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Mitochondrial DNA analysis of introgression between adjacent taxa of rock-wallabies, *Petrogale* species (Marsupialia: Macropodidae)

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Summary

Simple, inexpensive techniques were used to analyse the mtDNA of nine chromosomally distinct populations of *Petrogale*. Eight of these populations occur in sequence along the Great Dividing Range of eastern Australia; six have been described as species. Diagnostic mtDNA morphs were found throughout the latitudinal ranges of four of the described species. A fifth morph spanned the ranges of two described species and three additional taxa which have been designated chromosome races. These five mtDNA morphs, and others with local distributions, were used to assess interactions between the taxa. Limited introgression was indicated across the chromosomal boundaries of *P. penicillata*/*P. herberti* and *P. inornata*/*P. assimilis*; atypical mtDNA morphs were found within the *P. herberti* and *P. inornata* chromosomal distributions. No introgression was detected between *P. herberti* and *P. inornata*, whose distributions are separated by the Fitzroy River. Nor was there evidence of recent contact between *P. assimilis*, *P. herberti* and *P. purpureicollis*, despite the late occupancy of parts of the intervening area by unidentified *Petrogale*. These data, considered in the light of information obtained from previous studies on chromosomes, allozymes and parasites, have contributed to the decision to consider all the eastern representatives of the *lateralis–penicillata* group of *Petrogale* as being specifically distinct from each other.

1. Introduction

Rock-wallabies (*Petrogale*) are small (2–9 kg) macropodid marsupials, found across mainland Australia and on some offshore islands. They form colonies in suitable rocky areas which are often isolated from the nearest colony by distances of several kilometres, even when connected by continuous rocky terrain (Short, 1982).

Twenty-three forms of rock-wallaby have been described, generally on the basis of differences in morphology and pelage characters. Most forms were given specific rank, but some of the classifications have been disputed by various authors (see Briscoe *et al.* 1982).

Briscoe *et al.* (1982) examined the chromosomes and allozymes from most of the 23 named taxa and found what they considered to be 20 distinctly different karyotypes which they distinguished as those of 'chromosome races'. These included 5 previously unrecognized taxa. Briscoe *et al.* (1982) grouped chromosome races of similar morphology into provisional species if obviously closely related, and recognized 10 such provisional species. Calaby & Richardson (1988) listed the same 10 species, but in addition reinstated *P. assimilis* as a full species. Sharman *et al.* (1990) regarded the previously undescribed 'chromosome races', referred to by Briscoe *et al.* (1982), as subspecies of their nearest assumed related species, and retained the race nomenclature. They gave *P. assimilis* specific status but did not affiliate the Mt Claro and Mareeba races with a particular species. Eldridge *et al.* (1988), Bell *et al.* (1989), and Close & Bell (1990), however, included these races within *P. assimilis*.

Many of these taxa have parapatric or near parapatric distributions. Nine occur in sequence from Cape York, in north Queensland, south to Victoria (Fig. 1). Cytogenetic and electrophoretic studies by Briscoe *et al.* (1982) indicated gene flow across the borders of some taxa despite chromosomal differences,

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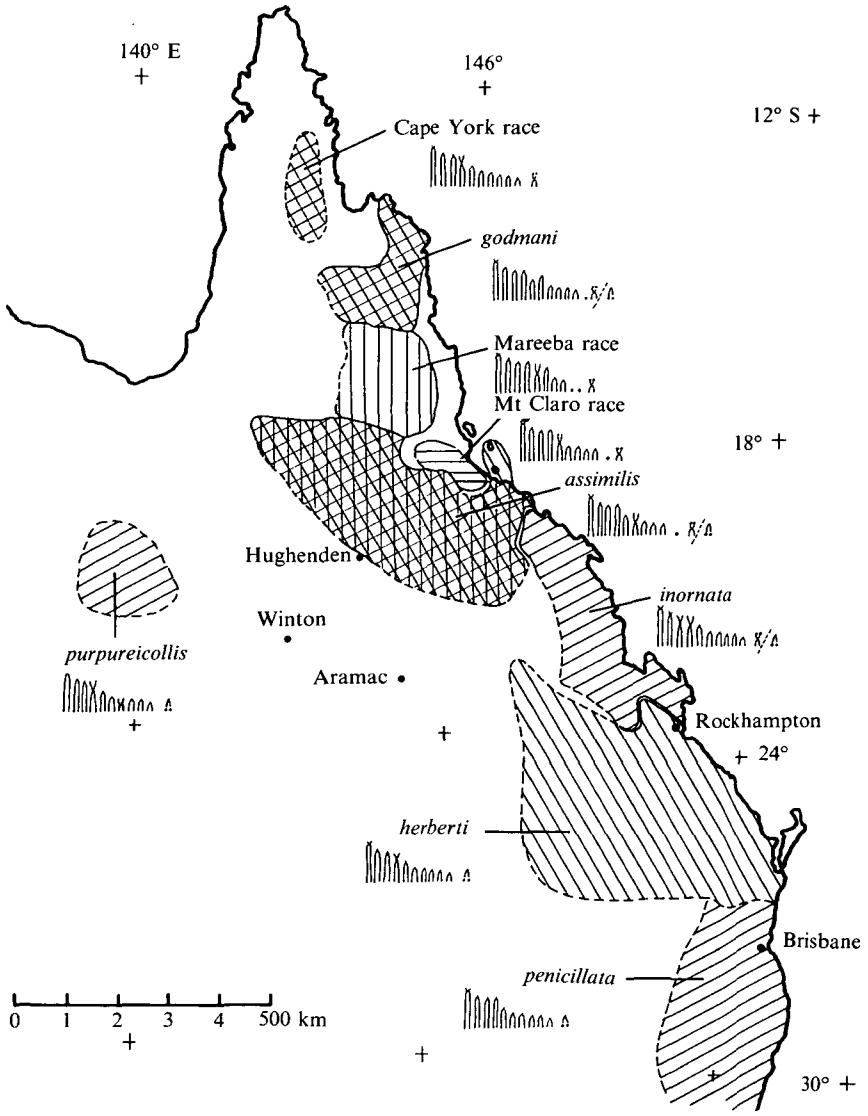

Fig. 1. Known distributions and haploid chromosomes of eastern taxa of the *lateralis/penicillata* group of *Petrogale*. The distribution of *penicillata* extends a further 1400 km south along the Great Dividing Range. The fifth chromosomes of *godmani*, *assimilis* and *inornata*, in addition, have an inversion involving most of the chromosome. Dotted border lines indicate that taxon boundaries are incompletely surveyed.

thus raising the possibility of similar flow between all contiguous taxa. This introgression is investigated in the present paper by examination of the distribution of different morphs of mitochondrial DNA (mtDNA) from chromosomally distinct taxa of *Petrogale* from eastern Australia.

Taxa of *Petrogale* are referred to by their original specific names if one was given. Where no specific name has existed, the name of the chromosomal race designated by Briscoe *et al.* (1982) is used. As the specific affinities of these chromosomal races are currently in question (Eldridge *et al.* 1990; Sharman *et al.* 1990), each taxon is named in the text without generic prefix and refers only to a taxon with a similar karyotype to that found at the relevant type locality or to the chromosome race named by Briscoe *et al.* (1982).

The qualities of mtDNA which make it suitable for studying closely related taxa are as follows:

(1) In recently diverged populations, genetic markers are more likely to be found in mtDNA than in nuclear DNA because the rate of sequence mutation exceeds that in nuclear DNA for all mammals so far studied (Avise *et al.* 1979; Brown *et al.* 1979). However, this relationship does not apply to all other animals (Moritz *et al.* 1987; Harrison, 1989).

(2) The little evidence available from rock-wallabies (unpublished data), is consistent with the general finding in mammals that mtDNA is maternally inherited (Lansman *et al.* 1983; Avise, 1986; although note Gyllenstein *et al.* 1991). Thus the mtDNA clone, from a female rock-wallaby that has produced at least partly fertile hybrid offspring with a neighbouring species, will persevere despite subsequent back-

