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The evolution of mitochondrial DNA in *Partula*

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The mitochondrial DNA of species of land snails of the genus *Partula* from the Society Islands has been analysed by means of restriction enzymes to determine relationships within the genus. Seventeen variable restriction sites were mapped onto the 14.5 kb mtDNA. These sites are highly variable within species. In *P. suturalis*, those genotypes occurring within a population are usually separated by single-step changes, and the differences between populations are geographically coherent. There is no detectable association between the distribution of mitochondrial genotypes and the occurrence of dextral or sinistral populations, confirming that chirality does not constitute a genetic barrier in this species. *P. taeniata* also shows a coherent geographic pattern of genotype distribution within and between populations. The mitochondrial differences between morphologically similar populations in the northeast and northwest suggest that the similarity may be the result of convergence. Despite the regular patterns of distribution within species, some genotypes are widely shared among species. One pattern was found in four species on Moorea and two species on the neighbouring island of Tahiti. Shared genotypes may represent ancestral forms or they may have resulted from hybridization. However we believe that the most attractive hypothesis is that they are subject to selection. Studies of *Partula* demonstrate that the evolution of the morphological, electrophoretic, and mitochondrial phenotypes occur at variable rates, independently of one another.

Keywords: mitochondrial DNA, *Patula*, population structure, restriction mapping, speciation.

Introduction

The land snails of the genus *Partula* provide unusually favourable material for the study of variation in natural populations. The genus contains about a hundred species distributed over the high (volcanic) islands of the South Pacific with the greatest diversity in the Society Islands of French Polynesia (Crampton, 1916, 1932). Each island in the Societies harbours a closely related group of species as we have shown by means of breeding studies, multivariate morphometric analysis, and protein electrophoresis (Murray & Clarke, 1968, 1976a, b, 1980; Clarke & Murray, 1969; Johnson *et al.*, 1977, 1986a, b). On the island of Moorea, for example, there are seven species. Two of these are the terminal members of a ring species, and two other pairs of species hybridize in some localities (Murray & Clarke, 1980). Within species there is extensive variation. Crampton (1932) recognized six morphological subspecies in *Partula taeniata* and two in *P. suturalis*. In addition, some populations of *P. suturalis* are dextral, some are sinistral, and some are polymorphic for chirality.

The electrophoretic analysis of enzyme variation supports the hypothesis that all the species from any one island form a monophyletic group, with the species from each successively younger island derived from those on the next older adjacent island (Johnson, Murray & Clarke, 1986a).

This conclusion conflicts with the widely accepted model of geographic speciation by multiple invasions in archipelagoes. This model predicts that morphologically similar species from different islands, such as *P. taeniata* from Moorea and *P. filosa* from Tahiti should be genetically more closely related than the morphologically and reproductively distinct *P. taeniata* and *P. suturalis* from Moorea.

In an effort to determine more exactly the relationships within the genus we have examined the restriction genotypes of the mitochondrial DNA of the species inhibiting the two youngest of the Society Islands, Moorea and Tahiti. By means of radiometric dating, these islands have been shown to have maximum ages of 1.5 and 1.0 million years respectively (Duncan & McDougall, 1976).

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Analysing restriction sites in mitochondrial DNA has proved to be useful for assessing the relationships between closely related species and between populations within species (Brown & Wright, 1979; Ferris *et al.*, 1983; Lansman *et al.*, 1983; Mulligan & Chapman, 1989; for reviews, see Avise, 1986; Avise *et al.*, 1987; Moritz *et al.*, 1987). The small size, high copy number, extra-nuclear location, and maternal inheritance combine to make mtDNA especially favourable material with which to measure genetic relatedness.

Some organisms, however, have raised special problems in the isolation of mtDNA. Molluscs are among the most difficult, and we have needed to devise particular methods for dealing with land snails (Stine, 1989). From these animals we are now able to make reliable preparations for analysis with restriction endonucleases.

In this study we have analysed the fragments generated with restriction enzymes to construct maps of the mtDNA of snails from 22 Moorean populations, including all seven species on the island, and from seven populations representing six of the Tahitian species. Two populations of an additional species from the island of Huahine have been included for comparison. These data provide new insights into the evolution of mtDNA and illuminate the relationships among the species of *Partula* and among the various kinds of characters used in phylogenetic studies.

Materials and methods

The stocks for this study were collected in the wild in 1980, 1982, or 1984. Each population (with one exception, *P. mirabilis*) is represented by a sample collected from an area of 10×10 m or less. Some of the specimens were freshly killed animals from the holding and breeding colony at the University of Virginia. Others were animals that had been killed by freezing at -80°C. We could not detect any difference between preparations from fresh and frozen specimens.

The isolation of the mitochondrial DNA of land snails has proved to be very difficult, but special methods for controlling nucleases and removing mucopolysaccharides devised by one of us (Stine, 1989) have made it possible to accomplish this goal. The foot, genitalia, and hepato-pancreas of individual snails were minced and then homogenized in 2 ml of ice-cold 0.25 M sucrose in TEK (50 mM Tris-HCl, pH 7.5, 10 mM EDTA, 1.5 per cent KCl) containing 140 μg/ml of ethidium bromide (to inhibit nuclease activity) in a Dounce homogenizer. Mucopolysaccharides were removed by centrifuging the sample through a layer of 1.1 M sucrose in TEK at 13,0000 ***g*** for 45 min at 4°. The pellet was resuspended and again centrifuged through the dense sucrose.

The mitochondria were disrupted by resuspension in ice-cold TEK containing 2 per cent NP40, lysing all membranes except for the nuclear membranes. The nuclei and tissue debris were pelleted by centrifugation. The supernatant was then extracted with phenol and chloroform, and the DNA recovered by ethanol precipitation.

