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Apparent introgression of mitochondrial DNA across a narrow hybrid zone in the *Caledia captiva* species-complex

Adam D. Marchant

Research School of Biological Sciences, Australian National University, Canberra, A.C.T., Australia.

Within the *Caledia captiva* grasshopper species-complex, the "Torresian" and "Moreton" taxa show extensive karyotypic and genic differences. They are parapatric, and form a narrow hybrid zone which has been shown to be stable and to be maintained by hybrid breakdown largely attributable to the chromosomal differences. The Moreton, "South East Australian" (SEA) and "Lake's Entrance" (LE) taxa, however, have similar genic characteristics and represent segments of a gradual continuous cline in the frequencies of chromosome morphs. A study of mitochondrial DNA (mtDNA) variation in these taxa, using fragment-length patterns generated by four restriction enzymes, has shown that Moreton, SEA and LE form one polythetic taxon, and that insects from certain sites within the Torresian range fall into a separate taxon. There is an area, however, within the range of the chromosomally and allozymically defined Torresian taxon, in which the mtDNA is found to be exclusively of the Moreton/SEA/LE type. This area of apparent introgression extends much further into the range of the Torresian taxon than any reported introgression of either chromosome or allozyme markers from the Moreton taxon. It is suggested that the hybrid zone (as defined by nuclear characters) has migrated southwards, somehow leaving Moreton-type mtDNA behind. If this is the case, then the absence of Moreton nuclear genes in these insects, whose mtDNA shows that they are descended from Torresian/Moreton hybrids, gives additional suppport to the concept of a "co-adapted genome" as a characteristic of a biological species.

INTRODUCTION

Hybrid zones which are maintained by a balance between selection against hybrids, and continual immigration and hybridisation (Bazykin, 1969), may provide a partial barrier to gene flow between two parapatric groups of organisms (Bigelow, 1965; Key, 1968; Barton and Hewitt, 1981). Such hybrid zones allow the investigation of (a) the genetic differences between the hybridising taxa that are the primary causes of reproductive isolation, and (b) the effects of introgression on the maintenance of genetic distinctness (and conversely, the resistance of genetically distinct groups of organisms to the introgression of foreign genes). These two factors represent, respectively, the formation and maintenance of "biological species".

This paper presents results from part of a study of mitochondrial DNA (mtDNA) variation in chromosomally-defined taxa of the grasshopper *Caledia captiva*, two of which form a hybrid zone. The "Torresian" and "Moreton" taxa differ from each other by large pericentric rearrangements involving most of the chromosomes within the complement ($2n = 22 + X0♂/XX♀$) (Shaw, 1976). Hybrid breakdown (complete embryonic inviability of F_2, and reduced survival of backcross hybrids) between the taxa has previously been demonstrated (Shaw and Wilkinson 1980), and shown to be due largely to the chromosomal differences (Coates and Shaw, 1982; Shaw *et al.*, 1982). The distributions of these and the other *Caledia* taxa are shown in fig. 1. Torresian and Moreton are parapatric, and two transects across their line of contact have been studied since 1976. Across both transects, there is a change from insects with predominantly Torresian to predominantly Moreton karyotypes within 200 metres, across an area in which *Caledia* (which appear to be mostly derived hybrids) are found in high abundance (Shaw *et al.*, 1980). In the southern transect, pure Torresian is replaced by pure Moreton within a distance of one kilometre (Moran 1978), while in the south, no Moreton elements are detected more than one kilometre west of the "null point" (50 per cent Torresian and

50 per cent Moreton chromosome frequencies) (Endler, 1977).

Allozyme studies have shown Torresian and Moreton to differ to the extent that characterises subspecies in other animals (Daly *et al.*, 1981). Across both transects, there is a sudden change in the frequency of several allozymes, from those characteristic of Torresian to those of Moreton. The allozyme null points correspond closely with the chromosomal null points (Moran *et al.*, 1980), but the extent of allozyme introgression is uncertain, as both taxa appear to display some endemic polymorphisms of the "diagnostic" alleles (Daly *et al.*, 1981).

The Moreton, "South East Australian" (SEA) and "Lake's Entrance" (LE) taxa represent segments of a gradual continuous cline in the frequencies of chromosome morphs (Shaw and Coates, 1983; Shaw, in prep.). The allozymic differentiation among these three taxa is characteristic of that of local populations within the same species (Daly *et al.*, 1981; Shaw and Coates, 1983; Coates and Shaw, 1984), so that they form a group among which gene flow is largely unhindered, and which show little differentiation apart from the major structural changes of their chromosomes.

