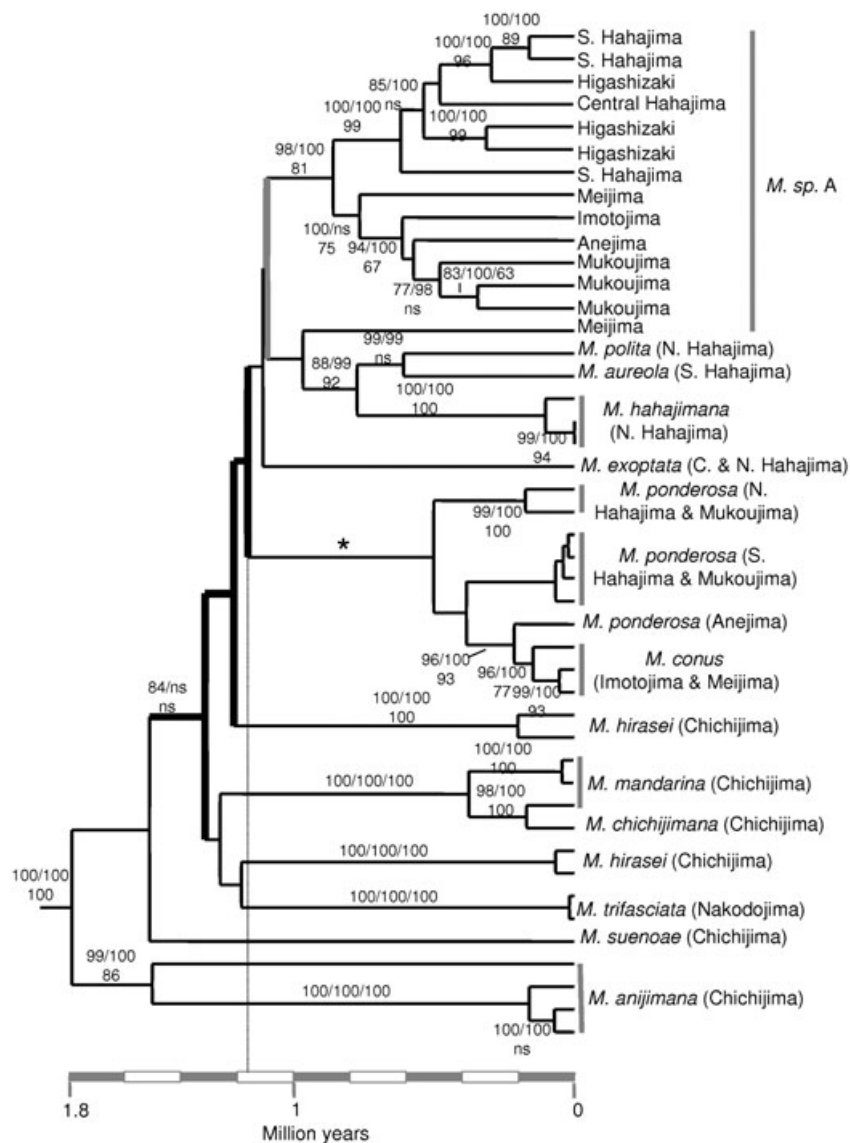
The lineage through time plot is convex for *Mandarina* (Fig. 6), which may be due to an early spate of lineage diversification, and results from the constant rates test are consistent with this ( $CR \gamma = -2.64$ ; significant). However, there was some evidence for phylogeny imbalance, with values approaching significance ( $N = 45$ ,  $B1 = 22.75$ , not significant;  $I_c = 0.22$ , significant, but marginal), and the relative cladogenesis statistic showing some variation across lineages (Fig. 4). While it is possible that the convex curve was due to incomplete lineage sampling, or unequal diversification across lineages, the interpretation is relatively robust to inaccuracies in the phylogeny, because all 20 SEQBOOT phylogenies showed the same convex pattern (not shown).

