Where and when does a ring start and end? Testing the ring-species hypothesis in a species complex of Australian parrots

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Speciation, despite ongoing gene flow can be studied directly in nature in ring species that comprise two reproductively isolated populations connected by a chain or ring of intergrading populations. We applied three tiers of spatio-temporal analysis (phylogeny/historical biogeography, phylogeography and landscape/population genetics) to the data from mitochondrial and nuclear genomes of eastern Australian parrots of the Crimson Rosella *Platycercus elegans* complex to understand the history and present genetic structure of the ring they have long been considered to form. A ring speciation hypothesis does not explain the patterns we have observed in our data (e.g. multiple genetic discontinuities, discordance in genotypic and phenotypic assignments where terminal differentiates meet). However, we cannot reject that a continuous circular distribution has been involved in the group's history or indeed that one was formed through secondary contact at the 'ring's' east and west; however, we reject a simple ring-species hypothesis as traditionally applied, with secondary contact only at its east. We discuss alternative models involving historical allopatry of populations. We suggest that population expansion shown by population genetics parameters in one of these isolates was accompanied by geographical range expansion, secondary contact and hybridization on the eastern and western sides of the ring. Pleistocene landscape and sea-level and habitat changes then established the birds' current distributions and range disjunctions. Populations now show idiosyncratic patterns of selection and drift. We suggest that selection and drift now drive evolution in different populations within what has been considered the ring.

Keywords: ring species; Crimson Rosella; *Platycercus elegans*; speciation; phylogeography; landscape genetics

1. INTRODUCTION

Speciation in the face of ongoing gene flow and the relative roles played by drift and selection in developing reproductive isolation that signals completion of speciation are still major issues in evolutionary biology (Schluter 2000; Coyne & Orr 2004; Dionne *et al.* 2008; Niemiller *et al.* 2008; Nosil 2008; Price 2008). Excellent opportunities to examine these issues are provided by 'ring species' or cases of 'circular overlap' (Mayr 1942, 1963; Irwin *et al.* 2001*a*; Irwin & Irwin 2002). Four criteria strictly define circumstances for an 'ideal' ring-species hypothesis of speciation: (i) two distinctive forms coexist today in sympatry, (ii) gene flow through a chain of populations has connected them before and since sympatry that came about through a range shift, (iii) the chain forms a complete ring, and (iv) the terminal differentiates are connected by gradual

geographical variation (Irwin *et al.* 2001*a*). Thus, differentiation between two reproductively isolated forms at geographical ends of the ring is hypothesized to have been through isolation by distance (IBD) while in primary contact and not through historical allopatry (Stejneger in Jordan 1905; Mayr 1942; Cain 1954; Irwin & Irwin 2002; Irwin *et al.* 2005; Martens & Päckert 2007). Almost all who have used the concept have emphasized that ring species inform about demographic history and divergence; furthermore, putative ring species can improve the understanding of how ecological divergence and geographical separation interact in speciation (Irwin *et al.* 2001*a*).

Evolutionary histories of only a few ring species have been addressed with molecular phylogenetic perspectives (e.g. Moritz *et al.* 1992; Crochet *et al.* 2002; Liebers *et al.* 2004). Fewer have been addressed with complementary phylogenetic and population genetic perspectives from both mitochondrial and nuclear DNA (nDNA; Wake & Yanev 1986; Moritz *et al.* 1992; Irwin *et al.* 2001*b*, 2005; Alexandrino *et al.* 2005). Prior studies have largely focused on using mitochondrial DNA (mtDNA) diversity to test whether members of a ring species are monophyletic, and