MATERIALS AND METHODS

Population sampling

The locations of collection sites are shown in fig. 2. All insects used were collected in the field except some from sites 3 and 5, which had been maintained in culture for three or four generations. All but one of the collection sites have previously been chromosomally characterised, in most cases over several seasons. References to this work are given in table 1. The single exception is site 23, which was found to be chromosomally Torresian, using the technique of mid-gut C-banding described by Shaw *et al.* (1976). Insects from the southern transect of the hybrid zone are part of the sample used in a study of chromosome and allozyme distribution reported by Shaw *et al.* (1985), and of ribosomal DNA variation (Arnold *et al.*, 1987). All insects were gutted and frozen in liquid nitrogen; most were stored in LN_2 or at −80°C before processing.

DNA preparation from individual grasshoppers

The insect was ground to a fine powder in a mortar, with LN_2. The LN_2 was allowed to evaporate, and the powder was added to 5 ml of a solution of 8 parts 0·05 M Tris, 0·1 M NaCl, 0·1 M Na_2EDTA, *p*H 7·0 with HCl; 1 part 5 per cent $SDS_{(aq)}$; 1 part 2mg/ml Proteinase $K_{(aq)}$ (freshly prepared). This suspension was incubated at 37°C for 1–3 hours, and then extracted with 3 ml of phenol equilibrated with 10 mM Tris, 1 mM EDTA *p*H 7·5 (TE). DNA was precipitated from the aqueous phase by adding approximately two volumes of a solution of 2·9 M sodium perchlorate in 80 per cent ethanol, mixing, and cooling to −20° for about an hour. Precipitated DNA was removed with a pasteur pipette or

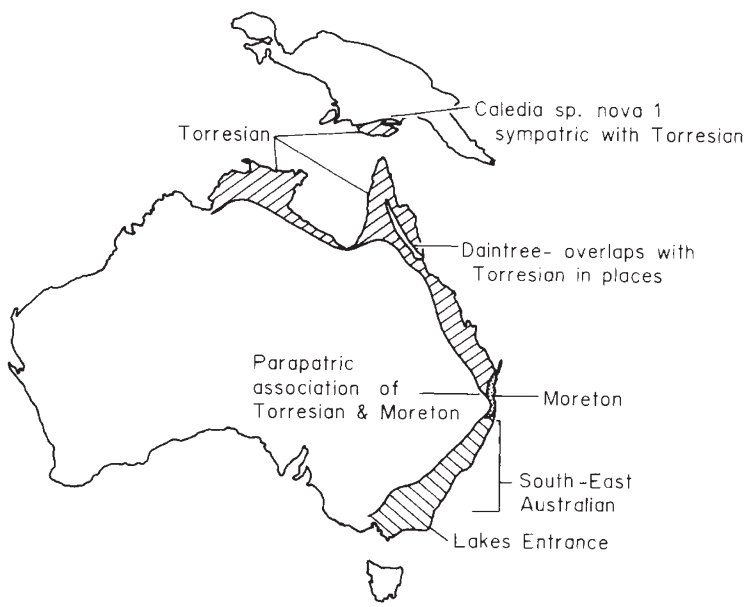

Figure 1 Distribution of *Caledia* taxa.