The exact history of the Bonin Islands is uncertain. While the three island groups arose independently, the order in which they arose is not known. The most basal lineages in the phylogeny are from Chichijima and Nakodojima (Fig. 3), which may mean that the



**Figure 3.** A, combined 16S and 12S rRNA maximum likelihood phylogeny showing the relationship between *Mandarina* from the Bonin Islands and *Euhadra*, the probable sister taxa on mainland Japan. Support values (*Euhadra* only; see Fig. 4 for *Mandarina*) are for maximum likelihood, Bayesian and neighbour-joining methods. In general, the topology of the *Euhadra* clade is well supported compared with *Mandarina* (see Fig. 4). B, ITS-2 maximum likelihood phylogeny showing the relationship between *Mandarina* and *Euhadra*, rooted on *Nesiohelix bipyramidalis*. The asterisk indicates support values of 100, 100, and 99 using maximum likelihood, Bayesian and neighbour-joining methods.



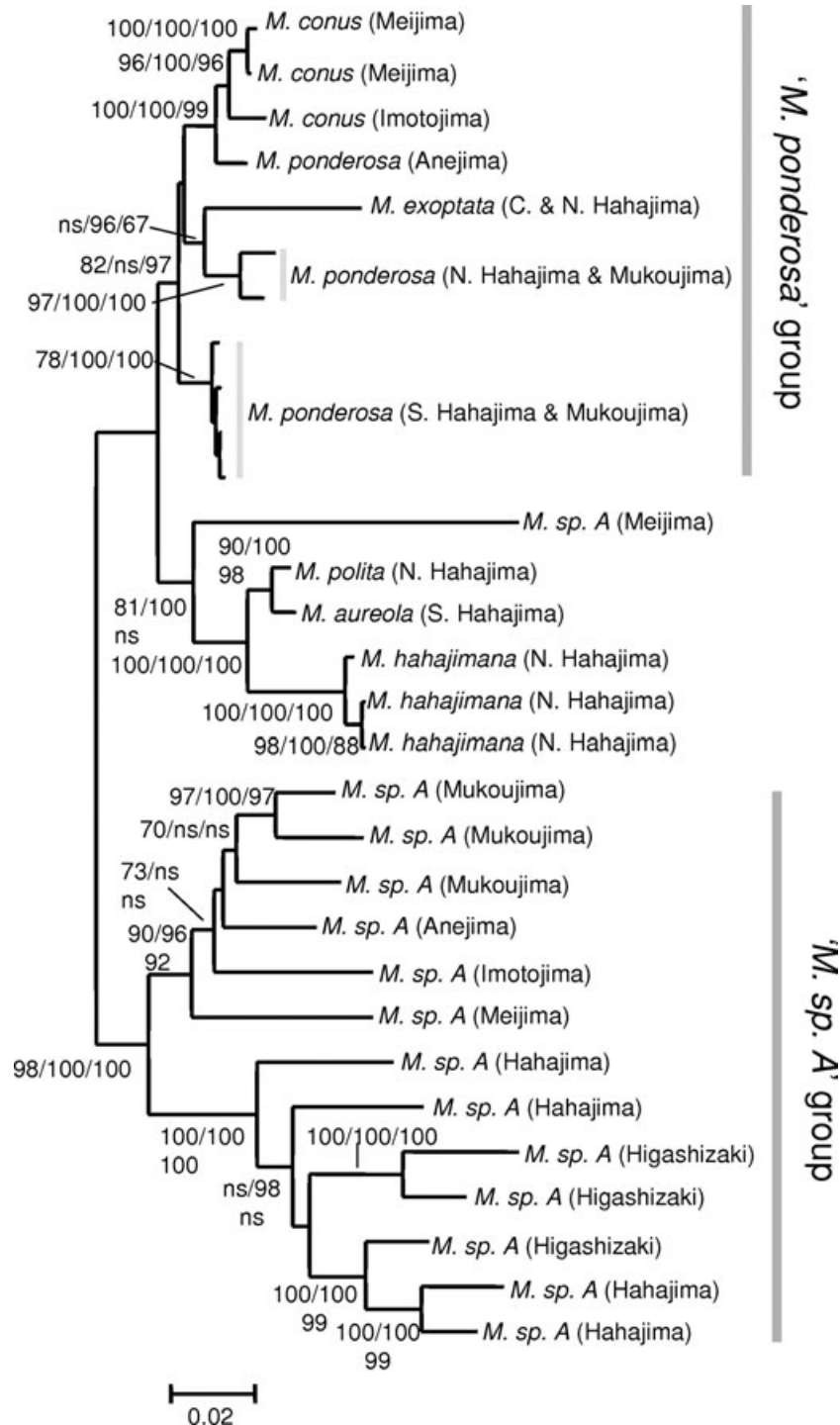


**Figure 4.** Non-parametric rate-smoothed maximum likelihood phylogeny. As in Fig. 3, support values are for maximum likelihood, Bayesian and neighbour-joining methods. The branch indicated by the asterisk is not supported in this phylogeny, but is strongly supported with long branch taxa excluded (Fig. 5). The scale bar is calibrated on the first emergence of the islands, approximately 1.8 Mya. Groups with higher than expected rates of cladogenesis are indicated by bars (black,  $P < 0.01$ ; grey,  $P < 0.05$ ).

Hahajima archipelago was the last to be colonized. If the initial colonization was  $\sim 1.8$  Mya, then the Hahajima archipelago could have been colonized  $\sim 1.1$  Mya (Fig. 4). Interestingly, this timepoint corresponds with the peak of lineage diversification (Fig. 6). However, caution is required because, while all three methods recovered lineages from the Hahajima archipelago as monophyletic, the topology was not supported by bootstrapping.

We investigated the relationships between the Hahajima archipelago lineages in depth (Fig. 5), but with caution because it is not proven that they form a