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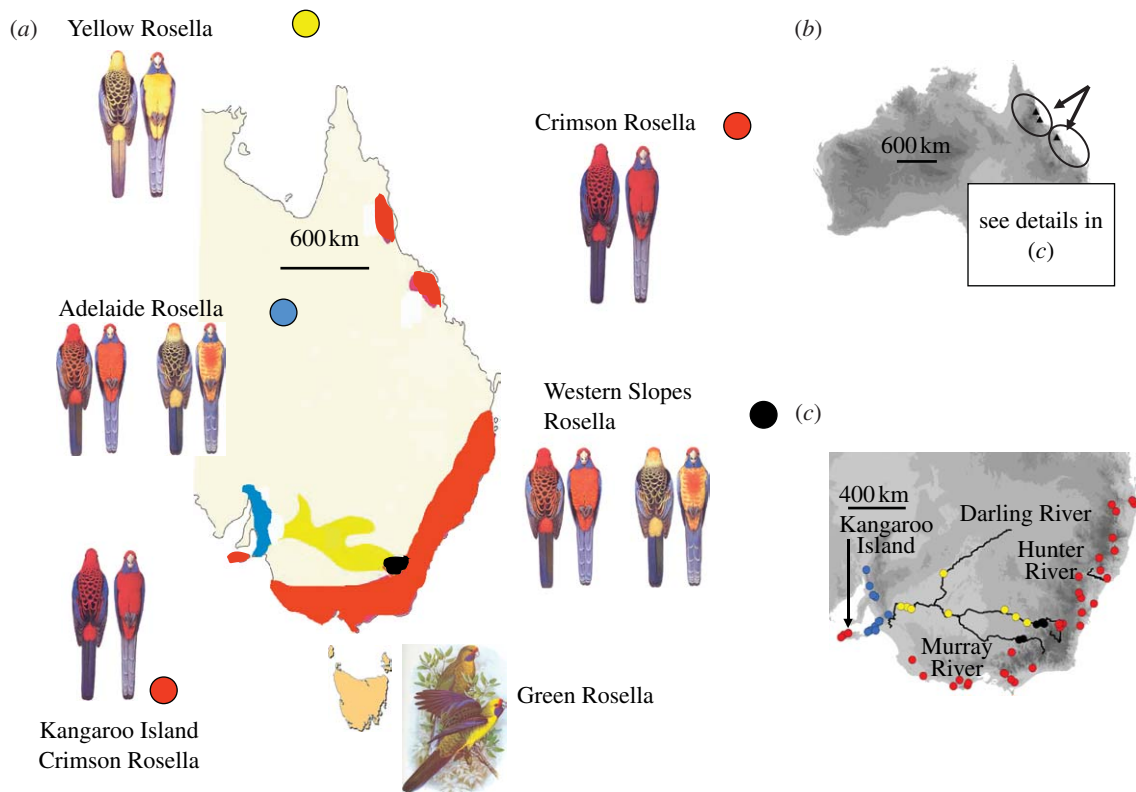
Figure 1. (a) Distribution and plumage phenotype variation in parrots of the Crimson Rosella complex in eastern Australia (from Forshaw & Cooper (2002) in this and subsequent figures). Note the narrow zone of unsuitable habitat currently separating Adelaide and Yellow (see the electronic supplementary material). (b,c) Sampling scheme of vouchered museum specimens for mtDNA analyses of the full mainland distribution of the complex.

on estimating where gene flow occurs in a ring (Irwin *et al.* 2001b; Martens & Päckert 2007). Here, we expand analyses of ring species by accessing three levels of spatio-temporal inference. The subjects for our analyses are eastern Australian parrots of the Crimson Rosella *Platycercus elegans* complex, which have long been considered an example of the ring speciation hypothesis (Cain 1955; Irwin & Irwin 2002; for details of distribution and taxonomy, see Schodde & Mason 1997; Higgins 1999; Christidis & Boles 2008). The geographical range of the entire Crimson Rosella complex in eastern Australia is shown in figure 1. We use combined analyses of mtDNA and nDNA genotype data to integrate deeper spatio-temporal perspectives from both phylogeny and historical biogeography with more recent ones from phylogeography and landscape genetics (Manel *et al.* 2003; Holderegger & Wagner 2006).

The populations in mainland southeastern Australia comprise the 'ring' distribution that is our focus (figure 1). They almost completely encircle unsuitable habitat of semi-arid vegetation. The group's two geographically terminal populations occur at the eastern side of the distribution. One of them is the Crimson Rosella (hereafter Crimson), predominantly red birds of wetter eastern Australian woodlands and forests. The other terminal differentiate (*sensu* Cain 1955; Irwin & Irwin 2002) is the Yellow Rosella (Yellow), predominantly yellow birds of drier, inland riparian habitats of inland southeastern Australia (figure 1). Crimson and Yellow approach each other geographically in the western slopes region of the Great Dividing Range in the southeast quarter of the distribution. Their contact was thought to be without hybridization (Cain 1955), but specimens of phenotypically

intermediate western slopes populations, here termed Western Slopes, have been collected since 1974 (see the electronic supplementary material). At the group's western limit approximately 800 km to the west of where Crimson and Yellow meet, an isolated population of Crimson occurs on Kangaroo Island (figure 1). It is separated by approximately 15 km of sea from nearby mainland populations known as the Adelaide Rosella (Adelaide). Adelaide is phenotypically intermediate between Crimson and Yellow. Adelaide is also isolated from mainland Crimson to its southeast by approximately 200 km of unsuitable habitat (figure 1). Adelaide is not known at present to be in contact to its east with Yellow and is separated from it by a narrow band of unsuitable habitat (details in the electronic supplementary material). We interpret the critical prediction of a ring speciation hypothesis when applied to the *P. elegans* complex to be that gradients in gene flow should be stepped only where the terminal Crimson and Yellow populations approach each other in the western slopes region (figure 1; the electronic supplementary material has further details of the distribution). Several natural range gaps of up to 200 km caused by approximately 15 km of sea and 200 km of otherwise unsuitable habitat for these birds (figure 1), not the alteration of suitable habitat since European settlement, mean that criteria (ii) and (iii) given above for a test of an ideal ring speciation hypothesis are compromised. More specifically, there is no clear prediction for what a test of IBD could show where a geographical discontinuity applies. It is unclear what the IBD test can reject in that case. Where a genetic discontinuity occurs at a geographical discontinuity, the outcome of an IBD test is not readily predicted. Expectations for what an IBD test would show if