Restriction digests were prepared with Bam HI, Hind III, Pst I, and Xho I. The yields of mtDNA from individual animals were small because of the small body size (<0.5 gram wet weight before dissection) and the extra steps necessary to isolate DNA from molluscs (see above and in Stine, 1989). Thus only three to seven digests per sample, depending on the size of the snail, could be prepared. The restriction fragments were separated by electrophoresis on agarose gels and transferred by Southern blotting to Gene

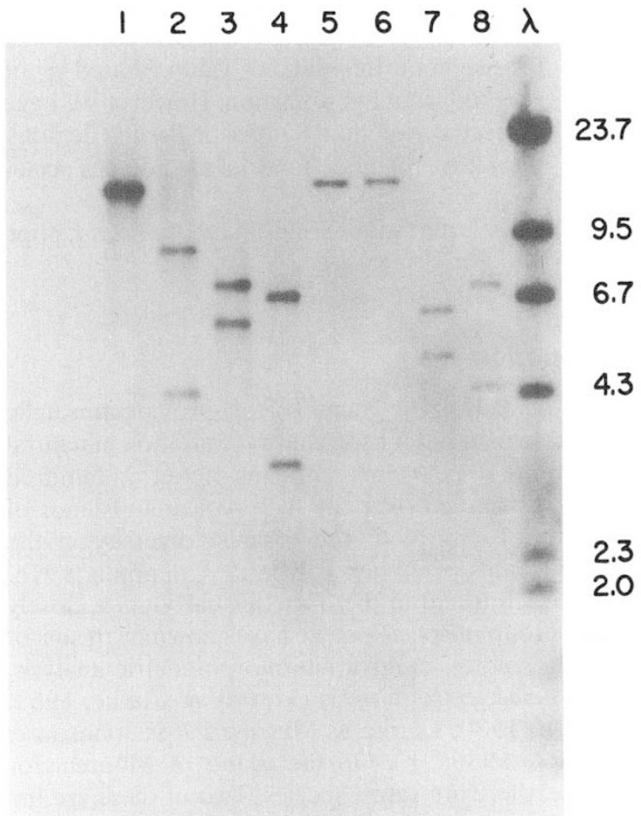

Fig. 1 An autoradiograph of restriction digests of *Partula* mtDNA probed with *Xenopus* mtDNA. Lanes 1 to 4 are from genotype J of *P. suturalis* from Mouputa (Bam HI, Xho I, Pst I, and Hind III; all fragments appear except for two from Hind III that are smaller than 1 kb). Lanes 5 to 8 are from genotype B of *P. taeniata* also from Mouaputa (Bam HI, Xho I, Pst I, and Hind III). Standards on the right are provided by lambda DNA digested with Hind III.

Screen Plus (New England Nuclear). The blots were probed with radioactively labelled mtDNA from the plasmid pXlm31, which contains the entire mitochondrial genome of *Xenopus laevis* (isolated by Igor Dawid). This probe successfully hybridizes with snail mtDNA at low stringency (see Fig. 1), since approximately 70 per cent of the sequence of many mitochondrial genes is shared by organisms from several different phyla (Howley *et al.*, 1979; Clary & Wolstenholme, 1985; Stine, 1989).

By using the restriction enzymes either singly or in pairs, it was possible to map the *Partula* mtDNA completely, thereby ensuring that the entire genome of the mitochondrion is accounted for. The resulting maps were analysed by treating the restriction sites as individual characters that were either present or absent in any particular genotypic map. PAUP (Swofford, 1985) was employed to search for the most parsimonious phylogenetic relationships among the genotypes represented in the populations in this study.

Results

We were able to isolate mitochondrial DNA and map the restriction sites from 227 snails in 31 populations of 14 species of *Partula*. The localities are listed in Table 1, and those on Moorea and Tahiti are shown on the maps in Figs 2 and 3.

The lengths of fragments in single and double digests established that the mtDNA was approximately 14.5 kilobases in size. Thus the length is similar to the size of the mtDNA of *Ascaris suum* (14,284 base pairs, Wolstenholme *et al.*, 1987). It contained 17 restriction sites for the four enzymes for which we have the most

Table 1 Localities in the Society Islands from which individuals were drawn for analysis of mitochondrial DNA

Island	Valley	Sample no.	Sample size	Species	Genotypes found
Moorea	Mouaputa	M639	5	*P. taeniata*	A, B
Moorea	Atimaha	26.2	6	*P. taeniata*	A
Moorea	Maramu	M750	5	*P. taeniata*	C, D
Moorea	Faatoai	25.1	17	*P. taeniata*	E, F, G
Moorea	Faamaariri	24.3	6	*P. taeniata*	H
Moorea	Paparoa	20.1	6	*P. exigua*	I
Moorea	Mouaputa	M639	34	*P. suturalis*	A, J
Moorea	Maharepa	21.1	3	*P. suturalis*	A, J
Moorea	Fanautaata	18.2	3	*P. suturalis*	J
Moorea	Paahonu	M766	4	*P. suturalis*	K, L, M
Moorea	Atimaha	M832	7	*P. suturalis*	N, O
Moorea	Vairahi	M699	6	*P. suturalis*	P, Q
Moorea	Urufara	M647	17	*P. suturalis*	R, S
Moorea	Haapiti	23.4	3	*P. suturalis*	T, U
Moorea	Tepatu	16.1	3	*P. 'dendroica'*	J
Moorea	Fareaito	27.2	4	*P. tohiveana*	A
Moorea	Mouaputa	M639	5	*P. 'olympia'*	A, V
Moorea	Fanautaata	18.3	3	*P. mooreana*	A, W
Moorea	Maatea	M682	6	*P. mooreana*	A
Moorea	Vaianai	M679	4	*P. mooreana*	A
Moorea	Fareito	M692 & 3	7	*P. mirabilis*	P
Moorea	Paparoa	20.7	4	*P. aurantia*	R
Tahiti	Tiarei	T811	7	*P. otaheitana*	P
Tahiti	Tipaerui	T859	9	*P. otaheitana*	X, Y
Tahiti	Tiarei	T811	6	*P. affinis*	P
Tahiti	Faone	T855	3	*P. jackieburchi*	P
Tahiti	Papehue	T801	4	*P. nodosa*	A, Z
Tahiti	Pirae	T858	13	*P. filosa*	AA, BB, CC, DD
Tahiti	Tiarei	T776	6	*P. hyalina*	A
Huahine	Turi, north	H589	9	*P. varia*	E
Huahine	Fitii	H586	12	*P. varia*	FF