monophyletic group. The advantage is that, by excluding lineages with long branches (e.g. *M. anijimana*), some of the effects of long-branch attraction are lessened, leading to more robust phylogenies. In fact, some of the inconsistencies between our phylogenies and those of an earlier study (Chiba, 1999a) are quite clearly due to the discovery of rare lineages with long branches. Using Hahajima archipelago lineages only, the lineage through time plots show the same pattern as before (inset to Fig. 6), indicating an early burst of lineage diversification, with a significant constant rate value ( $CR \gamma = -3.52$ ). Importantly, there was no

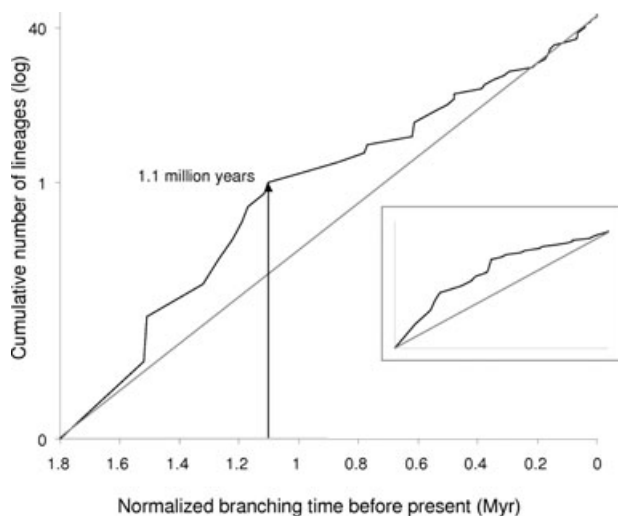


**Figure 5.** Combined 16S and 12S rRNA maximum likelihood phylogeny using *Mandarina* from the Hahajima archipelago. Support values are for maximum likelihood, Bayesian and neighbour-joining methods.

evidence for phylogeny imbalance or different rates of cladogenesis, so it is more certain that the convex lineage through time plot (Fig. 3B) is due to a burst of lineage diversification, rather than an artefact.

## DISCUSSION

The combined evidence from the phylogenies and ecological survey suggests that *Mandarina* have under-



**Figure 6.** Semi-logarithmic lineage through time plot for *Mandarina* from the Bonin Islands. The convex shape indicates a burst of cladogenesis early in their history, peaking around 1.1 Mya. Inset: the same plot using lineages from the Hahajima archipelago alone.

gone an adaptive radiation on the Bonin Islands, beginning from a single founding event. While *Mandarina* ecotypes are relatively invariant within populations (Chiba, 1996, 1999b), the same species at different locations can vary considerably in their shell characters, body colour and habitat use (Table 1). As most of the characters involved are inherited, then variation must represent genetic differences between populations. There is also good evidence for a burst of rapid cladogenesis in *Mandarina* from the Hahajima archipelago, early on in their history (Figs 5, 6), with somewhat weaker evidence for the Bonin Islands as a whole (Figs 4, 6). Therefore, as divergent selection has previously been implicated in causing differences between species in the same location (character displacement) (Chiba, 1996, 1999a, 2004), and theory predicts that most of the speciation should have taken place early in their history (Schluter, 2000), then the study adds to weight of evidence for an adaptive radiation by ecological speciation in *Mandarina*.