a geographical break is 10, 100, 1000 or 10 000 years old, for example, will depend on the age of the break, the rate of genetic drift, the markers used, and so on.

Several alternative hypotheses could explain the group's history. One is that ancestral Crimson and Yellow differentiated in allopatry and then secondary contact between them resulted in hybridization and phenotypic intermediacy of Adelaide and Western Slopes (Condon 1941; Serventy 1953; Cain 1955). This predicts that Adelaide and Western Slopes should be genetic admixtures of Crimson and Yellow. Another alternative is that an ancestral population was subdivided *in situ* into isolates. These are today's various populations of Crimson, Yellow, Adelaide and Western Slopes (figure 1). Each would have evolved in historical allopatry and so could be tested for genetic independence from each other.

Though not currently part of the focus of the ring speciation hypothesis, other isolated populations are part of the broader *P. elegans* complex and their historical connections need to be considered: two Crimson populations in tropical northeastern Australia and the Green Rosella *Platycercus caledonicus* (Green) of Tasmania and its offshore islands (figure 1). Remarkably, no major study of the *P. elegans* complex has appeared since Cain's (1955) plumage-based analysis. Earlier allozyme (Joseph & Hope 1984) and mtDNA (Ovenden *et al.* 1987) studies had limited taxon, population and nucleotide sampling and left many details of the group's evolution unresolved.

Our aim is to test the ring speciation hypothesis and the alternatives we have discussed as explanations of the history of differentiation and speciation in these parrots. We use a three-tiered approach. First, historical biogeographical and phylogenetic perspectives from mtDNA provide a broad evolutionary context in which the group's history is embedded. Second, phylogeographic analysis of mtDNA and microsatellite diversity within the group itself estimates its regional history and informs about the processes that have generated its patterns of neutral molecular diversity. Finally, landscape genetics describes contemporary patterns of gene flow in relation to present geography. It estimates the number of populations present and defines their spatial limits based on spatial and genetic data without other *a priori* categorization. We focus on whether phenotypic intermediacy of Adelaide and Western Slopes is due to gene flow from the terminal Crimson and Yellow populations at the ends of the range as it grew (the ring speciation hypothesis *sensu* Cain 1955) or past hybridization between ancestral Crimson and Yellow (e.g. differentiation in allopatry *sensu* Condon 1941).

2. MATERIAL AND METHODS

Figure 1 and the electronic supplementary material show localities of the specimens examined. Blood or tissue samples were collected from 307 unrelated adult rosellas from 92 localities throughout eastern and southeastern Australia between 2001 and 2005. In addition, 16 individuals of other *Platycercus* species and outgroup taxa were used in phylogenetic analyses (see the electronic supplementary material).

DNA extraction and PCR amplification of the mitochondrial *ND2* gene followed the method described by Joseph *et al.* (2002) and Joseph & Wilke (2006). Nucleotide diversities among mainland southeastern Australia populations comprising the ring were consistently low, mostly

below 0.25 per cent and maximum divergence within the entire *P. elegans* complex (figure 1) was 1.9 per cent. Accordingly, we used phylogenetic analyses (see the electronic supplementary material) only to test monophyly of the *P. elegans* complex, which was supported with 100 per cent bootstrap support (1000 pseudoreplicates were run after finding 20 equally parsimonious trees, tree-bisection-and-reconnection branch-swapping and random addition of sequences). Owing to low nucleotide diversities, we depict sequence relationships for all haplotypes within mainland southeastern Australian populations comprising the putative ring with an unrooted statistical parsimony network derived with TCS v. 1.18 (Clement *et al.* 2000). Nucleotide diversity statistics were generated in DNASP v. 4.10.2 (Rozas *et al.* 2003) as were mismatch analysis tests for population stability, increase or decline. These are the tests for reductions of old mutations and excesses of low-frequency younger alleles expected under a scenario of population growth (Fu 1997). The tests were done for Tajima's (1989) D, Fu's (1997) F_s and Ramos-Onsin & Rozas's (2002) R_2. Their results were broadly concordant, thus we present results for Fu's F_s only.