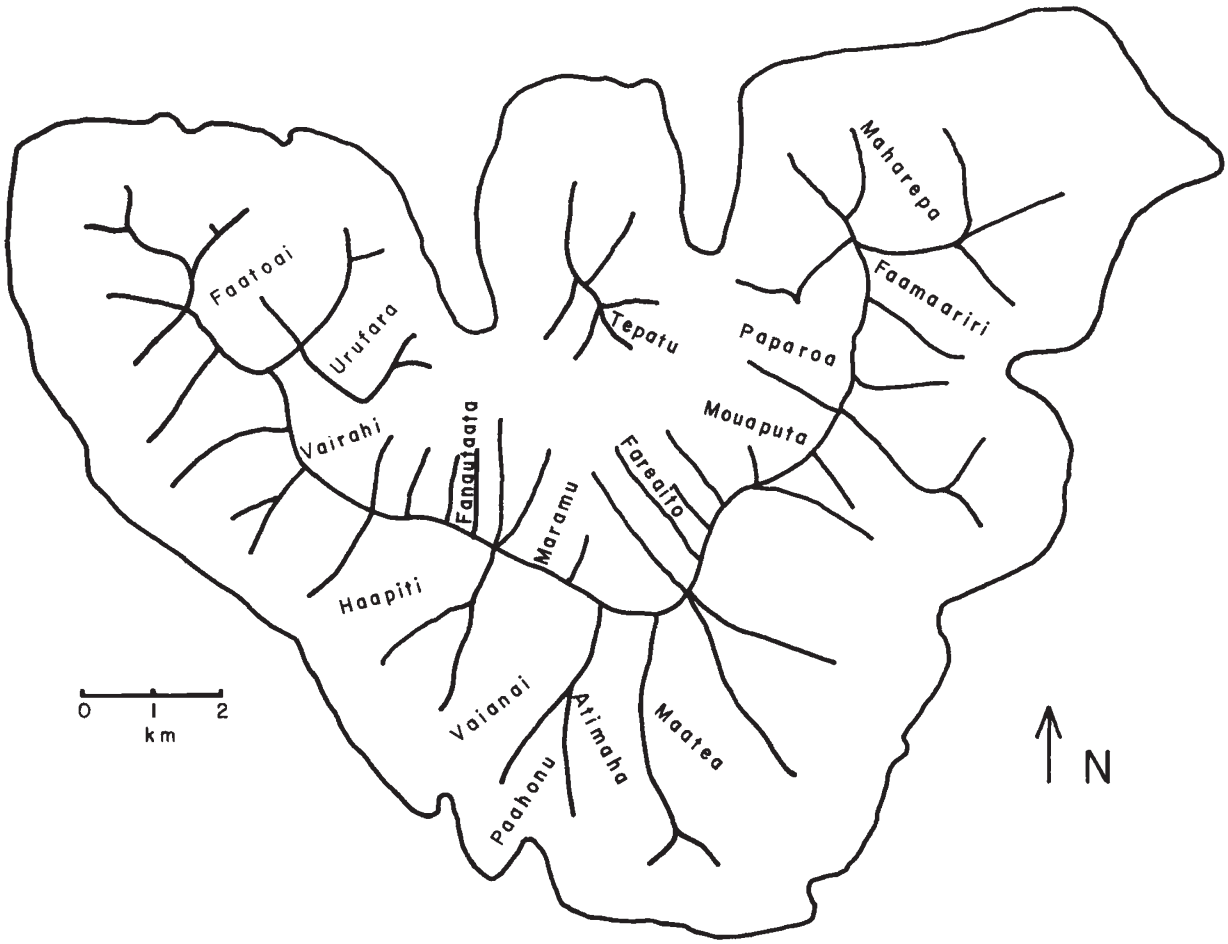

Fig. 2 The island of Moorea, Society Islands, showing valleys from which collections were taken. The interior lines indicate ridges.

complete data. In principle, 14 sites were phylogenetically informative, being present in at least two populations and absent from at least two others. Three sites were unique to single populations. The composite map of all the sites is shown in Fig. 4.

There is an unusual breadth of variation within the species of this genus. The distribution of the 32 observed mitochondrial genotypes is shown in Table 1, and their combinations of restriction sites are recorded in Table 2. Twelve populations were polymorphic, containing at least two different genotypes. Of these, two populations had three different genotypes, and one had four.

The relationships among the populations of *Partula suturalis*, the most extensively sampled species in the study, are summarized in Fig. 5. The nine populations contained 13 different genotypes, with two additional hypothetical ones being required to connect those actually observed. Seven of the populations were shown to be polymorphic, one containing three different genotypes. Despite the substantial variation within and between populations, two of the genotypes (A and J) were quite widely distributed, occurring in both sinistral and dextral populations of *P. suturalis*. One of these (J) was also found in the Tepatu population, which was formerly assigned to *P. dendroica* (Crampton, 1932), but which has been shown to be conspecific with *P. suturalis* (Murray & Clarke, 1980). The populations with the A and J genotypes are clustered together in the northeast and central valleys. There are two groups of highly variable populations, those in the south near Mt. Ahutau (K, L, M, N and O) and those in the west (P, Q, R, S, T and U). The similarities among genotypes within each of these geographic groups are higher than those between the groups, providing further evidence of geographic coherence of the variation.