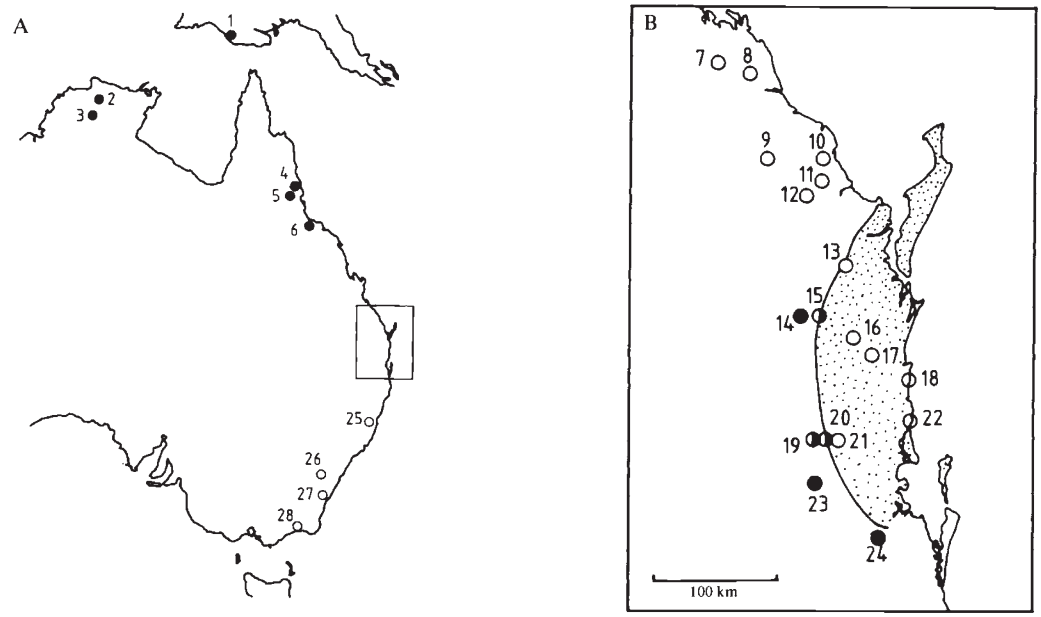

Figure 2 Locations of collection sites used in this study. Fig. 2(B) is an enlargement of the area indicated in fig. 2(A). ● = Torresian type mtDNA ○ = Moreton type mtDNA ◑ = mixed Torresian and Moreton mtDNA. The shaded area in 2(B) indicates the distribution of chromosomally Moreton insects.

forceps if it formed a visible mass, or by centrifugation at 11,000 rpm in a Sorval HB4 rotor for 15 minutes, if no fibrous DNA was visible. The DNA was washed with 70 per cent ethanol, allowed to air dry, then dissolved in 0·2 ml TE. The concentrations were determined by measuring the absorbance at 260 nm, and were then adjusted to 0·5 μg per μl.

Digestion, electrophoresis and Southern transfer

Four restriction enzymes were used: Hae III, Hind III, Msp I, and Xba I (Boehringer, New England Biolabs or Promega). Digestion of usually 2·5 μg DNA from each animal was carried out, using standard procedures. Electrophoresis in 0·6 per cent agarose was performed using materials and equipment described by Clark-Walker *et al.*, (1980). After electrophoresis, gels were treated with 0·5 M NaOH for 30 minutes (Reed, Clark-Walker, pers comm), and transferred to Pal Biodyne nylon filter, according to the method of Smith and Summers (1980).

mtDNA probes

mtDNA-specific probes were either of the following mixtures of cloned grasshopper mtDNA fragments, in equimolar proportions. No differences were observed between them in use.

(a) Four pUC8-derived plasmids containing Eco R1 fragments of *Locusta* mtDNA (McCracken *et al.*, 1987), and together representing the whole mitochondrial genome, were donated by G. R. Wyatt (University of Utah).

(b) Three pUC18-derived plasmids containing Sac 1 fragments of *C. captiva* mtDNA, again comprising the whole molecule. The construction of these plasmids will be described elsewhere.

The probes were labelled with α ^{32}P dATP by the random primer method (Clark-Walker and Sriprakash, 1981), hybridised to filters according to standard procedures, and mtDNA bands were visualised by autoradiography.

RESULTS

All enzymes produce detectable bands totalling in size to approximately 15 kbp (when compared to standards made from lambda DNA), known to be the size of mtDNA in *Caledia* (Marchant and Clark-Walker, unpublished). The number of different patterns observed to be generated by each of the enzymes was Hae III: 11; Hind III: 5; Msp I: 11; Xba I: 4. Each observed different combination of four patterns produced by these enzymes

