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Evolutionary biology

Gene flow despite complex Robertsonian fusions among rock-wallaby (*Petrogale*) species

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Complex Robertsonian rearrangements, with shared arms in different fusions, are expected to prevent gene flow between hybrids through missegregation during meiosis. Here, we estimate gene flow between recently diverged and chromosomally diverse rock-wallabies (*Petrogale*) to test for this form of chromosomal speciation. Contrary to expectations, we observe relatively high admixture among species with complex fusions. Our results reinforce the need to consider alternative roles of chromosome change, together with genic divergence, in driving speciation.

1. Introduction

Chromosome change has long been regarded as a driver of speciation [1,2]. This can happen in two ways: (I) underdominance—where hybrid individuals have reduced fertility resulting from missegregation during meiosis; and (II) recombination suppression—where genes associated with local adaptation within rearranged regions are linked as a result of reduced recombination within rearranged segments [3]; differences can then accumulate through time and cause reproductive isolation between chromosomally different populations (e.g. [4–6]). The former mechanism is commonly associated with Robertsonian changes and the latter most strongly with inversions.

Robertsonian rearrangements cause little disruption of meiosis when single fusions occur, fixing in populations under weak genetic drift or through meiotic drive [7–9]. However, hybrids between populations differing by multiple fusions with one or more arms in common (monobrachial homology) typically have severely reduced fertility owing to missegregation of complex multivalent chains (model I) [10–12]. Under this scenario, reproductive isolation occurs quickly leading to reduced gene flow between populations [7]. However, the effect of chromosomal rearrangements on gene flow is debated [4,6,13] and in systems with extensive monobrachial homology (e.g. *Rattus*, *Mus*, *Sorex*), the relationship of genic and karyotypic divergence is mixed [8,10,14,15].

Petrogale (rock-wallabies) have been regarded as a classic example of chromosomal speciation through meiotic breakdown (model I) [5,16,17]. The restriction of *Petrogale* populations to isolated rock outcrops with concomitant low dispersal and small population size has been assumed to facilitate the fixation of novel underdominant rearrangements via genetic drift [18]. Therefore, the use of *Petrogale* extends the scope of current model systems (e.g. rodents, shrews, *Drosophila* [4–6]), in which various models of chromosomal speciation can be explored and new theory developed—e.g. to incorporate drift into currently deterministic models. The six parapatric *Petrogale* species on the northeast coast of Australia show a range of autosomal chromosomal rearrangements, including Robertsonian fusions, inversions and centric shifts (figure 1 and table 1) [20]. The recent and rapid radiation of these taxa (0.44–1.58 Ma) [21] provides an opportunity to test for reduced gene flow in the presence of complex chromosomal changes