The relationships among populations of *Partula*

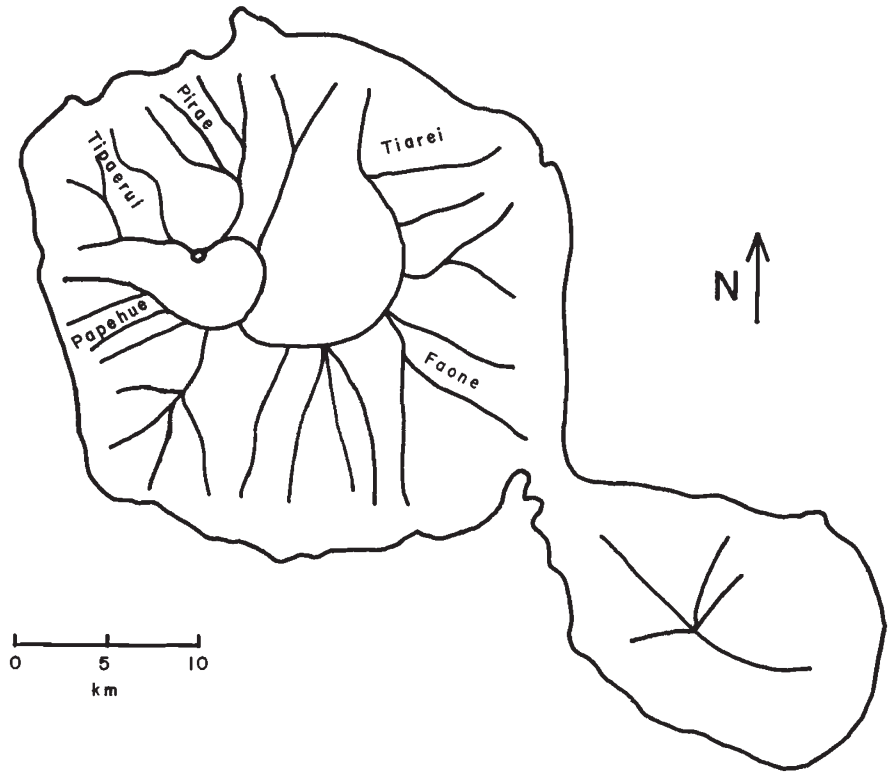

Fig. 3 The island of Tahiti, Society Islands, showing valleys from which collections were taken. The interior lines indicate ridges.

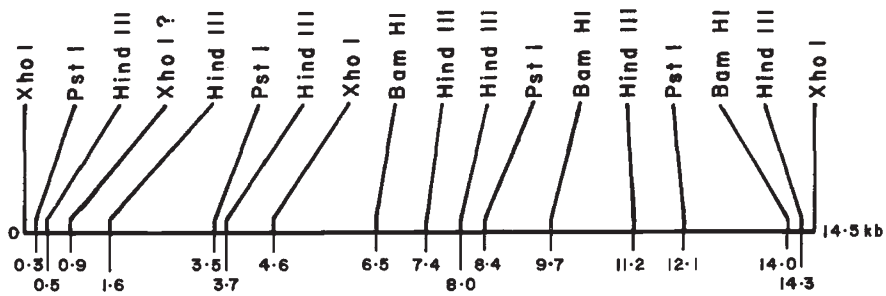

Fig. 4 A map of restriction sites detected in the mtDNA of *Partula* species. The site labelled Xho I? has not been definitively located; it is either at 0.9 (as shown) or at 3.7 on the map.

taeniata and the closely related *P. exigua* are shown in Fig. 6. Three of the six populations are polymorphic with 10 genotypes overall, three of them from a single population. Again there is a coherent geographical pattern, but it differs from that in *P. suturalis* in at least two respects. First, the southern population shares a genotype with one of the central populations. Second, it is the populations in the northeast, both *P. taeniata* (H) and the closely related *P. exigua* (I) that show large differences from each other and from the rest of the populations of *P. taeniata*.

The 32 genotypes found among the 14 species demonstrate that the mtDNA of *Partula* has undergone extensive changes during the evolution of the genus. Nevertheless some genotypes are shared quite widely within the genus. The most common (A) is found in *P. suturalis, P. taeniata, P. tohiveana*, and *P. mooreana* on Moorea and in *P. nodosa* and *P. hyalina* on Tahiti. A second genotype (P) is shared by three species on Tahiti and two on Moorea. These two genotypes are separated by two steps. We have not found the intermediate; but it is interesting that this pattern would

Table 2 Genotypes detected among populations of *Partula* from the Society Islands. Restriction sites are ordered as in Fig. 4. 1 = presence of site; 0 = absence; 9 = uncertainty (some animals have the site, some do not; but its association with other sites is uncertain)