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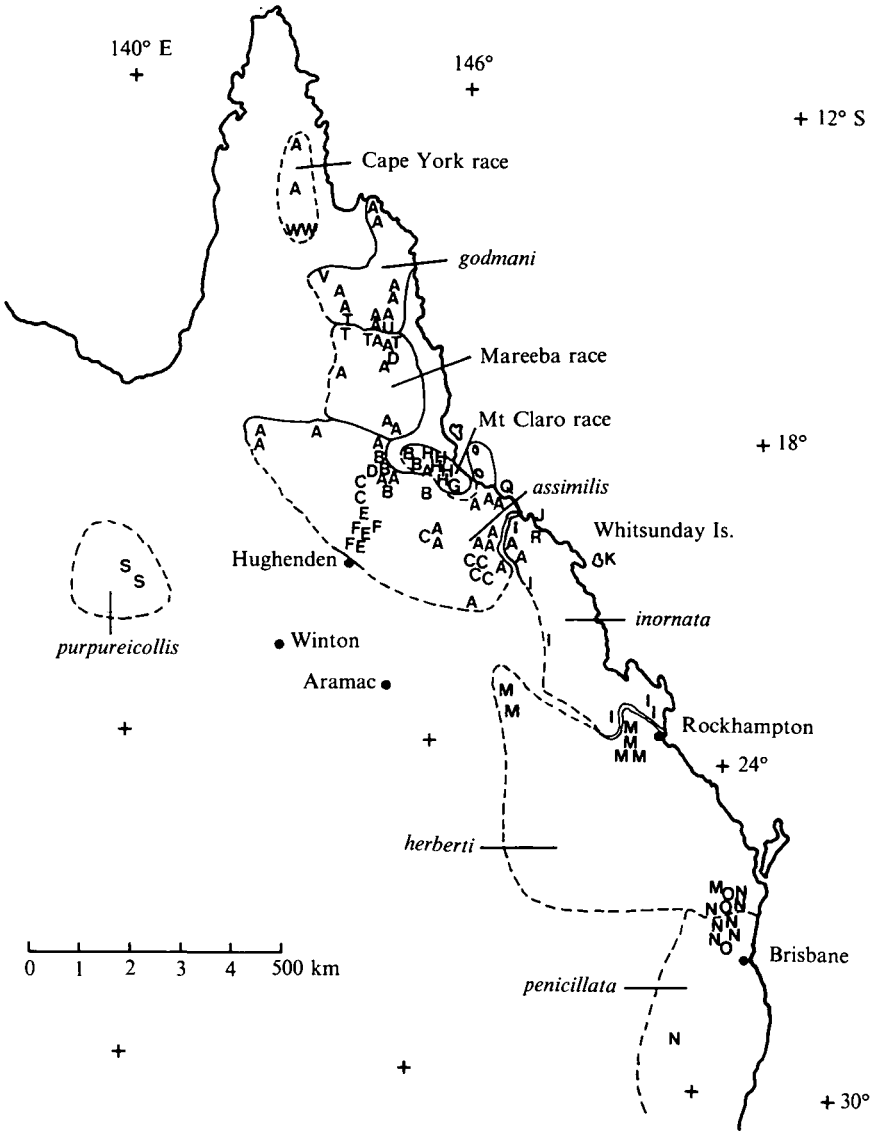

Fig. 2. The distribution of all mtDNA morphs found among the eastern taxa of the *lateralis/penicillata* group of *Petrogale*. The letters correspond to the composite mtDNA morphs shown in Table 1, and restriction morphs shown in Fig. 4.

crossing. On the other hand, chromosome and allozyme markers of the original female will be rapidly lost in the absence of favourable selection or continued migration. Thus mtDNA is a sensitive marker for introgression (see reviews by Takahata & Slatkin, 1984; Avise, 1986; Moritz *et al.* 1987; Harrison, 1989).

(3) Any mutations that accrue in the mitochondria of a female will be passed on to all descendants. Phylogenetic trees may then be compiled to estimate the relatedness of taxa. Such a study, for eastern *Petrogale*, will be described in a subsequent paper; here we deal only with introgression, hybridization and nomenclature.

The analysis of mtDNA has become increasingly popular in the study of species differences (Lansman *et al.* 1983; Bonhomme *et al.* 1989; Dowling & Brown, 1989; Lehman *et al.* 1991), and attempts have been made to simplify procedures to the stage where non-specialists can readily use them. In the present study, we have used those restriction endonucleases which produce mtDNA fragments large enough to be seen without radioactive labelling.

This paper describes the analysis of mtDNA obtained from six described species of *Petrogale* plus three additional 'chromosome races'. Only taxa within the closely related '*lateralis–penicillata*' group (Briscoe *et al.* 1982) from eastern Australia are considered (Fig. 1). Thus the Proserpine rock-wallaby, *P. persephone*, which Briscoe *et al.* (1982) included in the '*xanthopus*' group, is not considered here, even though its distribution lies within that of *P. inornata* (Maynes, 1982).

Specific aims were as follows:

(*a*) to determine whether each chromosomally defined taxon was associated with a particular mtDNA lineage;

(*b*) to trace the extent and direction of any introgression of mtDNA lineages across chromosomal borders;

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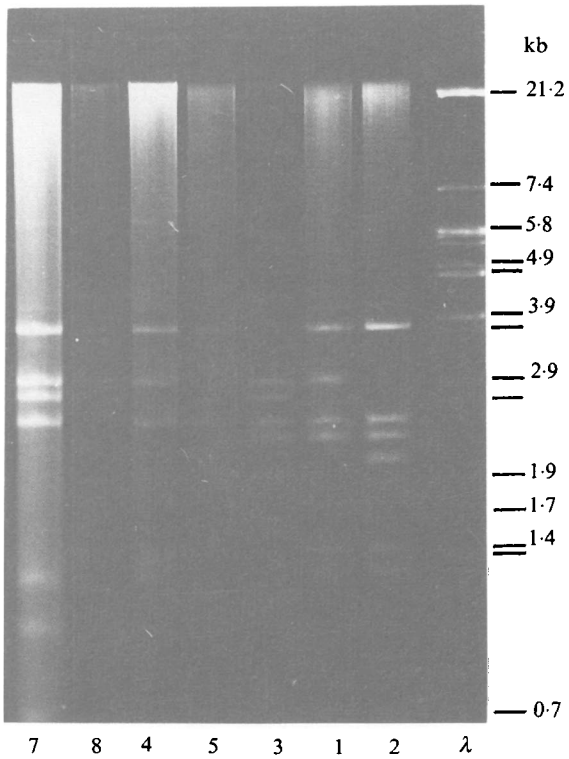

Fig. 3. Photograph of several restriction morphs produced by restriction enzyme *Hpa*II, stained with ethidium bromide and shown with λDNA as a standard. Morphs can be identified from Fig. 4.

(*c*) to seek evidence of reproductive isolation between adjacent taxa;

(*d*) to reassess the nomenclature of the *lateralis–penicillata* group of *Petrogale* from eastern Australia.

2. Methods

Rock-wallabies were collected in the field by shooting or trapping; skins and skulls were lodged with the CSIRO Museum in Canberra. Tissue samples were stored in liquid nitrogen within 2 h of death, and standard bone-marrow preparations for chromosome analysis were made and examined in the field and then stored at −15 °C.

Sample sizes were kept to a minimum, to reduce pressure on several species of rock-wallabies which have suffered declines in numbers since white settlement (Briscoe *et al.* 1982; Kinnear *et al.* 1988; Short & Milkovits, 1990). Moreover, in some localities, animals are difficult to obtain because of the isolated, rugged terrain. In this study mtDNA was obtained from 118 animals from 82 localities. Fig. 2 shows the location of all the different mtDNA types. In some cases, where two or more animals with the same mtDNA type were collected from the same colony, only one record appears in Fig. 2. Full details of numbers taken at each locality are provided in the relevant sections of the text.

Liver aliquots (7 g) were homogenized in 0·25 M sucrose, 1 mM-EDTA, 20 mM-HEPES, 0·05 mg/ml BSA, pH 7·5 and centrifuged 3 times to 1500 *g* for 10 min each. The supernatant was then centrifuged for 10 min at 15000 *g* and the pellet resuspended and recentrifuged for 20 min at 15 000 *g*.

The pellet was resuspended in 2·5 ml SSC (0·15 M-NaCl, 15 mM sodium citrate, pH 7·0), lysed in 1·25 ml SDS (2 % w/v sodium dodecyl sulphate, 0·1 M-EDTA, 0·5 M sodium perchlorate, 0·15 M-NaCl) and 1·25 ml

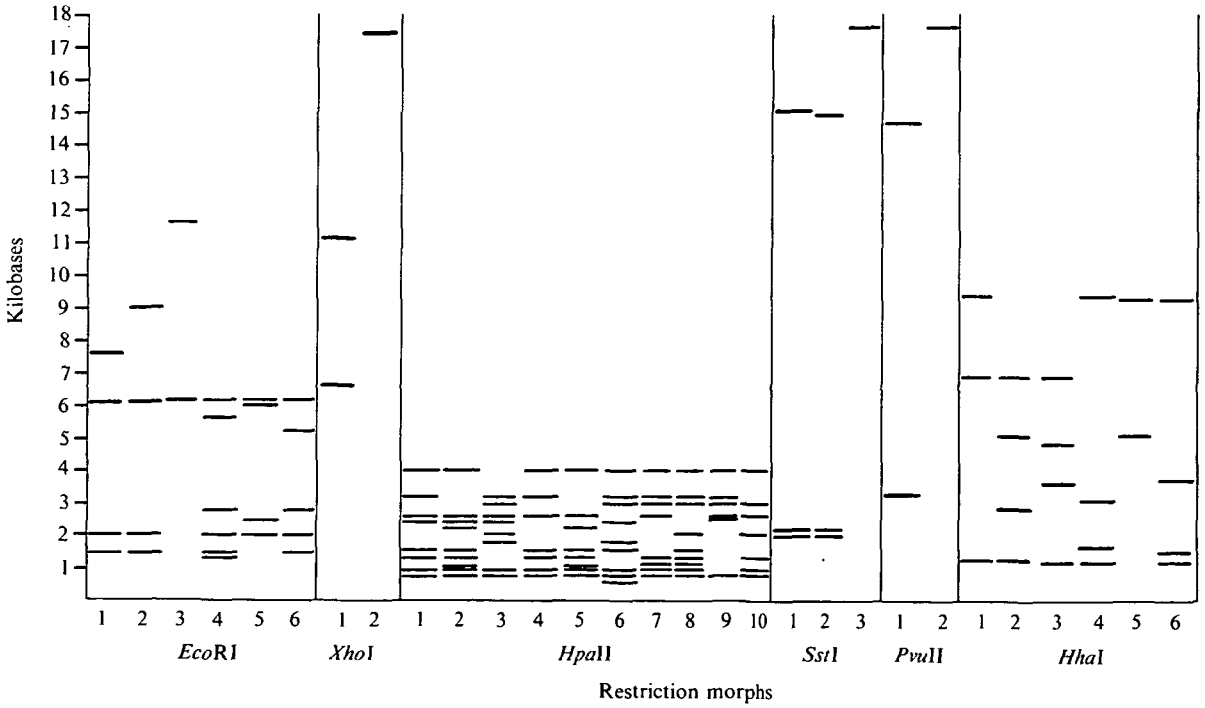

Fig. 4. Restriction fragment length polymorphisms of mtDNA from the *lateralis/penicillata* group of *Petrogale* of eastern Australia. The taxon in which each numbered polymorphism was detected is listed in Table 1.