Multiplex-ready PCR (Hayden *et al.* 2006) allowed microsatellite genotyping using published microsatellites and a standardized protocol (see the electronic supplementary material). Microsatellite allele frequency and sample spatial location data were analysed using GENELAND (Guillot *et al.* 2005a,b) because it can incorporate geographical information to detect spatial delineation of genetic discontinuities, where the number of population units is treated as an unknown parameter, and because it has been specifically designed for microsatellite data. The number of populations, k, was estimated from multiple runs with 1 million iterations and 100 000 burn-in generations. Results were produced (with k fixed) from the top 10 per cent of posterior densities of 30 runs with 2 million iterations and 200 000 burn-in generations (Coulon *et al.* 2006). MIGRATE-N v. 2.3 (Beerli & Felsenstein 1999, 2001) was used to estimate migration rates between all populations simultaneously and the effective size of each population. The microsatellite dataset was trimmed to include only loci fitting a stepwise mutation model ($n=12$). The starting parameters for both theta and migration rates were based on F_{st}-like calculations. The mutation rate was assumed constant across all loci (though we acknowledge a broad range of allele numbers) and the starting tree was a random tree. Several relatively short runs with different random seeds refined the fixed heating parameters to achieve adequate chain swapping and to ensure convergence. The final analysis used 10 short chains sampling 100 000 genealogies and four long chains sampling 1 000 000 genealogies. For both short and long chains, we used a burn-in of 10 000 generations and the results were averaged over four replicates of the long runs.

3. RESULTS
(a) *mtDNA*
Near-complete *ND2* sequences (1032 base pairs) were obtained mostly for 114 vouchered specimens of the *P. elegans* complex representative of its entire range (Crimson (45), Yellow (15), Adelaide (19), Western Slopes (13), Green (5)), and all 16 outgroup individuals (GenBank accession numbers EU407613–EU407719; DQ105447). All sequences were translated into amino acids with no unexpected stop codons. Deficiencies of guanine typical of avian mtDNA were observed ($A=0.32$, $C=0.36$, $G=0.08$
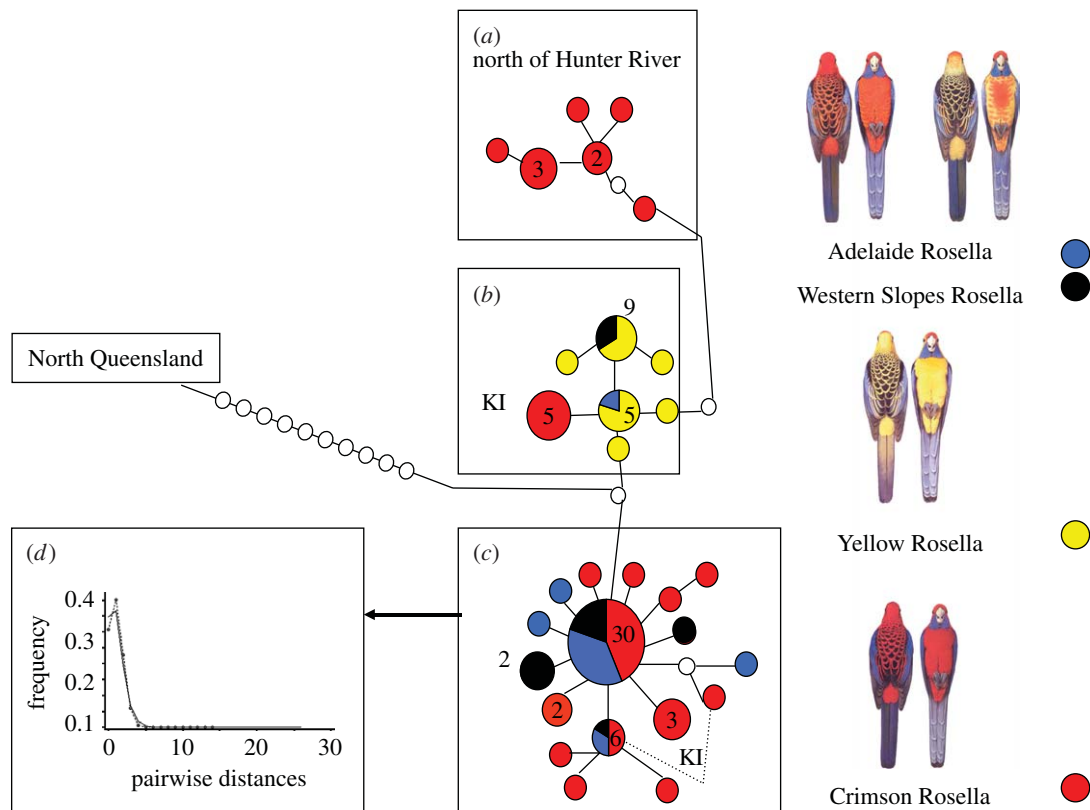
Figure 2. Unrooted network of mtDNA diversity in southeastern Australian populations of the Crimson Rosella complex, showing groups (*a*) 1, (*b*) 2 and (*c*) 3 as referred to in the text. Open circles indicate unsampled haplotypes. Colours in circles indicate the plumage phenotype of samples as in figure 1. The sample size for each haplotype is 1, unless otherwise indicated with a number. KI refers to haplotypes found in eight Kangaroo Island Crimson individuals. A solid line with open circles shows where the North Queensland populations join the network. A unimodal mismatch plot for (*c*) is shown in (*d*). (*d*) Dotted line, observation; solid line, expected.