	Restriction sites																
	X	P	H	X	H	P	H	X	B	H	H	P	B	H	P	B	H
A	1	1	1	0	1	0	1	1	0	0	1	1	0	0	0	1	0
B	1	1	1	0	1	1	1	1	0	0	1	1	0	0	0	1	0
C	0	1	1	0	9	0	1	1	0	0	1	1	0	0	0	1	0
D	0	1	1	0	9	1	1	1	0	0	1	1	0	0	0	1	0
E	0	1	1	0	1	0	1	1	0	0	1	1	0	0	0	0	0
F	0	1	1	0	1	0	0	1	0	0	1	1	0	0	0	0	0
G	1	1	1	0	1	0	0	1	0	0	1	1	0	0	0	0	0
H	0	0	1	0	1	1	1	0	0	0	1	1	0	0	0	0	0
I	1	0	1	0	1	1	1	0	0	0	1	1	0	0	1	1	0
J	1	1	1	0	1	0	1	1	0	1	1	1	0	0	0	1	0
K	1	1	1	0	1	0	1	1	0	9	0	1	0	0	0	1	0
L	1	1	1	0	1	0	1	0	9	1	0	1	0	0	0	1	0
M	1	1	1	0	1	0	1	0	1	0	0	1	0	0	0	1	0
N	1	0	1	0	1	1	1	0	1	0	0	1	0	0	0	1	0
O	1	0	0	0	1	1	1	0	1	0	0	1	0	0	0	1	0
P	1	0	1	0	1	0	0	1	0	0	1	1	0	0	0	1	0
Q	1	0	1	0	1	0	0	1	0	0	0	1	0	0	0	1	0
R	1	0	1	0	1	0	0	1	0	1	0	1	0	0	0	1	0
S	1	0	1	0	1	0	0	1	0	1	0	0	0	0	0	1	0
T	1	0	0	0	1	0	0	1	0	0	1	1	0	0	0	1	0
U	0	0	1	0	1	0	0	1	0	0	1	1	0	0	0	1	0
V	1	1	1	0	1	0	1	1	0	0	1	1	0	0	1	1	0
W	1	1	0	0	0	0	1	0	0	0	1	1	0	0	0	1	0
X	1	0	1	0	1	0	0	0	0	1	0	1	0	0	0	1	0
Y	1	0	1	0	1	0	0	0	0	1	0	1	0	0	1	1	0
Z	1	0	1	0	1	0	1	0	0	1	1	1	0	0	0	1	0
AA	1	0	0	0	1	0	1	1	0	1	1	1	0	0	1	1	1
BB	1	0	0	1	1	0	1	1	0	1	1	1	0	0	1	1	1
CC	1	0	0	1	1	0	1	1	0	1	1	0	0	0	1	1	1
DD	1	0	0	1	1	0	1	1	0	1	1	0	0	1	1	1	1
EE	9	0	1	0	1	0	0	0	1	0	0	9	9	1	0	1	0
FF	9	0	1	0	1	0	1	0	1	0	1	9	9	0	0	1	0

constitute a consensus sequence, since it would contain the most common state of each of the restriction sites over all the patterns.

We have used PAUP (Swofford, 1985) to search for the most parsimonious phylogenetic tree embracing the variation in Table 2. The minimum tree that we have found requires 43 steps, but there are many equally parsimonious trees that differ in detail. The principal problem is that changes at different sites are not very consistent with one another (consistency index = 0.37), implying that much of the change has not been progressively divergent. Only three restriction sites are completely consistent with the minimum trees, and two of these occur in the highly differentiated branch of *P. filosa*. Thus there must have been a considerable amount of reversal, parallelism, and/or convergence during the evolution of the group. Under these circumstances the relationships are best expressed as a network that allows us to show multiple similarities. Figure 7 embraces all single- and double-step changes together with those of higher order that represent minimum connections.

In spite of the uncertainties stemming from this inconsistency, clear systematic and geographic relationships are shown by the data at three levels. First, different genotypes found within the same population of a species tend to differ from one another by changes at single sites. In *P. filosa*, for example, there are four

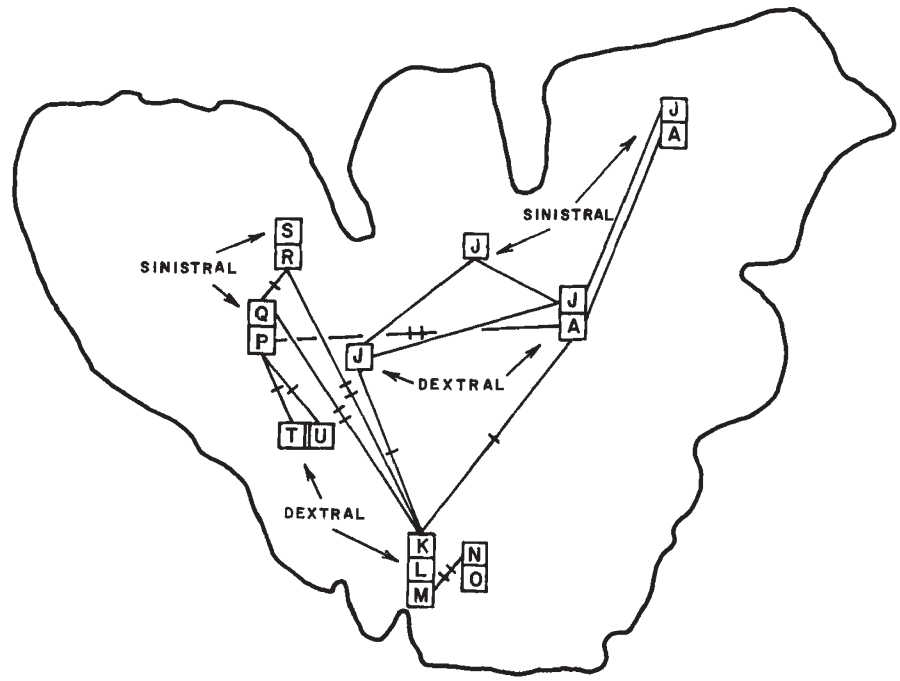

Fig. 5 A map of Moorea showing the geographical locations from which the various mitochondrial genotypes of *P. suturalis* were collected. Each change in a restriction site between genotypes is indicated by a crossbar. Genotypes within the same population are shown by contiguous squares. They are related by single-step changes with the exception of T and U, and also K, and M.
In each of these cases one two-step change is required.