In an adaptive radiation that has come about by ecological speciation, the expectation (by definition) is that closely related species should have quite different ecologies (Schluter, 2000). A contrasting approach as to how biological diversity arises is provided by community structure theory, which predicts low niche differentiation between species over evolutionary time scales, because rates of adaptation in environments outside of the fundamental niche might be slower than the extinction process (Peterson *et al.*, 1999; Ackerly, 2004). Recently, Losos *et al.* (2003) have bridged the gap between community evolution and adaptive radi-

ation. They suggest that most studies have reported low niche differentiation between species because they compared closely related taxa in allopatry. In a sympatric *Anolis* lizard community, the potentially stabilizing forces that inhibit niche shifts are opposed by natural selection, so that closely related species are no more ecologically similar than expected by chance (Losos *et al.*, 2003). In *Mandarina*, the situation may be similar to that of *Anolis*. Unfortunately, since multiple lineages were discovered within species, sometimes in quite different parts of the phylogeny (Fig. 3), then it is not clear how to test statistically the relationship between phylogeny and ecology in *Mandarina*. A more general point is that both our study and many other studies lack corroborating evidence from nuclear gene sequences (Rolan-Alvarez *et al.*, 2004 is a notable exception).

As has been shown in other model taxa for the study of speciation, in *Mandarina* the particular ecotype of each species in different populations is presumably due to a combination of the history of the lineage combined with the particular environmental circumstances and competition from other snails. There is clear evidence for character release in *Mandarina* (MacArthur & Wilson, 1967; Roughgarden, 1972), because populations on small islands are more often polymorphic (Table 1). Similar results have been observed for the sister taxa to *Mandarina* (*Euhadra*), that has recently (< 100 000 years) colonized the Japanese Izu islands (Hayashi & Chiba, 2004). Together, these results have a parallel with recent studies (Losos *et al.*, 2003; Rüber *et al.*, 2003; Gillespie, 2004), although it is unusual to record such a high degree of ecotype variation within a species. In the study of *Anolis*, closely related species are no more ecologically similar than expected by chance and some distantly related species are ecologically similar (Losos *et al.*, 2003). In Hawaiian spiders, ecotypes are thought to have originated within habitats by two mechanisms, either by *in situ* evolution of one ecotype from another, or else by dispersal from one locality to another, with or without speciation (Gillespie, 2004). Finally, in gobies, major habitat shifts have been shown to account for the acceleration in lineage splitting and speciation (Rüber *et al.*, 2003).

The general pattern of ecotype variation in *Mandarina* can be illustrated by the terrestrial snails of the Hahajima archipelago. On Hahajima, both forms of *M. ponderosa* (*NH* and *SH*) are tall-spined, dark and thick-shelled palm-litter specialists, whereas the sympatric congeners *M. aureola* and *M. polita* are more frequent in thin broad-leaved litter that is exposed to the sun (Fig. 1; Chiba, 1999b; except Kitakou and Higashizaki, where *M. polita* has a palm-litter ecotype, see Table 1). In contrast, *M. ponderosa* on Mukoujima is polymorphic for shell, body colour and

banding, and has more variation in spire height (A. Davison & S. Chiba, unpubl. data), so is found both in palm and broad-leaved litter (Fig. 1; Table 1). As *M. aureola* and *M. polita* are absent from Mukoujima, then the most parsimonious explanation is that monomorphic *M. ponderosa* moved into a vacant habitat on Mukoujima. Thereafter, natural selection produced a more generalist and polymorphic ecotype.

*Mandarina* sp. A is particularly interesting because it takes a huge variety of ecotypes across the Hahajima archipelago, yet is always recognizable from the form of the shell periphery and the morphology of the genitalia. In most parts, it is a pandanus and palm forest canopy specialist, only descending to the ground to deposit eggs (Chiba, 1996). In these circumstances, the shell is pale and low-spined; the body is also pale. In contrast, *M. sp. A* from Higashizaki and the islands of Meijima and Imotojima are relatively tall-spined and have a thick shell which is often also dark. On these islands the adults live on the ground. As Meijima and Imotojima are separated from Higashizaki by a long island chain (Fig. 2), then either the ground-living form of *M. sp. A* has evolved more than once, or else it is ancestral.