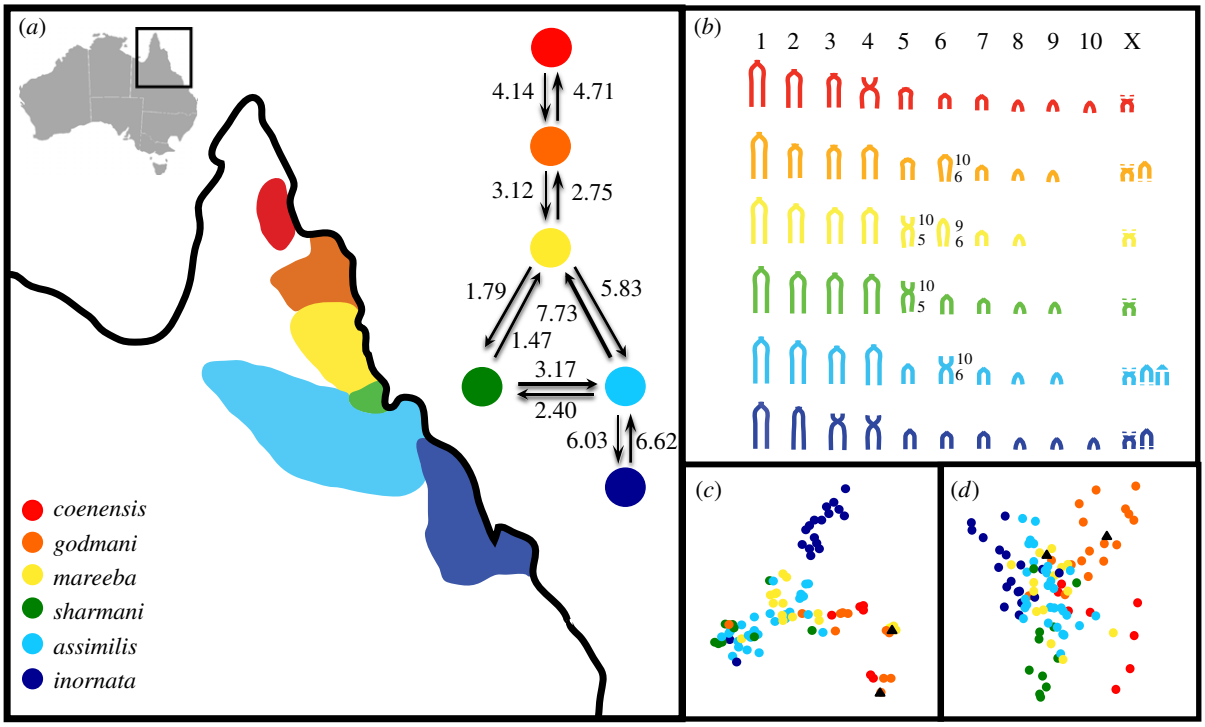

Figure 1. (*a*) Distribution of six *Petrogale* species in northeastern Australia (colours are species-specific for 1*a–d*; triangles represent natural *godmani* × *mareeba* hybrids) and relative mutation-scaled migration rates between adjacent species based on MIGRATE-N [19]. (*b*) Schematic of species karyotypes highlighting the diagnostic Robertsonian fusions (for more detail on rearrangements refer to electronic supplementary material, table S6). (*c*) MDS of mtDNA based on genetic distances (percentage of variation: PC1 = 9.23%, PC2 = 7.56%). (*d*) PCA of microsatellite data based on species genetic distance matrix of species in (*a*) (percentage of variation: PC1 = 21.29%, PC2 = 18.50%). PC1 is on the *X*-axis, PC2 is on the *Y*-axis.

Table 1. Summary of chromosome differences and known fertility for hybrids among the six northeast Queensland *Petrogale*. i, inversion; —, fusion; a, shift in centromere to acrocentric; FGD, fixed allozyme differences. Predicted levels of introgression are based on model I (see text).

hybrid	chromosomal changes	chromosome heterozygosity	fertility male	fertility female	FGD	predicted level of introgression
coenensis × *godmani*	simple (4)	3 5 6 10 3a 5i 6–10a	unknown	unknown	0	high
godmani × *mareeba*	complex (6)	5i 6–10a 9 5–10 6–9a	sterile	subfertile	2–4	low
mareeba × *sharmani*	simple (2)	6–9a 6 9	sterile	unknown	0	high
mareeba × *assimilis*	complex (5)	5–10 6–9a 5i 6–10 9	sterile	subfertile	0	low
assimilis × *sharmani*	complex (3)	5i 6–10 5–10 6	sterile	unknown	0	low
assimilis × *inornata*	simple (3)	3a 4a 6–10 3 4 6 10	sterile	unknown	1	high

(i.e. model I). Evidence from hybrids [16,22] indicated that genic as well as chromosomal differences may be involved in reproductive isolation (models I and II) and preliminary analyses using low-resolution methods also suggested introgression between species [23–25].

Here, we apply fine-scale genetic markers (mitochondrial DNA—mtDNA sequences and microsatellite genotypes) to (i) investigate the concordance between genetic and karyotypic structure across the chromosomally diverse eastern *Petrogale*; and (ii) test for reduced gene flow between parapatric taxa with complex monobrachial homology, compared to those with simple fusions (table 1) as expected under model I and generally assumed for this system.

2. Material and methods

One hundred and four samples were used in mtDNA and 99 in microsatellite analyses from across the distributions of six parapatric northeastern Australian *Petrogale* species: *P. coenensis* ($n = 8$); *P. godmani* ($n = 17$); *P. mareeba* ($n = 16$); *P. sharmani* ($n = 11$); *P. assimilis* ($n = 31$) and *P. inornata* ($n = 18$) (figure 1*a*; electronic supplementary material, figure S1 and table S1).