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Table 1. *Suite of restriction morphs comprising each composite morph. The order of enzymes is Eco*R I, *Xho* I, *Hpa* II, *Sst* I, *Pvu* II *and Hha* I. *Diagrams of each morph are shown in Fig. 4*

Composite morphs	Restriction morphs	Composite morphs	Restriction morphs
N	111211	F	327123
O	112211	D	427121
M	113211	B	327121
I	214121	H	228121
J	215121	G	227321
R	214122	T	627121
K	216122	U	227124
A	227121	V	229125
C	217121	W	229121
Q	527121	S	11 (10) 226
E	227123		

phenol/hydroxyquinoline (phenol saturated in TE buffer (10 mM-tris pH 8, 0·25 mM-EDTA) and 0·1% w/v 8-hydroxyquinone), incubated at 37 °C for 3 min, rotated for 20 min, then centrifuged at 10000 ***g*** for 10 min. An equivolume of chloroform/isoamyl alcohol (24:1) was added to the aqueous layer, rotated 10 min, then centrifuged at 10000 ***g*** for 10 min. The mtDNA was precipitated with 1·5 volumes of cold ethanol, incubated for 2–3 h at −20 °C and centrifuged for 10 min at 10000 ***g***. The pellet of mtDNA was dried, then resuspended in standard buffer (10 mM-tris, 1 mM-EDTA).

This method of extraction provides enough mtDNA of sufficient purity to allow analysis by restriction endonucleases without requiring further purification or radioactive labelling.

Resultant mtDNA was digested with restriction endonucleases in a total volume of 50 μl, and incubated for 4–6 h at 37 °C using conditions suggested by the manufacturers (Boehringer-Mannheim, Bethesda R. L., New England Biolabs). RNAase was added to reactions before the end of digestion (100 μg/ml final concentration). Samples of digested mtDNA were electrophoresed on 0·5 or 1·0% agarose gels with λDNA digested by *Hin*d III–*Eco*R I, and *Eco*R I–*Eco*R V mixtures, as molecular weight standards. Digested fragments were detected by staining with 10 mg/ml ethidium bromide for 30 min. Fig. 3 shows 7 samples digested with the enzyme *Hpa* II.

We follow Brown & Simpson (1981) and Lansman *et al.* (1983) in using the term 'restriction morph' to describe the pattern of restriction fragments obtained from the digestion by a single endonuclease of the mtDNA from one animal. For each enzyme, the different morphs were assigned an arbitrary number (Fig. 4). Results from all enzymes for each animal produced a 'composite morph' which was given a letter-code (Table 1). Animals which share an identical code belong to the same mtDNA clone with respect to those particular sites (Lansman *et al.* 1983). Table 2 lists which composite morph occurred in each taxon.

The mtDNA constitution of an animal in any taxon or locality can be determined by combined use of Tables 1 and 2 and Figs. 2 and 3. Chromosome differences between taxa can be found from Fig. 1, which shows gross karyotypes, and Table 3, which lists additional internal rearrangements of chromosomes, as well as available genetic data.

3. Results

When the circular molecules of mtDNA were cut once by a restriction endonuclease, the linear fragments formed were equivalent in size to marker fragments of 17·4 ± 0·5 kilobases. This value was repeated for the sum of fragments (17·3 ± 0·4 kb) produced by enzymes which cut the molecule in several positions (Fig. 4). Thus the value for this marsupial genus corresponds to that of 16·3 ± 0·4 kb for eutherian mammals (Brown & Vinograd, 1974).

Inspection of Fig. 4 shows that some morphs tally less than 17 kb. In these cases, additional bands may be superimposed on each other or are so small that the ethidium bromide staining was too faint to record them against the background DNA that occurred in some samples.

Nineteen restriction endonucleases were tested on mtDNA samples to find restriction morphs which could discriminate between the rock-wallaby taxa. Three enzymes (*Xba* I, *Bgl* I, *Kpn* I) did not digest the mtDNA, while *Alu* I produced too many fragments.

Table 2. *Composite morphs found in each taxon*

Taxon	Composite morphs	Taxon	Composite morphs
penicillata	N*O	Mareeba race	A*TD
herberti	M*NO	*godmani*	A*UVT
inornata	I*JRKA	Cape York race	AW
assimilis	A*QEFDBC	*purpureicollis*	S
Mt Claro race	AHGD		

* denotes a major morph, i.e. one found throughout the latitudinal range of the taxon.

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Table 3. *Summary of genetic and geographical data for taxa in contact or possible contact*

Adjacent taxa	Autosomal differences[a]	Known geographical separation	Introgression of mtDNA	Restriction site differences	Enzymes showing differences	Fixed gene differences (allozymes)[b]
A *penicillata*; *herberti*	(*a*) centric shift (CS) of No. 4 chromosome	Hybrid colony found	Yes	2	*Hpa* II (2)	4
B *herberti*; *inornata*	(*b*) different form of (*a*) above (*c*) CS of No. 3 (*d*) inversion of No. 5	20 km Fitzroy River	No	6	*Eco*R I (1) *Hpa* II (3)	3 *Sst* I (1) *Pvu* II (1)
C *herberti*; *assimilis*	(*a*) and (*d*) above (*e*) centric fusion (CF) of Nos. 6, 10	100 km of suitable but scattered habitat	No	8	*Eco*R I (1) *Hpa* II (4) *Sst* I (1) *Pvu* II (1) *Xho* I (1)	1
D *herberti*; *purpureicollis*	(*a*) and (*c*) above (*f*) pericentric inversion of No. 7	600 km, but unidentified colonies reported midway in last 50 years	No	7	*Hpa* II (5) *Pvu* II (1) *Hha* I (1)	5
E *inornata*; *assimilis*	(*b*), (*c*) and (*e*) above	10 km; Burdekin, Bowen Rivers	Yes	4	*Xho* I (1) *Hpa* II (3)	1
F *assimilis*; *purpureicollis*	(*c*), (*d*), (*e*) and (*f*) above	360 km; but unidentified colonies reported equidistant till 1979	No	9	*Xho* I (1) *Eco*R I (1) *Hpa* II (5) *Sst* I (5) *Hha* I (1)	4
G *assimilis*; Mt Claro race	(*d*), (*e*) above (*g*) CF of Nos. 5, 10	6 km. No observable barrier	Likely	1 (Morph H) 2 (Morph G) shared morphs (A, B)	*Hpa* II (1) *Sst* I (2)	0
H Mt Claro race; Mareeba race	(*h*) CS of CF of Nos. 6, 9	30 km; Burdekin River may separate	Possibly	1 (Morph H) 1 (Morph G) shared Morph A	*Hpa* II (1) *Sst* I (1)	0
I *assimilis*; Mareeba race	(*d*), (*e*), (*g*) and (*h*) above	20 km; no observable barrier	Possibly	Shared morphs (A) and (D)	—	0
J Mareeba race; *godmani*	(*d*), (*e*), (*g*) and (*h*) above; (*i*) CS of (*e*)	Hybrid colony; Mitchell River separates western colonies	Yes	Shared morphs (A, T)	—	4
K *godmani*; Cape York race	(*b*), (*i*), (*d*) as above	100 km of apparently suitable but scattered habitat	Possibly	Shared morph (A); Morphs (W) and (V) share unique *Hpa* II site	—	0

[a] Sharman *et al.* (1990); Eldridge *et al.* (1988, 1989, 1990, 1991). Restriction site differences are the minimum number of site gains or losses which distinguish the two taxa. Gains or losses are relative to composite morph N (see text and Fig. 5). The number of parentheses, following the restriction enzymes showing differences, is the number of site gains or losses for the enzyme.

[b] Allozyme data from Briscoe *et al.* (1982) and Briscoe (1991). Fixed gene differences include some enzymes for which alleles were found at low frequency in atypical taxa close to a hybrid zone.

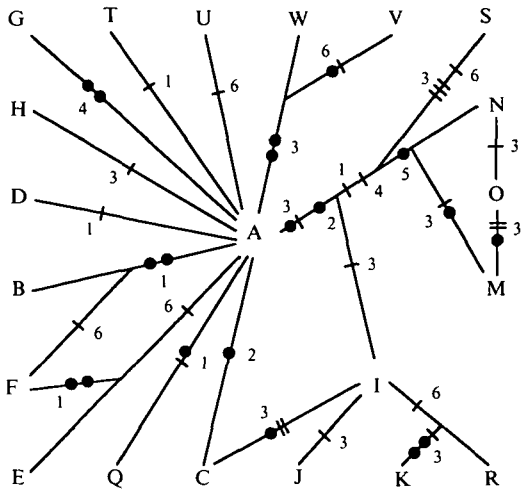

Fig. 5. Dendrogram showing relationships between mtDNA composite morphs (letter codes are listed in Table 2). Interlinking lines show the restriction site losses (+) or gains (●) which distinguish morphs; lines do not represent genetic distance. Gains and losses are all relative to composite morph N (see text). Numbers 1–6 represent restriction enzymes, as for Table 1, ie, 1 = *Eco*R I, 2 = *Xho* I, 3 = *Hpa* II, 4 = *Sst* I, 5 = *Pvu* II, 6 = *Hha* I. Gains and losses are all relative to composite morph N (see text).