Table 1 Mitochondrial DNA types observed at each collection site

Site number	Site name	Chromosomal type*	Reference	mtDNA variants present	Number of each variant	mtDNA type†
1	Papua	T	1	20	14	T
				24	1	T
2	Kakadu	T	1	18	5	T
3	El Sharana	T	1	19	6	T
				25	1	D
				26‡	2	
4	Yarrabah	T(Nth)	6	21	2	T
				22	5	T
5	Yungaburra	T(Nth)	6	23	1	T
6	Insulator Ck.	T or D§	6	16	19	T
7	Miriam Vale	T	6	1	5	M
8	Lowmead	T	6	1	1	M
9	Gin Gin	T	1	1	1	M
				4	1	M
10	Coonarr turnoff	T	2	1	1	M
				2	1	M
11	Goodwood	T	2	9	2	M
12	Childers	T	2	2	5	M
13	Tiaro	H	2	2	3	M
14	Bongmuller Ck.	T	5	1	1	M
				11	4	T
				12	10	T
				13	1	T
				15	2	T
15	Northern Hybrid Zone Transect	H	5	1	4	M
				5	1	M
16	Gympie	M	2	1	1	M
17	Cooran	M	2	1	1	M
18	Peregian	M	2	2	5	M
19	Neara Ck.	T	6	2	1	M
				6	2	M
				10	7	T
20	Southern Hybrid Zone Transect¶	H	4	1	2	M
				2	6	M
				3	10	M
				4	2	M
				10	20	T
21	Kilcoy	M	2	2	2	M
				3	1	M
				4	7	M
				7	1	M
22	Caloundra	M	2	8	2	M
23	Esk	T	1	10	4	T
24	Bullock Head Creek	T	6	10	5	T
				11	1	T
				14	1	T
25	Taree	S	6	1	1	M
26	Canberra	S	1	1	10	M
27	Araluen	S	3	1	3	M
28	Lake's Entrance	L	3	2	11	M

* T = Torresian, T(Nth) = Northern Torresian, differing from standard Torresian by a re-arrangement of chromosome 4, and allozymically distinct from other Torresian in Queensland ("Southern Torresian"), H = Moreton/Torresian hybrids, M = Moreton, S = South East Australian, L = Lakes Entrance. † T = Torresian-type mtDNA (fig. 3), M = Moreton-type mtDNA (fig. 3), D = Daintree-type mtDNA (this mtDNA, found only in insects from known Daintree-taxon sites—except for this individual—is distinct from both T and M, and will be described elsewhere). ‡ The mtDNA from these two animals differs from that of any other of the *Caledia* taxa. § Insulator Ck has been previously described as a Southern Torresian site (Shaw, personal communication). 18 of the animals used in this study were collected in 1980, and all had Torresian-type mtDNA. 8 of 10 animals collected in 1986, however, had Daintree-type mtDNA. Subsequent chromosomal analysis of some progeny from this collection showed them to have the Daintree karyotype (Contreras, personal communication). The karyotypes of the two individuals showing Torresian-type mtDNA (only one of which was successfully digested with all four restriction enzymes) are not known. ¶ Insects from the six sites along the hybrid zone transect (defined by Moran 1978 and 1979) have been pooled in this analysis. A detailed study of mtDNA variation along this transect is in preparation.

References 1: Shaw, 1976. 2: Moran and Shaw, 1977. 3: Arnold and Shaw, 1985. 4: Moran, 1978 and 1979. 5: Moran *et al.*, 1980. 6: Shaw, personal communication.

(referred to as a "variant") has been designated by a number. The variants observed at each site are shown in table 1, and a phenogram of these variants has been constructed using the single linkage method (Sneath and Sokal, 1973), based on the number of shared restriction-enzyme digestion patterns (fig. 3).

Two main clusters are apparent. One of these clusters contains mtDNA variants only from sites at which chromosomally and allozymically Torresian insects have previously been found, or from sites within the 1 km width of the hybrid zone. This cluster will be referred to in the following discussion as "Torresian type mtDNA". Within this type, smaller clusters correspond to allozymically distinguishable, geographically separated subtypes of Torresian (Southern, Northern, Northern Territory, Papuan) (Shaw *et al.*, 1980; Arnold and Shaw, 1985).

mtDNA variants in the second cluster were found in areas previously described as LE, SEA and Moreton, from the southern hybrid zone transect, and from an area to the north of the north-eastern end of the hybrid zone, extending at least up to Miriam Vale (site 7), which is approximately 200 km further into the Torresian territory than any introgressed Moreton elements have previously been reported. "Moreton type mtDNA" was also found in individuals from the hybrid zone transect with mixed karyotypes and allozyme genotypes, and in low frequencies in two populations (19 and 14), situated 3 and 16 kilometres (respectively) west of the hybrid zone. Populations from similar distances into the Moreton side of the hybrid zone do not indicate the presence of any Torresian-type mtDNA. This distibution pattern is shown in fig. 2(B).

Variation in the extent of distribution of Moreton-type mtDNA variants is considerable. Several variants were observed only in one or two animals, while variant 1 was seen from Araluen in southern N.S.W. (site 27) to Miriam Vale (site 7), and variant 2 was seen from Lake's Entrance (site 28) to site 10, near Bundaberg in South-East Qld.