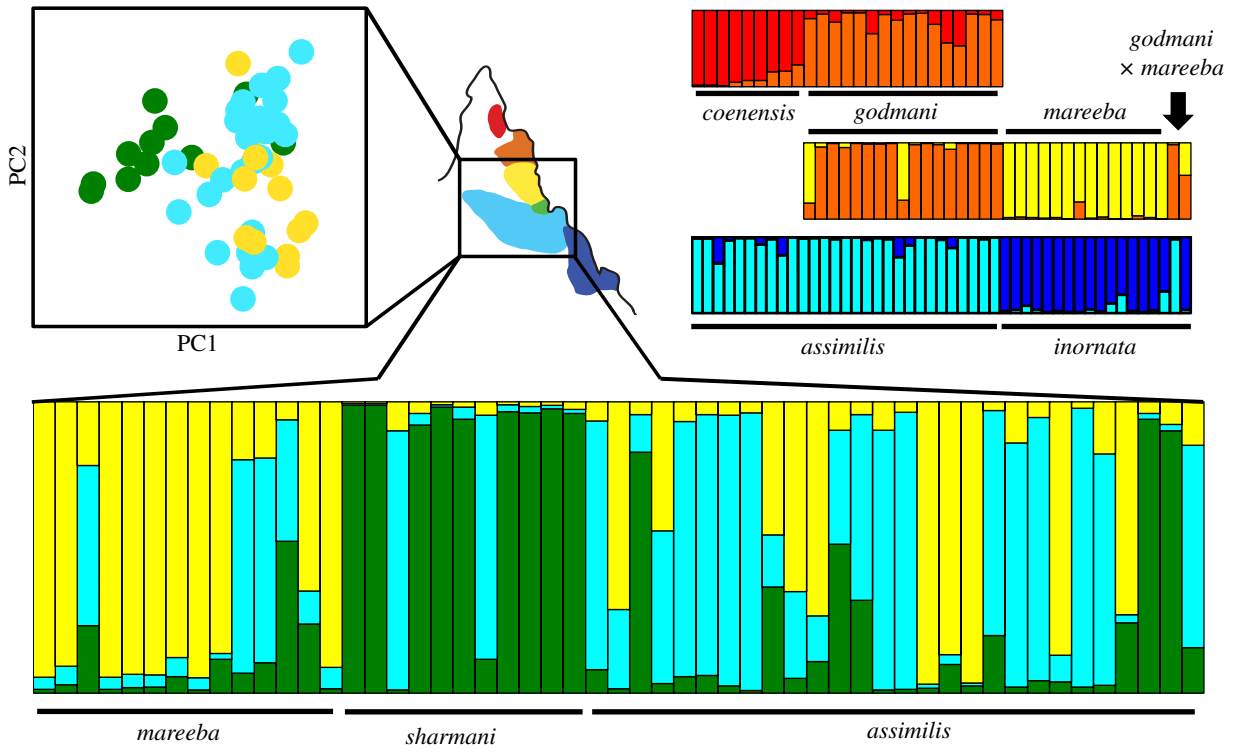

Figure 2. Genetic clustering of adjacent species of *Petrogale* (figure 1*a*) based on STRUCTURE [28] and PCA. Highlighted is the three-way comparison of *Petrogale assimilis* (A), *P. mareeba* (M) and *P. sharmani* (S) who share complex (A/M; A/S) and simple (S/M) rearrangements—table 1 (percentage of variation: PC1 = 21.74%, PC2 = 18.43%).

In addition, two naturally occurring F_1 chromosomal hybrids (*godmani* × *mareeba*) were included and a *P. penicillata* as an outgroup for phylogenetic analysis. Individuals were identified to species via karyotyping [16,24]. Methods for mtDNA sequencing and genotyping of 17 microsatellite loci are presented in the electronic supplementary material. Two of the 14 loci mapped to available *Macropus eugenii* data are on rearranged chromosomes in *Petrogale* (see the electronic supplementary material).

Multidimensional scaling (MDS) of sequence divergence among mtDNA haplotypes was conducted using the package ape [26] in R [27] based on Euclidean genetic distances to visualize genetic differentiation among species. In addition, a principal coordinate analysis (PCA) of microsatellite loci was analysed using a standardized covariance method in GenAlEx 6 [28]. The extent of recent admixture at microsatellite loci was also assessed using STRUCTURE (v. 2.1) ([29]; see the electronic supplementary material for details). For PCA and STRUCTURE, comparisons were conducted (i) for all species, and (ii) for comparisons between adjacent species, to assess admixture rates among geographically proximate taxa. Genic differentiation between species was also assessed using both Φ_{ST} (mtDNA) and F_{ST} (microsatellites) calculated in ARLEQUIN (v. 3.11) [30].