A further five (*Pst* I, *Bgl* II, *Bst*E II, *Bam*H I, *Apa* I) cut once while *Sal* I and *Sst* II cut twice and *Eco*R V cut thrice but produced the same morph for all taxa. Another (*Hin*d III), was apparently so sensitive to slight impurities in the preparations that many samples did not digest. Six enzymes (Fig. 4) produced clear fragments which discriminated between several taxa.

Fig. 5 is a dendrogram, prepared by comparing the RFLPs from Fig. 4, which links all the morphs according to the number of shared restriction fragments. For convenience, morph N of *penicillata* was arbitrarily considered to be the ancestral form, and all different sites are shown as gains or losses relative to that morph. For the 4-base enzyme *Hpa* II, however, some patterns involving two or more differences can have alternative interpretations. Those depicted, therefore, should serve only as a qualitative guide to the changes detected.

Morph A is shown as ancestral to all other morphs found in the five northern taxa. This decision is based on the widespread distribution of morph A throughout the latitudinal range of the five taxa, the localized distribution of the other morphs and because it is linked by the least number of site changes to all other morphs. For these reasons, morph A is hereafter called the 'major morph' for these taxa. Similarly morph I of *inornata*, morph N of *penicillata* and morph M of *herberti*, with distributions throughout the latitudinal ranges of each species, are also termed the 'major morphs'. Within the range of morph A, however, one of the other morphs may have been ancestral but now has a limited distribution. Morph C, for example, could be ancestral to both morphs A and I since it shares the *Xho* I pattern with morph I and the *Hpa* II pattern with morph A. Alternatively, independent mutations produced equivalent *Xho* I patterns in morphs I and C. Convergent mutations are also required to explain the relationship of morphs B, F and E.

(i) penicillata/herberti *contact zone*

Fig. 6 shows the localities of colonies of *penicillata* and *herberti* near Nanango, 100 km NW of Brisbane, and the number of mtDNA typings. These taxa differ by an apparent centromeric transposition of the number four chromosome (Fig. 1; Eldridge *et al.* 1990). The discrete colonies were located along thickly wooded creek gorges or rocky gullies at intervals of several kilometres. Chromosomal hybrids were found at one colony, on Yarraman Creek. Equal frequencies of each type of chromosome four were detected at this colony in 1976 from five animals (Briscoe *et al.* 1982) and again, in 1984 (this study), from three. Female hybrids carried pouch young, and a male hybrid had normal-sized tests and produced sperm. In meiotic spreads from these testes, a bridge and fragment was detected in about 25% of anaphase I cells (Sharman *et al.* 1990). All chromosomal hybrids were found to be backcross hybrids by electrophoretic analysis (A. A. Gooley and D. A. Briscoe, pers. comm.).

Only one restriction enzyme, *Hpa* II, distinguished the two taxa. This enzyme cuts a specific sequence of four bases, unlike the other enzymes used which cut sequences of six; hence, it is more sensitive to sequence changes. The major morph N of *penicillata* occurred at least 800 km south of Brisbane, while the major morph M of *inornata* was found as far north as Rockhampton (Fig. 2). Morph N of *penicillata* was found in two animals in the hybrid colony and in two others in a nearby colony of *herberti*. A third morph, O, had a curious distribution close to the chromosomal border; it occurred in two *herberti* and in one *penicillata* further south. Morph O differs by only one site change from morph N, but by three additional changes from morph M (Fig. 5). Morph M. of *herberti* was found in one *herberti* 40 km north of the chromosome border. No intervening colonies were found.

(ii) herberti/inornata *contact zone*

Fig. 7 shows collection localities, species borders and all mtDNA types found about the Fitzroy River which separates *herberti* and *inornata*. The karyotypes of the two species differ by rearrangements of the third and fourth largest chromosomes, and an inversion of the fifth (Eldridge *et al.* 1990). The respective mtDNA clones (major morphs M and I) have developed at least six site changes detected by four restriction endonucleases. No evidence of

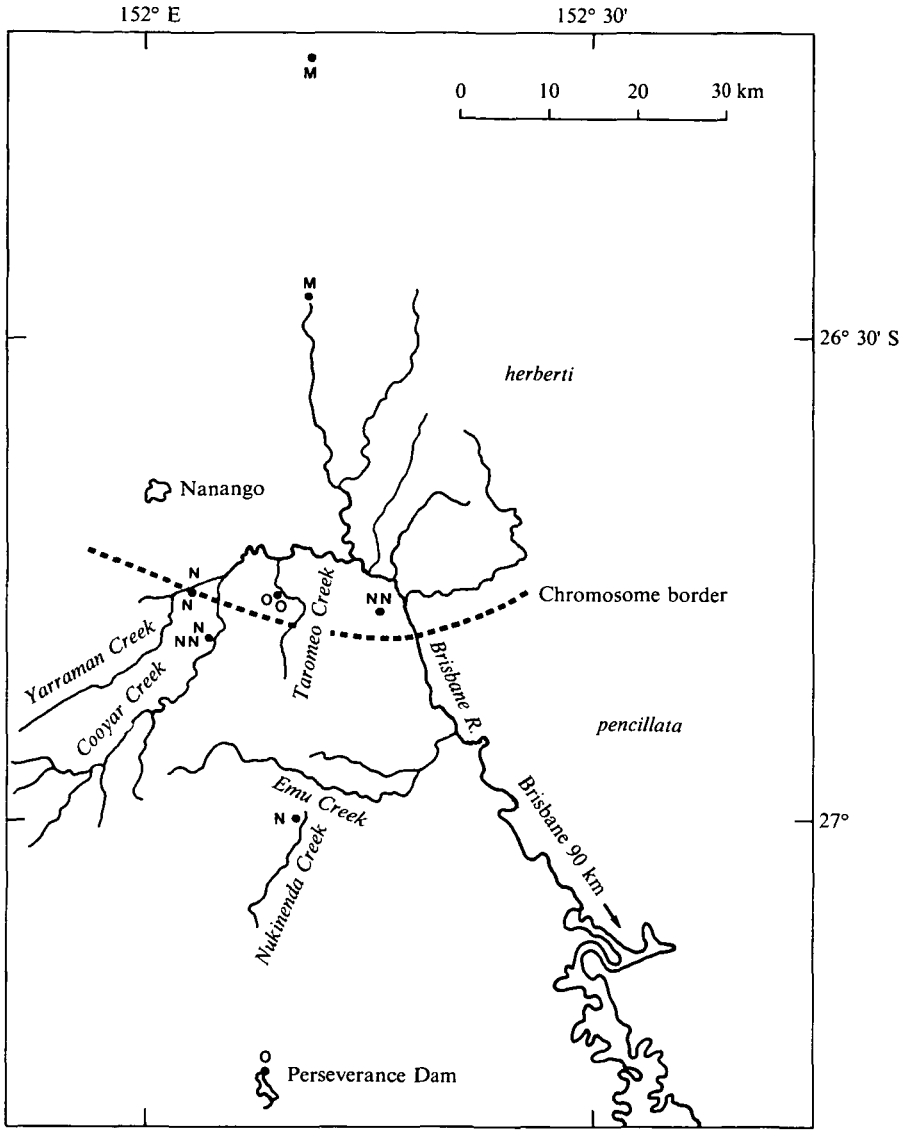

Fig. 6. The distribution of three mtDNA morphs (M, N and O) at the chromosome boundary between *penicillata* and *herberti*. The single colony containing chromosomal hybrids was found at Yarraman Creek.

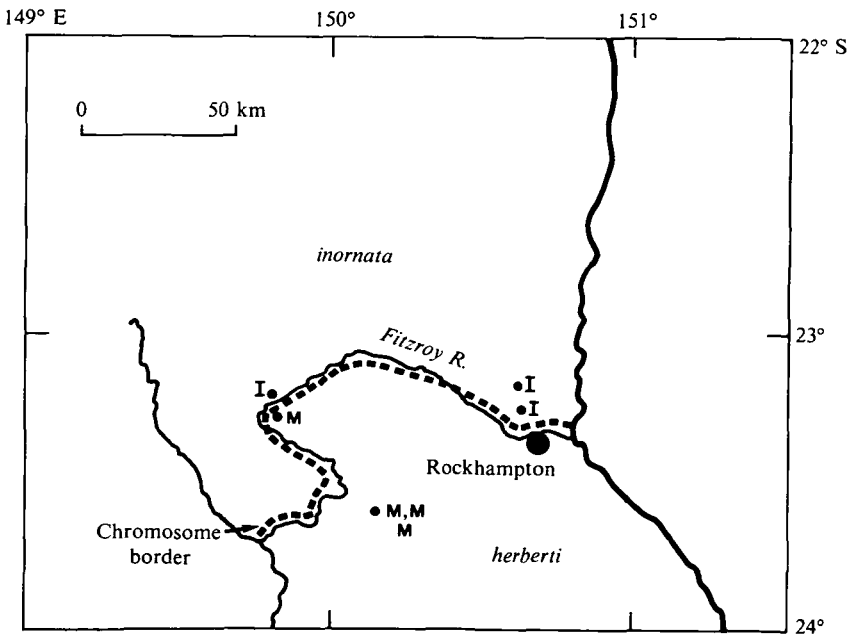

Fig. 7. The distribution of two mtDNA morphs (I and M) at the chromosome border between *inornata* and *penicillata*.