and $T = 0.24$). The complete dataset had 297 variable sites of which 248 were parsimony informative. The sequences we obtained are consistent with a mitochondrial origin and not nuclear copies of mtDNA.

The phylogenetic analysis (text and figure 1 of the electronic supplementary material) affirmed the monophyly of the *P. elegans* complex and of tropical northeastern Australian populations within it. We highlight five points from the unrooted network (figure 2): (i) haplotypes from nine mainland Crimson from the north of the Hunter River fell in a discrete group (group 1; figure 2*a*), (ii) haplotypes from phenotypic intermediates (Western Slopes and Adelaide) were spread across the other two groups of the network (groups 2 and 3; figure 2*b*,*c*), (iii) all but one Adelaide haplotype either shared the most common haplotype with Crimson from south of the Hunter River in group 3 or differed from it by single or, in one case, two substitutions, (iv) the one remaining Adelaide haplotype was shared with Yellow, and (v) haplotypes of eight Kangaroo Island Crimson were across groups 2 and 3 of the network.

Net nucleotide divergences among pairwise combinations of haplotypes from each of these groups ranged from 0.24 to 0.55 per cent. Group 3 had characteristics of a population that has recently expanded its size, i.e. a star-like network, a unimodal mismatch curve (figure 2), low nucleotide diversity at 0.10 ± 0.02 per cent and Fu's $Fs = -16.57$, which is well outside 95% confidence intervals of -4.01 and 4.51. Smaller sample sizes in groups 1 and 2 render these tests inappropriate.

(b) *Microsatellites*

We obtained microsatellite genotypes at 16 loci for 297 rosellas from 91 locations. Variability in the 16 loci is summarized in the electronic supplementary material. We tested conformance to Hardy–Weinberg equilibrium (HWE) expectations in samples from eight locations with large sample sizes (range 9–33). After the Bonferroni correction, locus *CP53D02* was removed from further analyses because it departed from HWE expectations in six out of the seven samples and had a relatively high estimate of the frequency of null alleles (see the electronic supplementary material). As a highly significant level of linkage was detected between locus *AgGT83* and locus *Ero08*, locus *AgGT83* was removed from further analyses.