Using microsatellite genotypes, we estimated relative mutation-scaled migration rates between adjacent species using coalescence in MIGRATE-N (see the electronic supplementary material). To evaluate whether high similarity among species could be the result of recent separation rather than migration, we used IMa [31] to contrast population-splitting models with and without migration, focusing on the *P. assimilis*, *P. mareeba*, *P. sharmani* (AMS) system (see the electronic supplementary material).

3. Results

Overall, there is no strong mtDNA structuring besides *P. inornata* (no reciprocal monophyly or separation in MDS—figure 1*c*; electronic supplementary material, figure S2). Similarly, the microsatellite loci reveal no clear genetic separation among species (PCA—figure 1*d*) and STRUCTURE results suggest only two genetic clusters, largely separating the northern taxa (*P. coenensis* and *P. godmani*) from the remainder (electronic supplementary material, figure S3, and tables S2 and S3).

Contrary to expectations, there is no general pattern of stronger suppression of gene flow between taxa with complex versus simple Robertsonian fusions (figures 1*a* and 2; electronic supplementary material, figures S2 and S3). This is especially evident across the three-way contact between AMS. Admixture and gene flow are highest between *P. assimilis* and *P. mareeba*, which differ by complex fusions, whereas *P. sharmani* clustered separately from *P. mareeba* despite only a simple chromosomal difference (figures 1*a* and 2; electronic supplementary material, table S4). IMa nested model comparisons reject the hypothesis that genetic similarity between AMS species is owing to ancestral polymorphism alone (electronic supplementary material, table S5). At other contacts, there is evidence for low admixture, with (*P. godmani*/*P. mareeba*) or without (*P. assimilis*/*P. inornata*) monobrachial homology of fusions (figure 2; electronic supplementary material, S3). In general, parapatric species pairs have the lowest genetic differentiation (F_{ST}, Φ_{ST}; electronic supplementary material, table S3) and to some extent greater gene flow (figure 1*a*; electronic supplementary material, table S4), despite some evidence of separation based on pairwise PCA comparisons between geographically adjacent species (electronic supplementary material, figure S3).

4. Discussion

The empirical results for *Petrogale* do not support a strong role of monobrachial homology in chromosomal speciation

(model I) as has long been assumed. Specifically, we did not observe consistently reduced admixture in the presence of complex (monobrachial) versus simple Robertsonian fusions. This is concordant with other systems (e.g. *Sorex*, *Mus*) where complex rearrangements have differential roles as gene flow barriers [8,32]. The (sub)-fertility of some female hybrid *Petrogale* may allow for backcrossing [33] and thus introgression between species, as has been noted in the well-studied *Mus* system [8].

In principle, high genetic similarity (and thus high rates of gene flow inferred using MIGRATE-N) can also result from recent separation, as could occur if the fusions resulting in monobrachial homology established quickly [14]. Retained ancestral polymorphism could explain some of the admixture seen, particularly for mtDNA. However, consistent rejection of models with zero migration using IMa (electronic supplementary material, table S5) highlights that recent separation alone cannot entirely explain our results.

The fact that our predictions associated with model I were not supported suggests that other mechanisms are acting independently or synergistically with the chromosomal rearrangements to drive speciation in this system (model II). The increasing scope and sophistication of theory relating chromosome change to gene flow suppression and speciation point to the need for incorporating population genomic data to test for suppression of gene flow and/or adaptive divergence specific to rearranged regions of chromosomes (model II). Such analyses for model systems with high-quality genomes have sometimes supported these predictions (e.g. [34,35]). However, there is a need to extend this approach to systems with diverse life histories to test generality, with an initial step to demonstrate that strong reproductive isolation owing to underdominance (model I) alone does not hold. We now expect that future comparative population genomic analysis of *Petrogale* would therefore illuminate how different effects of chromosome change and genic divergence interact to drive speciation, and so extend the generality of such studies.

Data accessibility. DNA sequences: GenBank accessions JQ62304, KT726229–KT726317. Microsatellite genotypes: electronic supplementary material.

Authors' contributions. S.P. participated in design of the study, carried out the molecular laboratory work, data analysis and wrote the paper. C.M. helped interpret results and with writing the manuscript. M.D.B.E. conceived the study, participated in interpretation of the results and helped draft the manuscript. All authors gave final approval for publication and agree to be held accountable for all aspects of the work.

Competing interests. We declare we have no competing interests.

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