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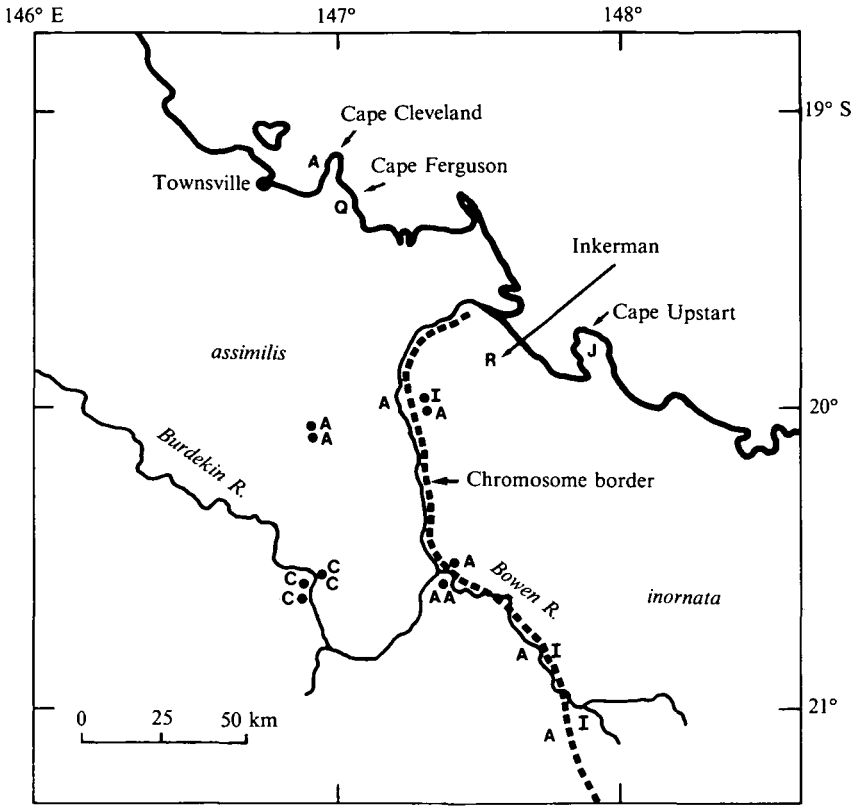

Fig. 8. The distribution of six mtDNA morphs (A, C, I, J, R and Q) at the chromosome border between *inornata* and *assimilis*.

mtDNA introgression was found at the junction of the two species.

(iii) herberti/assimilis *potential contact*

These species differ by an apparent centromeric transposition of chromosome four, a centric fusion of chromosomes six and ten and an inversion of the fifth (Table 3; Briscoe *et al.* 1982; Eldridge *et al.* 1990). Although the closest known colonies are 120 km apart, there is apparently suitable but isolated habitat midway between. The northern 60 km of this habitat has not yet been surveyed.

Adjacent colonies of *herberti* and *assimilis* each carried the major mtDNA morphs M and A of their respective species. All but one of the six restriction enzymes produced different patterns. At least six site differences were detected from five different enzymes. Introgression was not detected. However, colonies were scarce and individuals rare near the junction of the species. Only one *assimilis* and three *herberti* were collected.

(iv) herberti/purpureicollis *potential contact*

A distance of 600 km separates known colonies of these species (Fig. 1). However, local reports indicate that a colony of rock-wallabies existed at a gorge near Winton at least until 1979, and until 50 years ago near Aramac (Fig. 1). Consistent with the former claim was our discovery in 1984 of the distinctive *Petrogale* faecal pellets at the Winton site. These were old, dry and deep in the recesses of a rocky gorge. As the Winton and Aramac colonies are 375 and 125 km from the most westerly known colony of *herberti*, and 285 and 435 km from the nearest *purpureicollis* colonies, recent contact is possible. The two species differ by changes to two chromosomes representing three chromosomal rearrangements (Table 3; Briscoe *et al.* 1982; Eldridge *et al.* 1990).

The mtDNA composite morphs have identical restriction patterns for the enzymes *Xho* I, *Eco*R I, and *Sst* I. Differences in the patterns from the remaining three enzymes can be accounted for by four site changes (Fig. 5). There is no indication of hybridization or introgression.

(v) inornata/assimilis *contact zone*

These species differ by at least three chromosomal rearrangements (Table 3; Eldridge *et al.* 1990). The Burdekin and Bowen River systems separate the two species for a distance of 200 km but *inornata* occurs on the western bank 50 km south of the Burdekin/Bowen junction (Fig. 8). At the border of these taxa, the major morph A of *assimilis*, which extends a further 1000 km north, meets the major morph I of *inornata*, which extends 600 km to the southern limit of the species.

Of the eleven *assimilis* captured near the chromosomal border, seven carried morph A, and four morph C. Morph A, however, was also found in two animals, with chromosomes diagnostic of *inornata*, close to the border of the two species. Three other *inornata* from near the border had morph I. At least four mtDNA base changes, detected by the restriction enzymes *Xho* I (one change) and *Hpa* II (three changes), distinguish the two morphs (Fig. 5).

Five other morphs have local distributions. Morph C was found in four *assimilis* on either side of the Burdekin River 60 km upstream from the Bowen junction. This morph shares its *Hpa* II pattern with morph A, but has the same *Xho* I pattern as morph I. Along with morph A, it was also found in colonies 65 and 170 km to the north-west. Cape Upstart, site of the morph J (2 animals collected), is currently separated from the mainland by tidal flats, and would have been completely separated by higher sea levels 6000–6500 years B.P. (Flood, 1983). A single base change detected by the enzyme *Hpa* II (Table 1, Fig. 2), distinguishes morphs J and I, and may have arisen since the geographical separation of the two lineages. Morph R (one animal) was found immediately adjacent to Cape Upstart, at Inkerman. This morph is distinguished from morph I by a unique site gain for *Hha* I (Fig. 5). The same *Hha* I pattern was found 140 km further south at Whitsunday Island (Morph K) (Fig. 8). These island animals, however, have developed a distinctive *Hpa* II pattern. Barring convergent changes, therefore, the Whitsunday and Inkerman animals had developed their common *Hha* I site prior to the flooding of the straits which separated Whitsunday Island and the mainland less than 9000 years ago (see sea-level curve in Chappell (1983) and depth charts of the Australian Hydrographic Service). Presumably, the R morph reached its current mainland position after separation of Cape Upstart.

Cape Ferguson lies 100 km northwest of Cape Upstart and hosts *assimilis* (one animal collected) with a distinctive *EcoR* I site (Morph Q). Cape Cleveland, a further 10 km north, however, carries *assimilis* with the A morph (one animal). Like Cape Upstart, both these capes are separated from the mainland by salt pans (Barker & Close, 1990).

(vi) assimilis/purpureicollis *potential contact*

The closest known colonies of these species are separated by 360 km. Despite the present separation the species may have been in relatively recent contact, as rock-wallabies, of unknown origin, survived until at least 1979 near Winton (see (iv) above). Winton lies 200–300 km from the nearest known colonies of both species (Fig. 1).

Four chromosomal rearrangements distinguish the two species (Briscoe *et al.* 1982; Eldridge, 1991). However, a metacentric number 7 chromosome similar to that found in *purpureicollis* was found in 1977, and again in 1984, in a single colony of *assimilis* near Hughenden on the southwestern margin of the species' distribution (Briscoe *et al.* 1982). This general area includes the type locality of *P. puella*, which was described in 1926 and distinguished from *P. assimilis* mainly by its smaller size. At that time, *assimilis* was known only from Great Palm Island, near Townsville. Despite extensive searches in the area, we found no evidence of a population that was maintaining a separate gene pool from that of the taxon we attribute to *assimilis*. It appears that if *puella* ever existed it has now been absorbed by *assimilis* near its type locality.

However, in addition to the chromosome polymorphism described above, anomalies in the distribution of some allozymes, endo- and ectoparasites pinpoint the Hughenden area as one of unusual biological interest (Beveridge *et al.* 1989; Barker & Close, 1990; Briscoe, 1991). Two mtDNA polymorphisms were also found in the area: morph E is derived from the major morph A by an *Hha* I site change; morph F, in turn, shares the *Hha* I pattern of morph E, but has lost two *Eco*R I sites. This unusual genetic variation possibly represents the remains of a population, perhaps attributable to *puella*, which may still occur near Winton (see (iv) above). If so, examination of these animals should reveal the genetic markers found at Hughenden.

Four restriction enzymes produce the different patterns seen in the major mtDNA morph A of *assimilis* and morph S of *purpureicollis*, with a minimum number of nine detected changes in restriction sites separating the two lineages. The mtDNA data indicate no evidence of introgression.