In summary, the distributions of the Torresian type and Moreton type mtDNAs are largely congruent with the respective allozymically-defined taxa, and the two mtDNA types meet at the hybrid zone indicated by nuclear characters. However, there is apparent introgression of Moreton type mtDNA across the northern end of the zone, extending at least two hundred kilometres into Toressian territory. This contrasts strikingly with the very limited penetration reported of Moreton allozymes and chromosomal elements into the Torresians.

DISCUSSION

There have been several reports of mtDNA characteristic of one animal taxon being found in a sibling

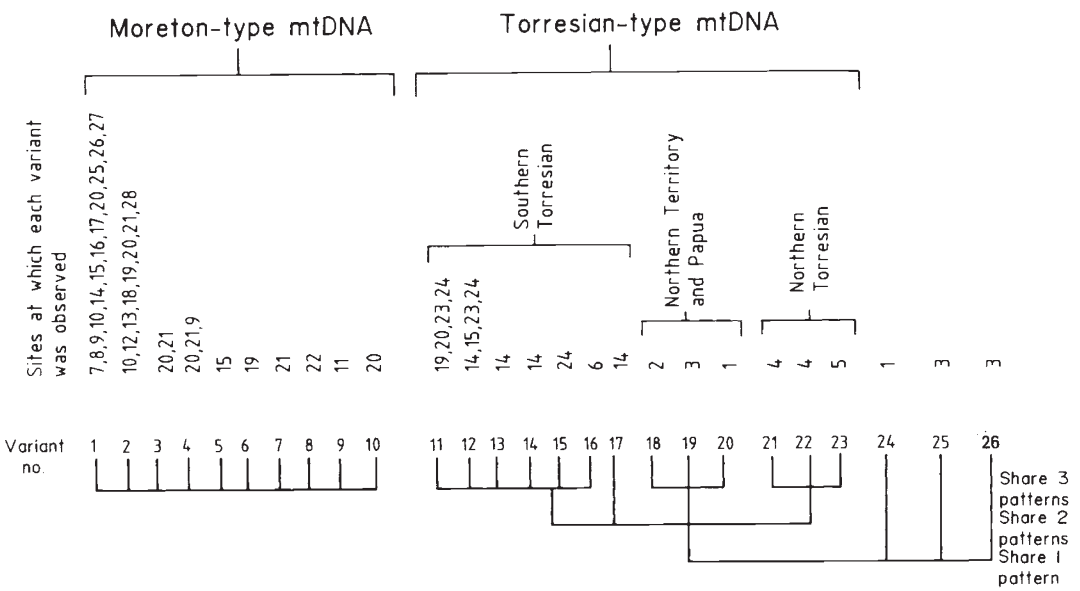

Figure 3 Cluster diagram of mtDNA variants, based on the restriction patterns produced by four enzymes, using the single linkage method of Sneath and Sokal (1973).

species or conspecific subspecies. Setting aside those cases where one taxon is hybridogenic or a parthenogen of hybrid origin (*e.g.*, Spolsky and Uzzell, 1986), there are always two possible explanations for these phenomena (Avise *et al.*, 1984): (1) the common mtDNA type is a shared ancestral character (the peculiar type(s), if any, may have appeared before or after the vicariance), and (2) mtDNA from one taxon has introgressed into the nuclear background of the other, partially or wholly replacing the endemic mtDNA of the second taxon.

In the case of *Caledia*, the shared ancestral hypothesis appears highly unlikely. Several mtDNA variants, which are identical according to the methods used here, are found in both the Torresian and Moreton chromosomal taxa. It might be argued that the vicariance event in which the Moreton taxon separated from the Torresian (or vice versa) involved a large mitochondrially polymorphic population, so that the new taxon inherited several mtDNA variants, but it is very improbable that these mtDNA variants have not changed at all while allozymic divergence between the taxa has progressed to the extent that they differ to a degree characteristic of subspecies (Daly *et al.*, 1981; compare with Powell, 1983).