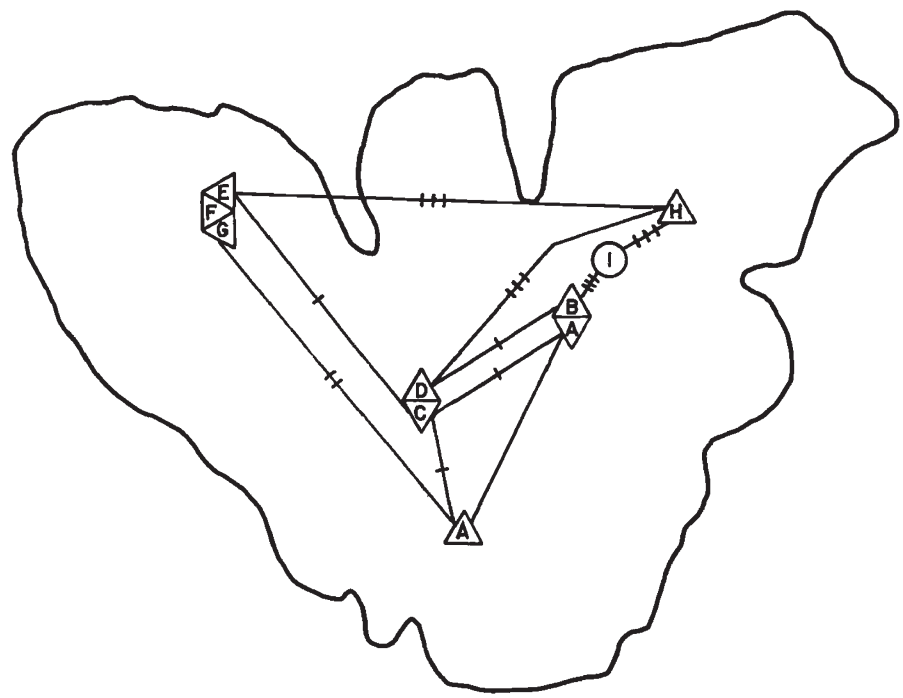

Fig. 6 A map of Moorea showing the geographical locations from which the various genotypes of *P. taeniata* and the closely related *P. exigua* were collected. Triangles = *P. taeniata*; circle = *P. exigua*. Conventions are as in Fig. 5.

genotypes separated by three single-step changes; and in *P. taeniata* from Faatoai there are three genotypes separated by two single-step changes. There are four exceptions to this generalization: two-site differences separate genotypes found in *P. suturalis* from Paahonu and from Haapiti; three-site differences, in *P. mooreana* from Fanautaata and in *P. nodosa*. However the sample sizes from these populations are small, and the intermediates may have been missed.

Second, genotypes tend to be closely related to their closest geographical neighbours. This pattern extends beyond the relationships already noted for *P. suturalis*

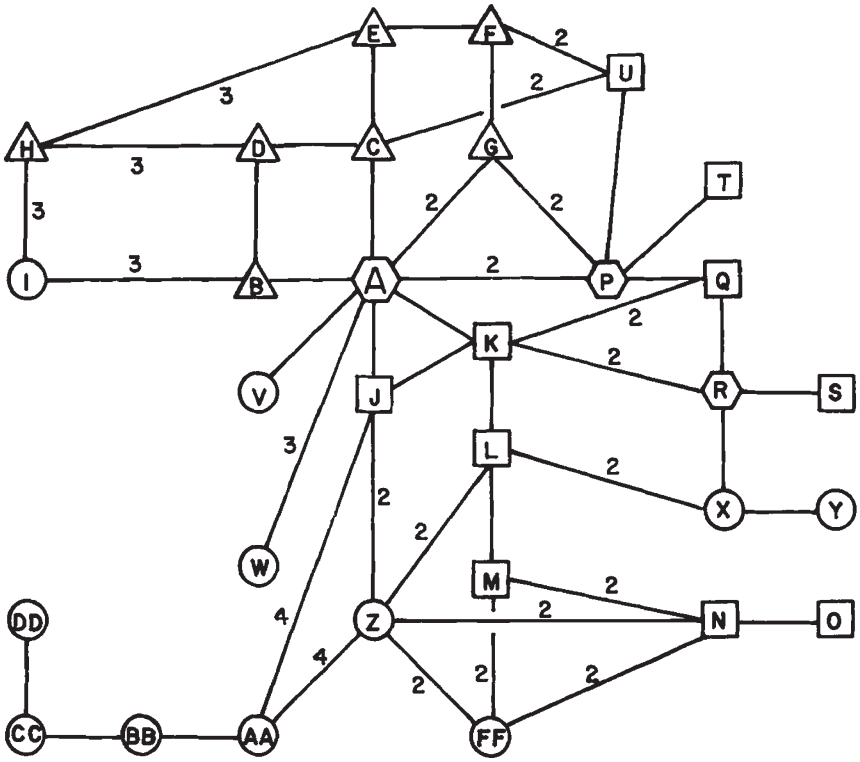

Fig. 7 A network showing the relationships of the genotypes found in this study. Squares = *P. suturalis* alone; triangles = *P. taeniata* alone; circles = other species alone; hexagons = genotypes shared by more than one species. All single and two-step relationships are given, together with greater differences when these are the closest relationships. There are a few omissions for the sake of clarity. EE is related by three steps each to FF, M, N, Q, and X. I is also related to N, V, Z, and FF by three; and AA, to T and V by four steps.

and *P. taeniata*. There is a close geographical association of the two *suturalis* populations containing the widely shared genotype A with the other species in which this genotype occurs, at least on Moorea. This genotype is found in two populations of *P. tohiveana* (including *P.'olympia'*), three of *P. mooreana*, and two of *P. taeniata* from the northeast to the centre and south of the island (see Fig. 8). The association is curious in the light of the occurrence of this genotype in two species on Tahiti.