(vii) assimilis/*Mt Claro race contact zone*

Adjacent colonies were found 6 km apart, with no obvious geographical barriers separating them (Fig. 9). However, as with all other taxa, each taxon has a unique bioclimatic profile (Sharman *et al.* 1990) as forecast by the bioclimate prediction system BIOCLIM (Nix, 1986). Although members of individual colonies can often be distinguished morphologically, so great is the variation within each taxon that the two groups in general can not be (unpublished data). Their karyotypes, however, differ markedly, with *P. assimilis* having a 6- to 10-centric fusion, and Mt Claro race a 5- to 10-centric fusion. Moreover, the chromosome 5 of the former is inverted relative to that of Mt Claro race (Eldridge *et al.* 1988). Although each taxon has unshared allozyme polymorphisms, no fixed gene difference was detected between the two groups (Briscoe *et al.* 1982).

Despite the close proximity of the two taxa, no chromosomal hybrids or colonies composed of members representing both karyotypes have been discovered in the wild. However, a male hybrid bred

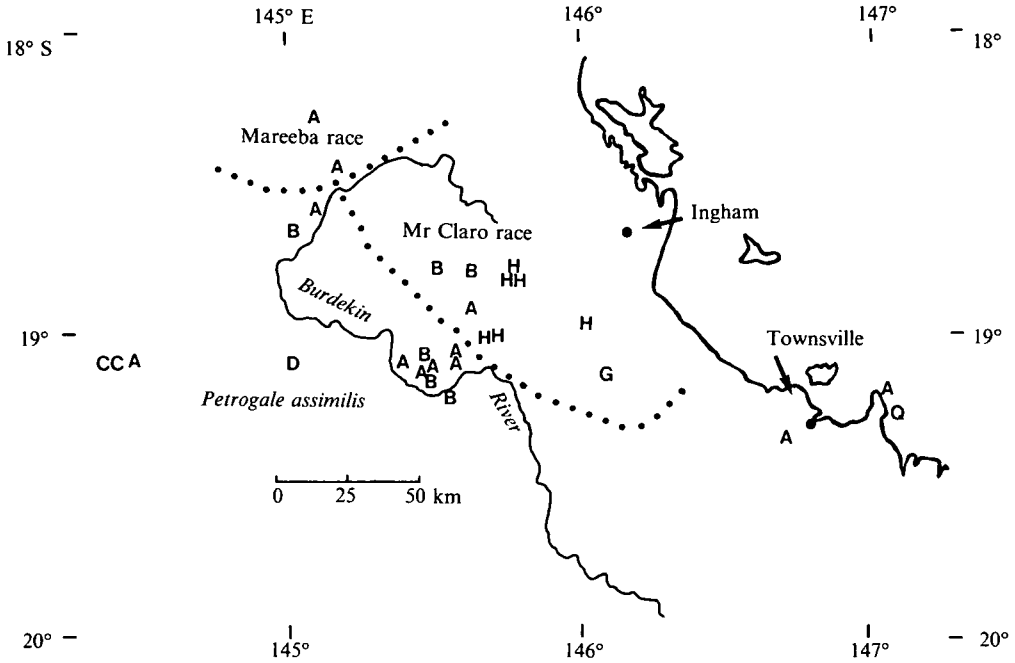

Fig. 9. The distribution of mtDNA morphs (A, D, H, G and B) at the chromosome border between *assimilis* and Mt Claro race.

in captivity was sterile (Sharman *et al.* 1990), and a male with aberrant testes was caught at the contact zone (Bell *et al.* 1989).

Among the 10 Mt Claro race animals examined there were four different mtDNA morphs (Table 1; Fig. 9). One animal, from close to the chromosome border, carried the major morph A of *assimilis*. The six animals from three different colonies carried morph H, which was unique to Mt Claro race but can be derived from morph A by one site change for the four-base enzyme *Hpa* II. Morph G, found in one animal, was also unique to Mt Claro race, and differs from morph A by two site changes for the six-base enzyme *Sst* I. The third morph, B, differs by two *Eco*R I site changes from morph A, and was found in two animals at two Mt Claro race colonies and also in four animals at three *assimilis* colonies close to the chromosome border (Fig. 9).

A fifth morph, D, which differs from morph A by at least two *Eco*R I site changes and from morph B by at least three changes, was found in one *assimilis* colony 30 km southwest of the chromosomal boundary. This morph was also found in a Mareeba race animal found near the more northerly contact zone between *godmani* and Mareeba race (Fig. 10). Of the eleven *assimilis* collected within 50 km of Mt Claro race, six had morph A, four B and one D.

Finding both morphs A and B on either side of the chromosome border may be evidence of hybridization of the two *Petrogale* groups. While the presence of morph A in Mt Claro race may predate the chromosome changes, morph B was only found within 30 km of the chromosomal border. The simplest explanation, therefore, is that some animals have hybridized.

(viii) *Mt Claro race/Mareeba race presumed contact zone*

The Mareeba race shares with Mt Claro race the 5- to 10-chromosome fusion, but in addition has a fusion of the 6 and 9 chromosomes which resulted from a centric fusion followed by a centric shift (Eldridge *et al.* 1988). Despite the chromosomal differences, the taxa cannot be distinguished reliably in the field on morphology alone. Furthermore, no fixed gene difference has been detected electrophoretically, although each taxon has unequal frequencies of polymorphisms (Briscoe *et al.* 1982).

The nearest known colonies are 40 km apart, separated by the Burdekin River. Although the Seaview and Gorge Ranges link known colonies and contain much rocky terrain, it was our impression that this area, in which no *Petrogale* were found, was considerably wetter than adjacent occupied areas. Vegetation surrounding the unoccupied rocky areas was thicker and more lush, and stands of rainforest were common. These observations are consistent with the predictions of BIOCLIM that distributions of *Petrogale* are defined by bioclimatic features (Sharman *et al.* 1990).

No chromosomal hybrids have been found in the wild. However, a male hybrid has been bred in captivity. This animal was sterile, having many abnormal spermatozoa (Sharman *et al.* 1990).

Although the mtDNA of only two Mareeba race animals from near the chromosome border has been examined, both samples carried morph A, which is found throughout the range of the taxon. Since this is also the major morph for *assimilis*, and is also found

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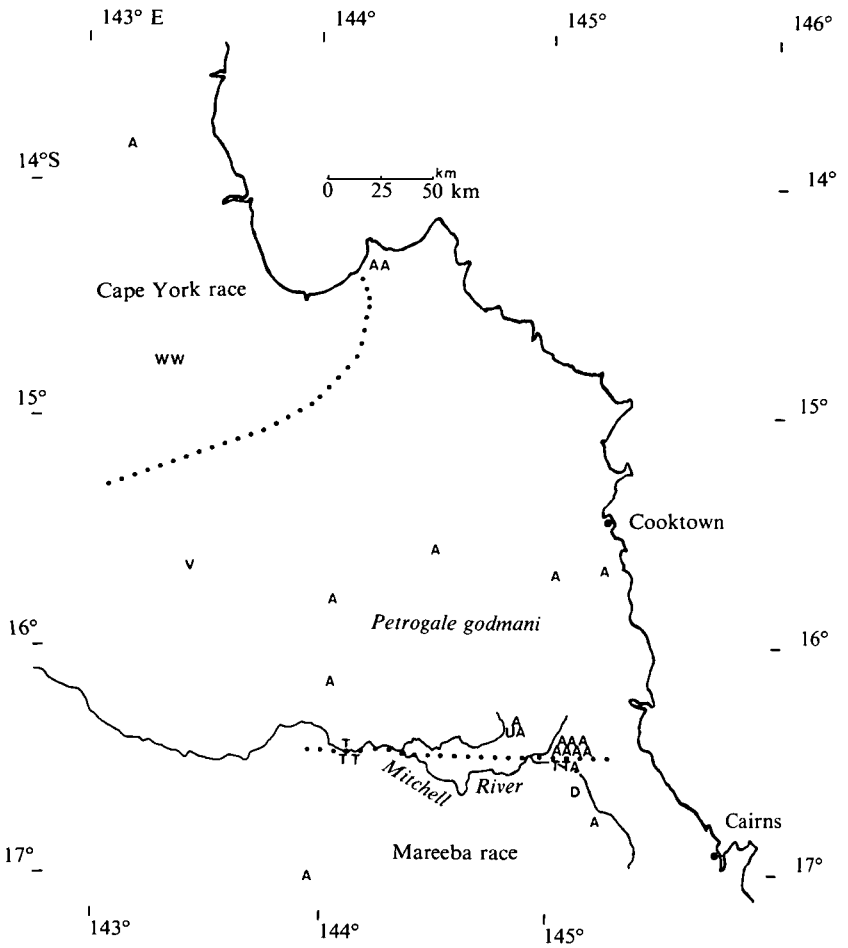

Fig. 10. The distribution of mtDNA morphs (A, D, T and W) at the chromosome borders of Mareeba race and *godmani*, and *godmani* and Cape York race.

in one Mt Claro race animal, its presence may predate the chromosomal changes or result from widespread introgression. Further studies are required to determine whether the unique mtDNA morphs of the Mt Claro race occur in border populations of the Mareeba race.

As described above, only morph A, which is also the major morph of *assimilis*, was found in Mareeba race animals close to the chromosome border (Fig. 9). Further collections are required to determine whether the nearby morph B of *assimilis* and Mt Claro race also occurs in Mareeba race.

(ix) *Mareeba race*/assimilis *contact zone*

These taxa differ by at least five chromosomal changes (Table 3), and although allele frequencies differ markedly for three allozymes, no fixed gene difference has been detected electrophoretically (Briscoe *et al.* 1982). The animals are morphologically similar, and are not separated by any obvious geographical or vegetation barrier. Indeed, colonies of both taxa were found on rocky areas of the western banks of the Burdekin River and its tributary, Anthill Creek, except in the 15 km which separated the colonies.