It appears more likely, therefore, that mtDNA has introgressed from the Moreton taxon into the Torresian. Numerous mechanisms can be proposed to explain asymmetrical introgression of mtDNA in hybrids. These might include asymmetries in the ease with which reciprocal intertaxon matings can occur, as has been observed in the frog species *Hyla cinerea* and *H. gratiosa*, and used to explain the mtDNA types found in a hybrid swarm (Lamb and Avise, 1986). Differential fitness of reciprocal hybrids may be involved—introgression of mtDNA between *Drosophila mauritiana* and *D. simulans* has been argued to be explicable by such a mating asymmetry coupled with the fact that F_1 male hybrids are sterile (as, to a lesser extent, are subsequent backcross males), while female hybrids are fertile (Solignac and Monnerot, 1986).

The mechanisms of asymmetrical introgression require considerable further investigations, but all which seem possible depend on nuclear genes (possibly in conjunction with cytoplasmic factors, as seen in the *P* element phenomenon (Crow, 1983)). So, even if a mechanism for asymmetrical introgression were to be demonstrated in Moreton and Torresian hybrids, it would not explain the penetration of mtDNA far beyond that of previously reported nuclear markers (that is, beyond the limits of the hybrid zone).

There are two *historical* explanations of the mtDNA introgression which seem plausible. Firstly, the Moreton-type mtDNA may have introgressed across the hybrid zone into the Torresian taxon, and then moved northwards. Secondly, the hybrid zone may have moved southwards over a period of time, somehow leaving Moreton mtDNA behind in the swept area (this hypothesis is opposite to that proposed by Moran (1978 and 1979), and Shaw *et al.*, 1979). To explain the penetration of mtDNA far beyond a (stationary) hybrid zone probably requires that a strong selective advantage be proposed for the invading mtDNA over the indigenous molecule; either an absolute superiority, a superiority in the recipient taxon's nuclear background, or a geographically local superiority. It seems unlikely that the original Torresian mtDNA, which is still present in the major part of this taxon's range, is absolutely inferior to that of the Moreton taxon. Unless the Moreton's mtDNA represents a major evolutionary advance in mitochondrial efficiency, it would have to be proposed that the Torresian mtDNA is somehow defective, and it is hard to envisage how an imperfect form of such a central element of an organism's metabolism could have become prevalent in an entire subspecies. Essentially similar arguments would hold if it were proposed that the selected entity is some other element in the cytoplasm, of which mtDNA is only a marker.

Similarly, while an incompatibility between nucleus and cytoplasm from different taxa would not be surprising, it seems improbable that a foreign mtDNA or cytoplasm (that derived from the Moreton taxon) could function better with the Torresian nuclear background than the original Torresian cytoplasm or mtDNA. Some of the animals used in this study were part of a large sample of insects from the southern transect of the hybrid zone, used in a study of karyotypic and allozymic analysis reported by Shaw *et al.* (1985). The chromosomal and allozyme details of all these individuals are known, and it does not appear from data so far available that there is any relationship between mtDNA type and either chromosomal or allozymic characters. This would tend to suggest that both types of mtDNA are equally good in any background. However, the frequency distribution of the two mtDNA types across this transect of the hybrid zone (Marchant *et al.*, in prep.) is very similar to that of ribosomal DNA variants reported by Arnold *et al.* (1987), and a statistical analysis is currently being carried out (Arnold and Rowell, in prep.) which will indicate if correlations between mtDNA type and any nuclear character exist.

If Moreton mtDNA does have an advantage over Torresian, it would seem most likely that it is a geographically local advantage, probably related in some way to the environment in the introgressed area.

Ferris *et al.* (1983) invoked a founder event to explain mtDNA introgression across a hybrid zone in *Mus* which is biogeographically similar to *Caledia*, but, at least in the present example, this does not appear to be likely, since (a) at least two Moreton-type mtDNA variants (1 and 2), with wide distributions in the Moreton/SEA/LE complex, are found in the introgressed area, and (b) in contrast with the *Mus* example, there is no known physical or ecological barrier between the Torresian and Moreton taxa at the point where the mtDNA appears to have transferred from one to the other, and so it is less likely that a single chance colonisation event (as suggested by Ferris *et al.*) could have had a major effect.

However, if the hybrid zone has moved (southwards, at the expense of the Moreton taxon), and a mechanism for asymmetrical introgression of mtDNA in the hybrids were to exist, then Moreton-type mtDNA could be left behind in the wake of the advancing zone. A selective introgression mechanism need not completely prevent Torresian mtDNA passing into the hybrids. Since mtDNA lineages are liable to rapid extinction through purely stochastic processes (Avise *et al.*, 1984), a moderate bias towards asymmetrical introgression of mtDNA might be sufficient to explain the complete fixation of the Moreton-type mtDNA in the area of interest. Computer modelling work is presently under way to test which conditions could be sufficient to explain the complete replacement of Torresian by Moreton-type mtDNA according to the moving hybrid zone model.