While the combined results of this study and previous studies strongly suggest a role for ecology in divergence and speciation (Chiba, 1996, 1999b, 2004), geography must have also played an important isolating role, especially as snails in general have low active dispersal abilities. Studies in other species have confirmed earlier suspicions that snails have highly structured populations (Schilthuizen & Lombaerts, 1994; Thomaz *et al.*, 1996; Davison & Clarke, 2000; Hayashi & Chiba, 2000). In *Mandarina*, different ecotypes of the same species are mostly on different islands, or at least separated by large distances from the main population. However, we argue that the reason for this is not that physical separation per se is required for genetic divergence between populations, but that it is the physical separation which brings about different historical contingencies. In particular, small islands (e.g. Meijima) generally have fewer species, and are more dependent upon chance colonizations and stochastic extinction (MacArthur & Wilson, 1967; Ricklefs & Bermingham, 2004).

In other species, there is plentiful evidence that divergent selection can lead to the evolution of new forms, sometimes very rapidly (Nossil, Crespi & Sandoval, 2002; Feder *et al.*, 2003a; Rolan-Alvarez *et al.*, 2004). In some of these species, the genetics of isolation is relatively well understood (Feder *et al.*, 2003b; Wilding, Butlin & Grahame, 2001). Although the genetics has yet to be investigated in *Mandarina*, we speculate that relatively few genes are involved in divergence, because shell banding and colour are determined by a few loci in other snails. As it is pos-

sible to model shell shape using as few as four parameters (Raup, 1961), then a few genes of major effect may also determine shell shape.

Previously, Chiba (1999a) suggested that the same *Mandarina* ecotypes have evolved repeatedly on different archipelagos, as a result of convergent evolution. We believe that this study provides the necessary complement to that pattern, in providing evidence that the same species can take many different ecotypes. However, a cautionary note regarding convergent evolution is that the mitochondrial study has not yet been corroborated by independent phylogenies produced from nuclear loci (although there is no doubt that it is a monophyletic radiation; Fig. 3). Recent experience has indicated that mitochondrial genes may introgress much more easily than nuclear genes (Sota & Vogler, 2001; Shaw, 2002). Moreover, while the *Mandarina* ITS-2 and mtDNA phylogenies indicate that the Bonin Islands were colonized during a single founding event (Fig. 3), they both provide insufficient resolution to finally establish if each archipelago was colonized only once (Figs 3, 4). The observed pattern of cladogenesis is also that expected if the radiation on the three archipelagos took place almost simultaneously (Fig. 6).

It is conceivable that introgressive hybridization of mtDNA could explain why apparently closely related species (by mtDNA) have different ecologies. However, for introgressive hybridization to explain these results, then gene-flow must have been between species in different niches (e.g. between arboreal and terrestrial snails), whereas in other species hybridization has been shown to occur more often between species in the same niche (Rundle *et al.*, 2000). Moreover, introgressive hybridization can not explain the finding that the same species can take many different ecotypes. A more satisfactory explanation is that local patterns of natural selection on shell characters and behaviour have led to divergence between populations.

So what are the putative agents of selection? There is probably strong disruptive selection on habitat use, arising from environmental selection on shell and body colour (affecting body temperature), shell shape (differences in movement), as well as the effects of predation by the resident thrushes *Turdus* sp. and land crabs (selection for crypsis, frequency-dependent selection, selection for strengthened shells). Presently, however, the relative role of each remains to be tested. A critical question for the future is whether reproductive isolation is a pleiotropic effect of divergent selection.

Finally, it is interesting to compare the relatively well understood role of natural selection in causing divergence between populations of *Mandarina* and other organisms (Schluter, 2000) with the more uncertain role for sexual selection (Albert & Schluter, 2004; Knight & Turner, 2004; Seehausen & Schluter, 2004).

In *Mandarina*, different species are recognized by their characteristic genital morphology, and the penis size of some species differs by up to five times, even though the snails are much the same size. However, the degree to which sexual selection is implicated in *Mandarina* speciation remains unknown.

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