No chromosomal hybrids have been found in the wild, but three hybrids have been bred in captivity. The male hybrid was sterile, producing no sperm, while a female hybrid produced two young (Sharman *et al.* 1990). A full sister of the latter produced no offspring, and inspection of its reproductive system revealed abnormalities of the uterus and ovaries (unpublished data).

(x) *Mareeba race*/godmani *contact zone*

In *godmani*, the number 6 and 10 chromosomes are fused to form an acrocentric, which Eldridge *et al.* (1989) have shown to be derived from a centric fusion, as in *assimilis*, followed by a centric shift. Thus the chromosome differences between this species and the Mareeba race are extensive. The border between the two taxa extends generally along the Mitchell River for a known distance of 100 km (Fig. 10).

Chromosomal hybrids were found on a small hill located 13 km and 5 km from parent colonies. Allozyme studies of the zone detected four fixed gene differences which distinguish the taxa and showed the hybrids to be backcrosses, and also detected four alleles with markedly different frequencies on either side of the zone (Briscoe *et al.* 1982; Briscoe, 1991).

Despite these chromosomal and genic differences, the mtDNA morph A found in the Mareeba race also extends throughout the latitudinal range of *godmani*

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(Fig. 10). An additional morph, T, was found at two locations. At one, it was carried by a *godmani* and also by two Mareeba race animals on adjacent banks of the Mitchell River. At the other, it was found in two Mareeba race animals 100 km to the east and at the closest point to the hybrid colony. This morph can be derived from morph A by one *Eco*R I site change. A third morph U was detected only 10 km from the hybrid colony in a single *godmani*. This morph can be derived from morph A by at least two *Hha* I site changes. Of the 12 *godmani* found within 30 km of the chromosome border, 10 were morph A, 1 was T and 1 U. Of the 7 Mareeba race, 3 were A and 4 T. Further from the border, 5 *godmani* were A and 1 was V. Hence the distribution of morph T appears unequally distributed around the border.

(xi) *Cape York race*/godmani *presumed contact zone*

A gap of 100 km separates the closest known colonies of these taxa, despite intervening rocky country. This is the only apparent break between taxa in over 3000 km of the Great Dividing Range. At least four structural changes distinguish the chromosomes of the taxa, yet the allozymes are similar (Eldridge *et al.* 1989; Briscoe, 1991). Specimens of Cape York race were difficult to obtain and were found only in widely scattered, small colonies throughout its range.

The mtDNA morph A also spans the distribution of the Cape York race, giving that morph a range of 950 km across five taxa (Figs. 2, 10). Other morphs, however, occur on the chromosome borders: morph W, with at least two *Hpa* II site changes, occurs in the Cape York race, while morph V, with the same *Hpa* II sites, but with two additional *Hha* I site changes, was found in the nearest colony of *godmani*. Either the change that produced morph W preceded the chromosomal changes, or introgression of morph W occurred in one or other direction, followed by the changes required to produce morph V in *godmani*.

4. Discussion

The mtDNA of *Petrogale* consists of $17{\cdot}4 \pm 0{\cdot}5$ kilobases, a figure similar to that recorded for other mammalian species (Brown & Vinograd, 1974). Six restriction enzymes produced five unique composite morphs by which four of the nine taxa could be recognized. These unique or 'major' morphs extended throughout their latitudinal ranges.

Five taxa, *assimilis*, *godmani*, and the Mareeba, Mt Claro and Cape York races shared a fifth major morph. Fig. 5 shows that 10 minor morphs with localized distributions can be derived from this shared morph A by changes detected by single enzymes. Two additional morphs V and F can be derived from adjacent minor morphs. Even the unique minor morphs H and G of Mt Claro race are more closely related to morph A than to each other. Thus even if one of the minor morphs, such as morph C, is ancestral and has survived in a small area, morph A has spread most widely and is undoubtedly ancestral to most local morphs. Similarly, morph I of *inornata* has the widest distribution and is most closely related to all other morphs in the species.

Branch lengths shown in Fig. 5 are not measures of genetic distance. If the original populations were polymorphic, actual evolutionary pathways can differ significantly from those constructed from DNA sequences, particularly in recently diverged species (Pamilo & Nei, 1988). The value of Fig. 5, therefore, when used in conjunction with Fig. 2, is to illustrate the significance of the different morphs at borders between species.

Differences detected between the major morphs, and the presence of derived morphs at boundaries, allowed the hypothesis of hybridization and subsequent introgression to be tested. Finding the major morph of one taxon in animals which carry the chromosomes diagnostic of another is good evidence that animals with different karyotypes have hybridized. Alternative hypotheses are that convergent changes in the mtDNA account for the similarity, or that ancestral mtDNA sequences predate the chromosomal changes. Introgression is the most likely explanation where the anomalous morphs were only found close to the borders of the two taxa.

Such anomalies involving major morphs were found at two of the contact zones examined: (1) *penicillata*/*herberti*, (2) the *inornata*/*assimilis* (Table 2). Anomalies with minor morphs were found at two other contact zones: (3) *assimilis*/Mt Claro race; (4) Mareeba race/*godmani*.

Introgression was indicated at zone (1), where the major morph N of *penicillata* was found in two animals with the *herberti* karyotype 20 km east of a known hybrid colony (see Results, i). A minor morph O, only one restriction site different from morph N, was also found on either side of the chromosomal border (Fig. 6).

Unless morph O is an ancestral form predating the divergence of morphs N and M, its distribution would result from introgression. Since morph O differs by only one site change from the *penicillata* morph N, and by three from the *herberti* morph M (Fig. 6), the latter hypothesis is more likely to be correct. Admittedly, few animals were sampled, and further collections might detect a wider distribution of morph O. However, allozyme data obtained from the animals are consistent with the hypothesis of introgression. Here relatively high frequencies of alleles typical of *penicillata* were found within 25 km of the hybrid zone, and low frequencies of *herberti* alleles were found in *penicillata* animals (Briscoe, 1991). Furthermore, results of electrophoresis of lice carried by the same animals indicate a succession of contacts and separations between the two taxa (Barker & Close, 1990).

Maternal inheritance of mtDNA requires that for mtDNA morphs to introgress across chromosomal borders, females must invade, then produce fertile hybrid offspring. Similar distribution patterns, however, would arise if males of one species had moved across the previous chromosomal border, hybridized with the original females, and subsequently with their own daughters and granddaughters. In this way, chromosomes of the invaders could dominate the colony, leaving the mtDNA unchanged. Thus the chromosome border may have moved southwards as *herberti* males established in *penicillata* colonies.

Similarly, at contact zone (2) the *inornata/assimilis* chromosomal border may be moving northwards leaving *assimilis* mtDNA to mark the former distribution (Fig. 7).

Two animals bearing the major morph A of *assimilis*, yet with the chromosomes of *inornata*, were found at two adjacent localities within 10 km of the Burdekin/Bowen Rivers, which separate the two species for almost 200 km. These anomalous A morphs may have arisen independently from the I morph of *inornata* by four convergent site changes. It is also possible, as discussed in Results (v), that both morphs might be derived from morph C (Fig. 5). Alternatively, female *assimilis* and male *inornata* have produced fertile hybrids. Subsequent backcrossing to *inornata* would then lead to animals with the homozygous karyotype of the latter species but with the mtDNA of the former.

At contact zone 3 (see Results, vii) morph B was found in colonies of both Mt Claro race and *assimilis* collected close to the border, but nowhere else (Fig. 9). On the one hand, the presence of morph B in both taxa may be due to introgression. On the other, high frequencies of H in Mt Claro (6/10) and of A (6/11) in *assimilis*, and the absence of H in *assimilis* and the paucity of A in Mt Claro (1/10) indicate that introgression may be limited. These data are consistent with allozyme studies where allele frequencies differed widely between the two taxa, although no fixed gene difference was detected (Briscoe *et al.* 1982; Table 3).

At contact zone 4 (see Results, x), the finding of morph T in both *godmani* and Mareeba race is likely to be the result of introgression. Morph T was discovered in a single *godmani* on the opposite bank of a small, boulder-strewn river to two Mareeba race animals also with morph T (Fig. 10). Had the existence of morph T predated the extensive chromosome and allozyme changes which distinguish the taxa (Table 3), its distribution would have been expected to be more extensive.

Hybrids and backcross hybrids were discovered at one of nine colonies at the Mareeba/*godmani* contact zones. This discovery demonstrates at least partial fertility of hybrids, although a male hybrid was sterile (Sharman *et al.* 1990).

Hence introgression is indicated at the Mareeba/*godmani* zone, although the distribution of morph T suggests it is not extensive. Four fixed gene differences, four unequally shared polymorphisms from a total of 50 electrophoretic loci and at least six chromosome differences mark this border (Briscoe *et al.* 1982; Table 3). Moreover, the distribution of lice species in this area suggests that contact between the two host species has been relatively recent (Barker & Close, 1990). Yet morph A spans the distribution of these taxa and three others besides, over a distance of 950 km.