Within southeastern Australia, the peak of the posterior distribution in GENELAND for the number of clusters was $k = 4$. As Crimson north of the Hunter River were a separate group with respect to the rest of southeastern Australia (figure 2) and with the geographical range of populations north of the Hunter being outside the geographical ring, further GENELAND runs excluded samples from the north of the Hunter River (figures 2 and 3). With *k* now fixed to $k = 3$, the highest 10 per cent posterior density of 30 GENELAND runs converged on the same result. All but three individuals were clearly assigned to one of three clusters with 100 per cent confidence. The variance between runs of the remaining three individuals was low (average $= 1.29 \times 10^{-5}$). Three clear clusters were recognized for all but three individuals and the assignment
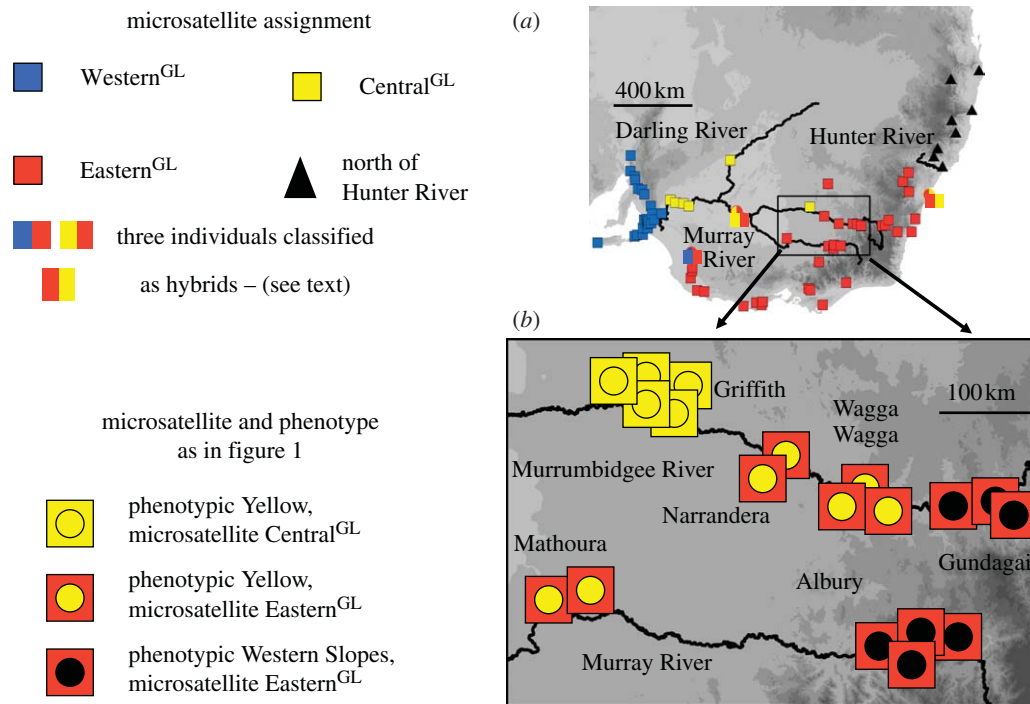
Figure 3. (*a*) Summary of microsatellite analyses of southeastern Australian populations of the Crimson Rosella complex. Coloured squares are microsatellite-based GENELAND classifications of individuals as described in the text (Eastern[GL], Central[GL] and Western[GL]). Bicoloured symbols show the three individuals that were classified as 'hybrid' assignments of 0.61 Eastern[GL]/0.38 Central[GL], 0.05 Eastern[GL]/0.95 Central[GL] and 0.16 Eastern[GL]/0.84 Western[GL]. (*b*) The map details sampling for microsatellites in the upper and middle reaches of the Murray River system and shows discordance between microsatellite-based assignments (squares) and plumage phenotypes (circles).

of individuals by GENELAND to genetic clusters was not concordant with the plumage-based classification: (i) Western[GL], which comprised phenotypic Adelaide and Kangaroo Island Crimson, (ii) Central[GL], which comprised phenotypic Yellow except its easternmost samples at Mathoura, Narrandera and Wagga Wagga (figure 3), and (iii) Eastern[GL], which comprised phenotypic Yellow from Mathoura, Narrandera and Wagga Wagga, all Western Slopes and phenotypic Crimson south of the Hunter River. The voucher specimens from Mathoura, Narrandera and Wagga are Yellow with little or no red in their ventral plumage (see details in the electronic supplementary material). Three individuals classified as hybrids indicated some admixture of the clusters (bicoloured symbols in figure 3) and had assignments of 0.61 Eastern[GL]/0.38 Central[GL], 0.05 Eastern[GL]/0.95 Central[GL] and 0.16 Eastern[GL]/0.84 Western[GL]. Total genetic diversity among mainland southeastern populations now under study was significantly apportioned according to these three assignment clusters (R_{st}- and F_{st}-values ranged from 0.007 to 0.06 and significance values were up to 0.0003; see also figure 2 of the electronic supplementary material).

In the highest 10 per cent posterior density runs, Kangaroo Island Crimson were consistently assigned with 100 per cent confidence to the same cluster as Adelaide, i.e. Western[GL]. Eighty-four per cent of runs that identified a geographical break between Western[GL] and Eastern[GL] did so concordantly with respect to location and individual plumage phenotype. Corresponding figures for breaks between Western[GL]/Central[GL] and Central[GL]/Eastern[GL] were 47 and 26 per cent, respectively. Estimates of gene flow in each direction between adjacent GENELAND clusters were similar, differing by a maximum of 0.13 for the Central[GL] to Western[GL] comparison.

Table 1 shows simultaneously estimated relative levels of gene flow scaled by the mutation rate among all GENELAND-defined groups derived from MIGRATE. We highlight that the hypothesized eastern ring terminus between Central[GL] and Eastern[GL] in the western slopes region is less of a barrier to gene flow than the geographical disjunction of 200 km of unsuitable habitat between Eastern[GL] and Western[GL] (figure 1). Gene flow from Central[GL] to Eastern[GL] is greater than that from Eastern[GL] to Western[GL], with barely overlapping 95% credibility intervals. The same trend is seen in gene flow in the opposite direction. Furthermore, gene flow across the western genetic discontinuity between Western[GL] and Central[GL] is similar to the hypothesized eastern terminus. Population size estimates, which are again relative estimates scaled by the mutation rate, were similar with overlapping 95% credibility intervals; the trend among them was Eastern[GL] < Western[GL] < Central[GL].