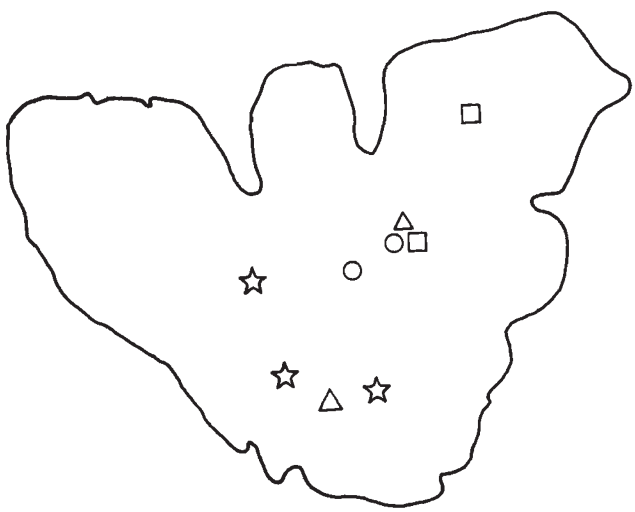

Fig. 8 Populations in which the shared genotype A has been found. Squares = *P. suturalis*; triangles = *P. taeniata*; circles = *P. tohiveana* (including *P. 'olympia'*); stars = *P. mooreana*. Not shown are *P. nodosa* and *P. hyalina* from Tahiti.

The second widely shared genotype (P) is found in *P. otaheitana, P. affinis*, and *P. jackieburchi* from Tahiti and in *P. suturalis* and *P. mirabilis* from Moorea. Two of the Tahitian species come from the same locality. There is some question about the status of the three Tahitian taxa. Crampton (1932) considered *affinis* to be a 'sympatric sub-species' of *otaheitana*. Kondo (1980) originally described *jackieburchi*, on account of the structure of the genitalia, as a member of the related genus *Samoana*. However the evidence from the electrophoretic analysis of enzyme polymorphisms suggests that all three of these taxa are very closely related (Johnson *et al.*, 1986c), but we do not yet know whether they exchange genes.

Discussion

The results of our study of the mtDNA in *Partula* present us with patterns of variation that are quite familiar in some respects and very surprising in others. Although the extent of the variation within individual species is large for species with such restricted distribu-

tions, the geographic coherence of the variation is what we have come to expect from other studies of this sort (Avise, 1986). Genotypes within the same population tend to differ from one another by single-step changes, and neighbouring populations are more similar to one another than are those from further away.

However, when we compare the patterns of mitochondrial relationships among species with those derived from the study of morphological and electrophoretic characters, something quite different emerges. No two character sets provide congruent estimates of the phylogeny of the group. Our first consideration, therefore, must be whether or not the new data are reliable.

Two lines of reasoning strongly support the reliability of these data. First, the ability to map each restriction site on mtDNA of a uniform size rules out the possibility that our results stem either from loss of fragments or from mistaken similarities. Under these circumstances the use of a heterologous probe for the detection of restriction fragments appears to be entirely justified. Although it has been shown that evolutionary changes involving about 30 per cent of the mitochondrial genome occur very rapidly (Brown *et al.*, 1979), the remaining 70 per cent, containing the genes encoding the large and small subunits of ribosomal RNA, the three cytochrome oxidase subunits, cytochrome b, and subunit 8 of the ATPase, are sufficiently similar across phyla to permit hybridization of mtDNA at low stringency (Clary & Wolstenholme, 1985; Howley *et al.*, 1979). In particular, the *Xenopus* probe has been shown to be effective in detecting restriction fragments from molluscan mtDNA (Stine, 1989).

Second, the rates of change required to produce the observed degree of differentiation in *Partula* are not excessively high. Following Nei & Li (1979) the minimum average similarity among the mtDNA genotypes of *Partula* on Moorea and Tahiti is 0.6, corresponding to a sequence divergence of 0.0426. Since the total time allowed by the geological age of the islands is 1.5 million years, the estimated rate of sequence divergence is 2.8 per cent/MY, very close to the 2 per cent/MY estimated for primates (Brown *et al.*, 1982).

Nor is it unusual to find mtDNA genotypes widespread within a species. Such genotypes have been observed, for example, in *Homo sapiens* (Johnson *et al.*, 1983) and *Agelaius phoeniceus* (Ball *et al.*, 1988). What is remarkable about the *Partula* results is the extent to which the common genotypes are distributed across species boundaries.

The shared genotypes and their meaning. The most common genotypes in our survey tend to be shared among more than one species. For example, pattern A has been found on Moorea in two populations of *P. suturalis*, two of *P. tohiveana* (including one originally described by Crampton as *P. olympia*), three of *P. mooreana*, and two of *P. taeniata*. It also occurs on Tahiti in populations of *P. nodosa* and *P. hyalina*. These include species that are very different in their morphology, their ecology, their breeding biology, and their genetics as assayed by the electrophoresis of enzymes (Murray & Clarke, 1980; Johnson *et al.*, 1986b). There are at least three hypotheses that might explain this extraordinary similarity.

The first is that this identity represents the retention of an ancestral genotype in the several species. This explanation is attractive because of the central position of the shared genotypes in the minimum-length network (Fig. 7). Neigel and Avise (1986) have modelled the process of mitochondrial sorting in lineages derived from populations with more than one type of mtDNA. With reasonable assumptions they have shown that two species may retain shared ancestral mitochondrial genotypes for approximately 4N generations. It is conceivable, therefore, that some of the genotypes shared by closely related species of *Partula* may be explained simply by delay in the sorting out of ancestral genotypes. However, the process of sorting in recently separated lineages can hardly account for the common genotypes in all the species, especially those occurring on Tahiti and Moorea, such as *P. nodosa* and *P. hyalina*, which differ substantially from the Moorean species in their allozymes (Johnson *et al.*, 1986a).