This distribution would result if morph A was ancestral and predated fixation of the diverse chromosome rearrangements and distinctive alleles of the different taxa. Given the 5- to 10-fold more rapid rate of mutation in mtDNA than in nuclear DNA (Ferris, 1983*a*) during the time required for fixation, considerable changes would be expected in the mtDNA. However, only those mutations which generate new restriction sites or change existing ones will be detected by the enzymes used in this study. For the latter class of mutations, the 15 enzymes which cut the DNA (32 sites) would sample only 176 base pairs (1% of the genome). Thus the mtDNA of the various populations undoubtedly differ by many mutations not detected by the suite of enzymes. Saitou & Nei (1986) suggested that 2600–2700 nucleotides would have to be examined to produce a reliable evolutionary tree from the mtDNA of primates.

On the other hand, the suite of enzymes was able to distinguish the mtDNA of all other rock-wallaby taxa. Only one of these distinct morphs, I, is not geographically separated from morph A. An alternative hypothesis, therefore, is that morph A has introgressed from one taxon to the next via female dispersants. Introgression has already been predicted by Sharman *et al.* (1990) and Eldridge *et al.* (1991) to account for the chromosome anomalies of eastern rock-wallabies.

Introgression on such a large scale has been noted by Ferris *et al.* (1983*a*) for mtDNA across boundaries between the European mice *Mus musculus* and *M. domesticus*. As with *godmani* and Mareeba race, fixed gene differences were noted on either side of the border, and sterility has been observed in male hybrids (Briscoe *et al.* 1982; Ferris *et al.* 1983*a*). Moreover, Barton & Bengtsson (1986) have argued that a gene can penetrate an apparently impervious border and spread rapidly through the opposing taxon given sufficiently strong selection.

The five northern taxa, then, share morph A either because it was ancestral or because it has introgressed across chromosomal boundaries. In developing likely phylogenies from chromosome data for the eastern *Petrogale* radiation, Eldridge *et al.* (1991) have accounted for several karyotypic homoplasies by assuming that chromosome changes have introgressed across lineages. Those authors place *inornata*, *godmani* and *assimilis* on one clade because of their shared, inverted fifth chromosomes. Cape York race, with its

ancestral fourth chromosome, diverges earliest from another clade containing *penicillata*, *herberti* and the Mt Claro and Mareeba races. Introgression is required between the clades after the Cape York race divergence in the one, and after separation of *inornata* from *assimilis* and *godmani* in the other clade to account for the acrocentric 3 and 4 chromosomes in the latter two species.

Thus if morph A was ancestral to all 5 northern taxa it must have been shared also by the ancestors of *herberti* and *penicillata*, at least as a polymorphism. If no further introgression has occurred, further RFLP analysis would test the accuracy of the phylogeny proposed by Eldridge *et al.* (1991). The mtDNA of Mareeba and Mt Claro races (taxa which share a fusion of chromosomes 5 and 10) on one clade should be most similar, as should that of *assimilis* and *godmani* (taxa which share a 6–10 fusion) on the other. MtDNA of Cape York race should be most dissimilar because its chromosomal lineage diverged before the proposed chromosome introgression between clades.

However, the different chromosomal forms may have arisen relatively recently. The tendency of *Petrogale* to live in small, isolated, deme-structured colonies with individual longevities of up to 12 years in the wild (R. Delaney and W. Davies, pers. comm.), provides optimal conditions for rapid fixation of chromosomal rearrangements (Bush *et al.* 1977). Similar homogeneity of mtDNA among populations of mice homozygous for different chromosomal fusions was reported by Ferris *et al.* (1983*b*). Like rock-wallabies, mice have a social organization ideal for rapid fixation of nuclear mutations; Ferris *et al.* (1983*b*) proposed a rate of fixation of chromosomal changes in mice ten times that of other mammalian species.

No evidence of mtDNA introgression was detected at the *herberti/inornata*, *herberti/assimilis*, *herberti/purpureicollis* or *purpureicollis/assimilis* potential contact zones. Of these, only *herberti* and *inornata* have distributions known to be parapatric (Fig. 1). The river barrier between the two is non-perennial, but physical contact between them is indicated by the exchange of their respective species of lice at two of five colonies examined along the border (Barker & Close, 1990). Although these two species are morphologically similar at their common border, the mtDNA results are consistent with the chromosome and allozyme data which indicate isolation of the two gene pools (Table 3). Hence, they can be regarded as 'good' biological species in the sense of Mayr (1963). However, there is no information on the fertility of hybrids.

Other currently allopatric species might hybridize if given the opportunity through climatic or geographical changes. The birth in captivity of a fertile female offspring from a female hybrid of two morphologically, geographically and ecologically distinct species, *P. xanthopus* and *P. persephone* (Sharman *et al.* 1990; unpublished data), indicates that a significant period of allopatry is not in itself sufficient to cause complete sterility of hybrids. However, the differences in morphology, chromosomes, allozymes and mtDNA of those other taxa, listed above, which are in possible contact, are sufficient to consider them distinct species.

The disputed question of whether limited gene flow can occur between recognized species is obviously relevant to this study. If the position is taken that there can be no gene flow between species, perhaps all or most forms of *Petrogale* should be lumped into one species. This is clearly unworkable considering the range of morphologically and chromosomally distinct forms (Briscoe *et al.* 1982; Sharman *et al.* 1990). The alternative position would then require that a certain degree of gene flow be arbitrarily declared as the watershed between the taxonomic levels of species and subspecies.

Deciding whether adjacent taxa are different species is therefore difficult where limited introgression has been detected, as at the *inornata/assimilis* border. Briscoe *et al.* (1982) provisionally placed *assimilis* as a subspecies of *P. inornata*, and the presence of apparently introgressed mtDNA close to the border indicates that fertile hybrids can be produced (Fig. 8). Moreover, the major mtDNA morphs of each species differed at only three restriction sites. On the other hand, only 2 animals among the 11 with *inornata* karyotypes that were tested close to the chromosomal boundary carried mtDNA morphs typical of *assimilis*. Besides the chromosomal differences there is also a fixed gene difference (Briscoe, 1991) and the Burdekin/Bowen Rivers separate the two taxa for a distance of 200 km. Where *inornata* does cross the Bowen River, no sign of hybridization was detected. At that point, however, the species of louse normally associated only with *assimilis* was found on *inornata* at the closest colony to *assimilis* (Barker & Close, 1990). This indicates physical contact between the two *Petrogale* taxa. There is at least some degree of hybrid infertility, as spermatogenesis was defective in a captive-bred hybrid (Sharman *et al.* 1990). It appears, then, that there is little gene flow between the two taxa and each therefore deserves full specific rank.

Limited introgression was also detected at the *penicillata/herberti* contact zone (Fig. 5). Although originally described as separate species, most authors have considered *herberti* to be a subspecies of *P. penicillata* (Calaby & Richardson, 1988). Despite evidence of some fertility of chromosomal hybrids in the field (Sharman *et al.* 1990), the following observations indicate that, despite hybridization, the two taxa are maintaining separate identities: (1) The mtDNA major morphs of each taxon differ by two detected site changes. (2) The hybrid colony was apparently stable over 8 years. (3) No chromosomal hybrid was detected in four colonies adjacent to the hybrid colony. (4) The genetic data showed that gene flow was restricted, with

apparent introgression of mtDNA being in only one direction, and paralleled by the distribution of alleles of blood and tissue proteins typical of each taxon (D. A. Briscoe & A. A. Gooley, pers. comm.). (5) Endoparasite communities differed in animals on either side of the chromosome border (Beveridge *et al.* 1988). (6) Electrophoretic differences detected in lice of the two taxa indicate cycles of parapatry, allopatry and renewed parapatry (Barker *et al.* 1991).

Decisions on the specific classification of these two taxa are particularly difficult. On one hand, some introgression has been demonstrated, and at least partly fertile backcross female hybrids were found; yet on the other hand, all individuals of each taxon can be recognized (by their chromosomes) from those of the other, and gene flow is restricted. The level of gene flow between these taxa must approach that arbitrary watershed that separates species from subspecies.

Classification of the Mareeba and Mt Claro races and *assimilis* is also difficult. Male hybrids between taxa were sterile, the chromosomes of *assimilis* differ markedly from the other two races (Sharman *et al.* 1990; Eldridge *et al.* 1988), and the most common mtDNA morph of Mt Claro race was not found outside the chromosomal borders (Table 3). On the other hand, one of two female hybrids (*assimilis* X Mareeba race) was at least sub-fertile (Sharman *et al.* 1990), and two mtDNA morphs of Mt Claro race are shared with *assimilis*.

Similarly, *godmani* and Cape York race are difficult to classify. Their karyotypes are distinct (Eldridge *et al.* 1989) yet their mtDNA, as determined from the restriction enzymes used in this study, has not markedly differentiated. Nevertheless, these two taxa, like the others described above, are maintaining their genetic identity despite apparent contact and have discrete distributions.

This study has shown that the use of non-radioactive techniques to analyse mtDNA can yield valuable information on the interaction of several closely related taxa of rock-wallabies. The distributions of different mtDNA morphs at contact zones between taxa have indicated no introgression between some taxa, and limited amounts between others. This information, when combined with data on chromosomes and allozymes, can assist in assessing the taxonomic state of each taxon. We believe that all the taxa of *Petrogale* examined above are specifically distinct from each other since they meet all the following criteria: (1) all members of a taxon are distinguishable, such as by karyotype; (2) each taxon has a discrete distribution; and (3) there is evidence of non-geographical barriers to gene flow between adjacent taxa. These criteria are consistent with the evolutionary species concept of Wiley (1981), and will be used in a revision of the taxonomy of the genus. This approach will clarify the previously confused and much disputed nomenclature.

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