4. DISCUSSION

With a broad phylogenetic and biogeographical perspective established from our molecular genetic analyses, we tested whether a ring speciation hypothesis applies to mainland southeastern Australian populations of parrots of the Crimson Rosella *P. elegans* complex (figure 1). Both mtDNA and microsatellite molecular genetic datasets indicated that birds north of the Hunter River (figure 1) are a separate group relative to all others to the south and west (figures 2 and 3). While this result is of historical biogeographic interest (Ford 1987; Schodde & Mason 1997; Adams *et al.* 2006), it directs our focus on where a ring speciation hypothesis can be examined in mainland southeastern Australian populations south and west of the Hunter River.

Table 1. Coalescent-based estimates scaled by the mutation rate of population size and the relative migration rate from analyses using MIGRATE.

	population size			migration					
	Western^{GL}	Central^{GL}	Eastern^{GL}	Western^{GL} to Central^{GL}	Central^{GL} to Western^{GL}	Eastern^{GL} to Western^{GL}	Western^{GL} to Eastern^{GL}	Eastern^{GL} to Central^{GL}	Central^{GL} to Eastern^{GL}
maximum likelihood estimation	0.98	1.02	0.95	0.63	0.76	0.50	0.45	0.73	0.78
95% credibility intervals	0.93–1.03	0.95–1.09	0.91–0.99	0.49–0.80	0.60–0.94	0.37–0.66	0.34–0.59	0.56–0.92	0.62–0.98

Our key finding, we stress, is that contrary to the prediction of the ring speciation hypothesis of a single genetic discontinuity, we observed three genetic clusters (figure 3a) and, consequently, three genetic discontinuities (figure 2 of the electronic supplementary material). A genetic discontinuity was predicted by the ring speciation hypothesis in the western slopes region but was observed within Yellow rather than further east, where it was expected in the zone of morphological transition between Crimson and Yellow. This was in the absence of any geographical or genetic break throughout the region inhabited by the phenotypically intermediate Western Slopes birds, which define that morphological transition. Later work could test whether introgression from Crimson into Yellow occurs with selection maintaining the Yellow plumage phenotype. Further west, a second genetic discontinuity between Adelaide and Yellow was spatially concordant with the present geographical discontinuity between them but was not predicted by the ring hypothesis. A third genetic discontinuity, again not predicted by the ring hypothesis, was between Adelaide and Kangaroo Island Crimson on the one hand and mainland Crimson on the other. That is, our data did not detect the approximately 15 km sea gap between Adelaide and Kangaroo Island Crimson as a genetic discontinuity (figure 3), whereas they did do so for the 200 km of unsuitable habitat along the mainland coast that isolates Adelaide from mainland Crimson (figure 1). Similar relative values of gene flow (scaled by the mutation rate) across these discontinuities (table 1) show that while the discontinuities do not totally impede gene flow, they are of sufficient magnitude to effectively isolate the clusters they define.

Thus, not only are there three geographical discontinuities involving Adelaide with Yellow and Crimson, but those discontinuities also have a complex relationship with the three genetic discontinuities that we inferred. This key finding contrasts with a single concordant genetic and geographical discontinuity predicted by the ring speciation hypothesis in the western slopes region, a prediction not upheld by our data (figure 2 of the electronic supplementary material). In some other long-standing avian examples where a traditional and strict formulation of the ring speciation hypothesis has been tested, it at best, partially explains the relevant group's history (Martens & Päckert 2007). Similarly, we find that for our results, as we have summarized them, the ring speciation hypothesis does not fully explain the patterns in our data. This contrasts with its adequacy in the ring formed by *Ensatina* salamanders (Moritz *et al.* 1992; Jackman & Wake 1994; Alexandrino *et al.* 2005) and *Phylloscopus trochiloides* warblers (Irwin *et al.* 2005).

Nonetheless, while we reject the ring speciation hypothesis for these rosellas based on the contemporary patterns of the distribution of genetic diversity, we discuss three alternatives. Two of these involve the simpler phenomenon of divergence in allopatry, and are as follows.

(i) *Incomplete ring speciation.* Crimson and Yellow evolved as terminal differentiates of a ring distribution, but that some of the evidence has been lost through climatic and environmental changes during the Plio-Pleistocene, or that the speciation process did not reach completion before the ring was joined in the east.