Evidence that the Torresian/Moreton hybrid zone has in fact moved might come from data on past climatic distributions, which should show that the area favourable to the Moreton taxon extended further north at some time in the past than it does now. Other evidence would be the presence of other Moreton elements in the area. Data on these subjects is currently being collected (B. Kohlmann, M. Arnold, P. Wilkinson, personal communication).

If the hypothesis can be substantiated, then the absence of Moreton nuclear elements in the area passed over by the hybrid zone would suggest the action of selection against (nuclear) genes in foreign genetic backgrounds (as also argued by Powell, 1983). This system would then corroborate the theory of the co-adapted (nuclear) genome, and, in conjunction with the evidence available for co-adaption of karyotypic structure (Shaw *et al.*, 1985), and within-parental-set genic coadaptation (Shaw and Coates, 1983) in *C. captiva*, this strengthens the case for "biological species" being natural entities of evolutionary importance, rather than just artifices.

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REFERENCES

ARNOLD, M. L. AND SHAW, D. D. 1985. The heterochromatin of grasshoppers from the *Caledia captiva* species complex. II Cytological organisation of the tandemly repeated DNA sequences. *Chromosoma*, *93*, 183–190.

ARNOLD, M. L., SHAW, D. D. AND CONTRERAS, N. 1987. Ribosomal RNA-encoding DNA introgression across a narrow hybrid zone between two subspecies of grasshopper. *Proc. Natl. Acad. Sci. USA.*, *84*, 3946–3950.

AVISE, J. C., NEIGEL, J. E. AND ARNOLD, J. 1984. Demographic influences on mitochondrial DNA lineage survivorship in animal populations. *J. Mol. Evol.*, *20*, 99–105.

BARTON, N. H. AND HEWITT, G. M. 1981. Hybrid zones and speciation. In Atchley, W. R. and Woodruff, D. S. (eds.) *Evolution and Speciation*, Cambridge University Press, pp. 109–145.

BAZYKIN, A. D. 1969. Hypothetical mechanisms of speciation. *Evolution*, *23*, 685–687.

BIGELOW, R. S. 1965. Hybrid zones and reproductive isolation. *Evolution*, *19*, 449–458.

CLARK-WALKER, G. D., SRIPRAKASH, K. S., McARTHUR, C. R. AND AZAD, A. A. 1980. Mapping of mitochondrial DNA from *Torulopsis glabrata*. Location of ribosomal and transfer RNA genes. *Current Genetics*, *1*, 209–217.

CLARK-WALKER, G. D. AND SRIPRAKASH, K. S. 1981. Sequence rearrangements between mitochondrial DNAs of *Torulopisis glabrata* and *Kloeckera africana* identified by hybridisation with six polypeptide encoding regions from *Saccharomyces cerevisiae* mitochondrial DNA. *J. Mol. Biol.*, *157*, 367–387.

COATES, D. J. AND SHAW, D. D. 1982. The chromosomal component of reproductive isolation in the grasshopper *Caledia captiva*. I. Meiotic analysis of chiasma distribution patterns in two chromosomal taxa and their F_1 hybrids. *Chromosoma*, *86*, 509–531.

COATES, D. J. AND SHAW, D. D. 1984. The chromosomal component of reproductive isolation in the grasshopper *Caledia captiva*. III. Chiasma distribution patterns in a new chromosomal taxon. *Heredity*, *53*, 85–100.

CROW, J. F. 1983. Hybrid dysgenesis and the *P* factor in *Drosophila*. *Jap. J. Genet.*, *58*, 621–625.

DALY, J. C., WILKINSON, P. AND SHAW, D. D. 1981. Reproductive isolation in relation to allozymic and chromosomal differentiation in the grasshopper *Caledia captiva*. *Evolution*, *35*, 1164–1179.

ENDLER, J. A. 1977. *Geographic Variation, Speciation, and Clines*, Princeton University Press.

FERRIS, S. D., SAGE, R. D., HUANG, C., NIELSEN, J. T., RITTE, U. AND WILSON, A. C. 1983. Flow of mitochondrial DNA across a species boundary. *Proc. Natl. Acad. Sci. USA.*, *80*, 2290-2294.