Murray & Clarke (1984) have estimated the approximate generation time in *Partula* to be three years and the effective population sizes to be between 400 and 2000 individuals. These statistics would establish an upper limit for the time of separation of the six species sharing the A genotype of about 24,000 years. Such a short time is quite incompatible with estimates of the evolution of enzyme polymorphism in these species. Using the maximum time allowed by the geological ages of the islands it has been shown that the change in Nei's D (Nei, 1978) has been at least twice as large as one might expect from other studies (Johnson *et al.*, 1986a; Maxson & Maxson, 1979). Accepting the recent separation of the *Partula* species would require rates of enzyme evolution about 100 times faster than 'normal'.

An additional argument against the sorting hypothesis is that if N is small, then diversity within populations should be low. Our observations show that even with relatively small sample sizes, multiple genotypes are common within populations from localities no larger than 10×10 m.

The second hypothesis is that the genotypes have

been shared through hybridization. It has been suggested that this process is responsible for similarity of mtDNA is closely related species (Ferris *et al.*, 1983). A number of *Partula* species have been shown to be able to exchange genes. *P. suturalis* and *tohiveana* are the terminal members of a circular overlap which includes the intermediate from *P. 'olympia'* (Murray & Clarke, 1980). Individuals from all three of these taxa share the A genotype. Other pairs of species known to hybridize are *P. suturalis* and *aurantia, P. taeniata* and *exigua*, and *P. taeniata* and *mirabilis*. Thus there are abundant opportunities for mitochondria to cross species boundaries. On the other hand, *P. suturalis, P. taeniata*, and *P. mooreana*, which share the A genotype, have been shown to be reproductively quite distinct (Murray & Clarke, 1980). These observations and the occurrence of shared genotypes in species from both Moorea and Tahiti argue against the hypothesis of hybridization.

A final hypothesis, as suggested by Whittam *et al.* (1986) for *Homo sapiens*, is that selection favours the common genotype or genotypes (see also Adams & Rothman, 1982; Aquadro *et al.*, 1984). The two widely shared genotypes (A and P) differ by changes at only two positions. The missing intermediate could be considered a consensus genotype since its sequence would contain the most common state of each restriction site scored over all the observed patterns. Moreover, the analysis has shown that convergence is a prominent feature of the evolution of these sites. Taken together, the wide divergence, the conservative retentions, and the evidence of reversal argue that the common genotypes are ones that have been modified and reconstituted more than once. We therefore suggest that the common genotypes probably present selectively favoured stable states, with individual sites departing from and returning to the norm.

Mitochondrial phylogeny. Taking into consideration the evidence for reversal and convergence, any phylogenetic analysis must be approached with caution. There are a number of trees, differing in their details, that have the same number of steps. The most conservative approach to phylogenetic analysis in this case is therefore to base our conclusions on a network of relationships rather than on a conventional tree. The network in Fig. 7 clearly shows the reasons for this choice.

The network has, of course, no root. We originally included the species *P. varia* from the Leeward Island of Huahine in the expectation, based on allozyme analysis (Johnson *et al.*, 1986*a*), that it would provide a useful outgroup. However, *P. varia* is extremely polymorphic, and we were therefore unable to establish individual maps unequivocally. Thus its point of connection to the tree is uncertain. Another possible candidate for the root would be the consensus genotype, which lies between the two widespread genotypes A and P.

Within this framework some of the phylogenetic relationships are clear. The connection of *P. taeniata* to the rest of the species is almost certainly through the A genotype. The minimal network for the group (see Figs 6 and 7) provides an interesting comparison with the morphological relations of populations within the species. In *P. taeniata* there is a cline in shape from long, thin shells (ssp. *elongata*) in the south to short, fat shells (ssp. *nucleola and strigata*) in the north (Crampton, 1932). According to the mitochondrial relationships, the northeastern (H) and northwestern subspecies (E–F–G) represent separate branches of the tree. If this represents the true relationships within the species, then the similarity of the short, fat shells is convergent.

For *P. suturalis* the network shows clearly that neither the dextral nor the sinistral populations can be monophyletic. The sinistral populations in the northeast (J–A) and northwest (R–S) are more similar to adjacent dextral populations than they are to each other (see Figs 5 and 7). Moreover the dextral population in Haapiti (T = U) is more similar to the sinistral populations of the northwest than to the other populations of dextrals. This lack of congruence between the coils and the mtDNA confirms the independence of chirality and the other genetic characteristics of the population (Johnson, 1987; Johnson *et al.*, 1987).

mtDNA and allozymes. An additional instructive comparison is that between the degree of differentiation in two different classes of biochemical characters. The analysis of allozymes (Johnson *et al.*, 1986b) shows extreme divergence in the populations of both *P. taeniata* and *P. suturalis* from the far south of Moorea. Indeed each is more different from other conspecific populations than those populations are from some other species in the genus. Either the southern populations were isolated before further speciation occurred in the group or else rates of change in these enzymes are not uniform. The data from mtDNA allow us to answer that question, since the results from the two species are very different. In *P. taeniata* the southern populations are the most conservative, showing the A genotype whch links this species to the other members of the genus. On the other hand, the mtDNA from *P. suturalis* in this same area is very different from that of other populations of the species. We cannot say whether the genotypes found there are ancestral or derived, since that distinction depends heavily on how the network is rooted, but it is quite clear that isolation

alone cannot explain the observed results in both *P. taeniata* and *P. suturalis*. We conclude therefore that the data from allozymes and from mtDNA can only be reconciled by accepting the hypothesis of variability in the rates of evolution of these biochemical characters.

The information that we have obtained on variation of the mtDNA in *Partula* reinforces our view that the species in this genus form a very closely related group. The substantial intraspecific genetic variation in this character set is consistent with the results from the genetics of colour patterns (Murray & Clarke, 1976a, b), morphology (Murray & Clarke, 1980), and protein electrophoresis (Johnson *et al.*, 1986b). However, the different character sets evolve independently and at variable rates. This mosaic pattern of evolution can only occur if natural selection plays a role in the genetic differentiation of *Partula*.

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