KEY, K. H. L. 1968. The concept of stasipatric speciation. *Syst. Zoo.*, *17*, 14-22.

LAMB, T. AND AVISE, J. C. 1986. Directional introgression of mitochondrial DNA in a hybrid population of tree frogs: the influence of mating behaviour. *Proc. Natl. Acad. Sci. USA*, *83*, 2526-2530.

McCRACKEN, A., UHLENBUSCH, I. AND GELLISSEN, G. 1987. Structure of the cloned *Locusta migratoria* mitochondrial genome: restriction mapping and sequence of its ND-1 (URF-1) gene. *Curr. Genet.*, *11*, 625-630.

MORAN, C. 1978. *Population studies of* Caledia captiva (*Orthoptera*: *Acridinae*) *in South East Queensland.* Ph.D. Thesis, Australian National University, Canberra.

MORAN, C. 1979. The structure of the hybrid zone in *Caledia captiva. Heredity*, *42*, 13-32.

MORAN, C., WILKINSON, P. AND SHAW, D. D. 1980. Allozyme variation across a narrow hybrid zone in the grasshopper, *Caledia captiva. Heredity*, *44*, 69-81.

POWELL, J. R. 1983. Interspecific cytoplasmic gene flow in the absence of nuclear gene flow: evidence from *Drosophila. Proc. Natl. Acad. Sci. USA*, *80*, 492-495.

SHAW, D. D. 1976. Population cytogenetics of the genus *Caledia* (Orthoptera: Acridinae). I. Inter- and intraspecific karyotype diversity. *Chromosoma*, *24*, 221-243.

SHAW, D. D. AND WILKINSON, P. 1980. Chromosome differentiation, hybrid breakdown, and the maintenance of a narrow hybrid zone in *Caledia. Chromosoma*, *80*, 1-31.

SHAW, D. D. AND COATES, D. J. 1983. Chromosomal variation and the concept of the coadapted genome—a direct cytological assessment. *Kew Chromosome Conference II*, Allen and Unwin.

SHAW, D. D., COATES, D. J., ARNOLD, M. L. AND WILKINSON, P. 1985. Temporal variation in the chromosomal structure of a hybrid zone and its relationship to karyotype repatterning. *Heredity*, *55*, 293-306.

SHAW, D. D., MORAN, C. AND WILKINSON, P. 1980. Chromosomal reorganization, geographic differentiation and the mechanism of speciation in the genus *Caledia. Royal Entomological Society*, Symposium 10, paper 11, Blackwell, Oxford, pp. 171-194.

SHAW, D. D., WEBB, G. C. AND WILKINSON, P. 1976. Population cytogenetics of the genus *Caledia* (Orthoptera: Acridinae). II. Variation in the pattern of *C*-banding. *Chromosoma*, *56*, 169-190.

SHAW, D. D., WILKINSON, P. AND MORAN, C. 1979. A comparison of chromosomal and allozymal variation across a narrow hybrid zone in the grasshopper *Caledia captiva. Chromosoma*, *75*, 333-351.

SHAW, D. D. AND WILKINSON, P. 1980. Chromosome differentiation, hybrid breakdown, and the maintenance of a narrow hybrid zone in *Caledia. Chromosoma*, *80*, 1-31.

SHAW, D. D., WILKINSON, P. AND COATES, D. J. 1982. The chromosomal component of reproductive isolation in the grasshopper *Caledia captiva*. II. The relative viabilities of recombinant and non-recombinant chromosomes during embryogenesis. *Chromosoma*, *86*, 533-549.

SMITH, G. E. AND SUMMERS, M. D. 1980. The bidirectional

SPOLSKY, C. AND UZZELL, T. 1984. Natural interspecies transfer of mitochondrial DNA in amphibians. *Proc. Natl. Acad. Sci. USA*, *81*, 5802-5805.
transfer of DNA and RNA to nitrocellulose or diasobenzyloxymethyl-paper. *Anal. biochem.*, *109*, 123-129.

SNEATH, P. H. A. AND SOKAL, R. R. 1973. *Numerical Taxonomy.* W. H. Freeman, San Francisco.

SOLIGNAC, M. AND MONNEROT, M. 1986. Race formation, speciation and introgression within *Drosophila simulans*, *D. mauritiana*, and *D. sechellia* inferred from mitochondrial DNA analysis. *Evolution*, *40*, 531-539.