(ii) *Allopatric differentiation with secondary hybridization.* The ancestral Crimson and Yellow differentiated in allopatry and then secondary contact between them resulted in hybridization and phenotypic intermediacy of Adelaide and Western Slopes (Condon 1941; Serventy 1953; Cain 1955). This predicts that Adelaide and Western Slopes should be genetic admixtures of Crimson and Yellow.

(iii) *In situ vicariant differentiation.* An ancestral population was subdivided *in situ* into isolates, which became today's various populations of Crimson, Yellow, Adelaide and Western Slopes (figure 1). Each would have evolved in historical allopatry and so could be tested for genetic independence from each other.

Critical to our discussion of these alternatives will be three premises. First, the temporal framework for the group's evolution is well within the Pleistocene. This inference is based on the low mtDNA diversity within the group (mostly less than 0.25%) and our finding that Adelaide and Crimson are part of an inferred recent population expansion (Fu's *Fs*; figure 2; see Drovetski *et al.* 2004; Weir & Schluter 2008 and caveats of Peterson 2006; Ho 2007). Our second premise arises from the observation that Crimson and Yellow are isolated from each other today (figure 1) by unsuitable semi-arid vegetation, where Plio-Pleistocene Lake Bungunnia, a 33 000 km^2 freshwater lake, existed from 3.5 to 0.7 Myr ago (Stephenson 1986; Zhisheng *et al.* 1986; Bowler *et al.* 2006). That is, Lake Bungunnia and the increasing aridity of Plio-Pleistocene glacial cycles (Bowler *et al.* 2006) were potential agents initiating the isolation and differentiation of Crimson and Yellow from each other in the south and the north, respectively, of southeastern Australia. Third, the intermediate Adelaide phenotype results from hybridization on secondary contact between Crimson and Yellow. We do consider an alternative below, but the present premise is supported strongly by the similar intermediate plumage phenotypes of Western Slopes birds resulting from hybridization between Crimson and Yellow since the anthropogenic habitat change occurred within their range in the last 100 years (see the electronic supplementary material).

(a) *Incomplete ring speciation*

Building on our data and these premises, we first consider that ancestral Crimson (south) and Yellow (north; figure 1), having already differentiated by the mechanisms suggested or indeed by any other mechanism, eventually both spread westwards. mtDNA diversity suggests that a higher rate of spread is plausible in Crimson (Fu's *Fs*; figure 2), so Crimson may have been more likely than Yellow to have occupied more than its present range at some time in its immediate past history. This may have included part or all of what is Adelaide's present range (cf. Ford 1977). Note that the sea level at the last Glacial Maximum, 18–20 000 yr ago, was 120 m lower than now (Williams 2001; Yokoyama *et al.* 2001). Potentially, this would have facilitated westwards spread of Crimson and a connection between mainland Crimson and Kangaroo Island Crimson. Eventually under this scenario, Crimson and Yellow came into secondary contact somewhere at the west of the distribution as a result of their respective

ranges' spreading (cf. Cain 1955) and before they attained reproductive isolation from each other. Adelaide would have resulted from that contact. Note also that there were intermittent land connections between Kangaroo Island and the adjacent mainland before their current isolation was established 10 000 years ago (Twidale & Bourne 2002). This scenario explains several key elements of the data: (i) Adelaide retains primary mtDNA (figure 2) and microsatellite (figure 3) signals of shared ancestry with mainland and Kangaroo Island Crimson, respectively, (ii) Adelaide shows evidence of contact and/or introgression from Yellow (figures 2 and 3), (iii) Adelaide has a strong plumage signal of ancestry from both Crimson and Yellow, and (iv) divergent mtDNA haplotypes of Kangaroo Island Crimson (figure 2). Adelaide's present latitudinal cline in phenotype would have evolved later in response to environmental gradients within its geographical range (see table 3 of the electronic supplementary material). The microsatellite data either are not yet detecting the separation of Adelaide and Kangaroo Island Crimson from each other or there is gene flow between them. Under this scenario, Pleistocene environmental changes were critical in sculpturing past and present-day genetic and geographical continuities and discontinuities in this part of the distribution (e.g. Adelaide and Kangaroo Island Crimson, geographically discontinuous and genetically continuous; Adelaide and Yellow, genetically and geographically discontinuous). Secondary contact at the east of the distribution, as we noted above, followed a very recent anthropogenic change (see the electronic supplementary material). An alternative is that ancestral Crimson spread south and west along the coast of mainland southeastern Australia as far as and including the present range of Adelaide, then along the inland rivers of the Murray–Darling Basin (figure 1) back towards the east, with subsequent severing of the ring southwest, northwest and southeast of Adelaide's range (figure 1). The Yellow phenotype would have arisen in the west and gone to fixation under selection along the inland rivers.

(b) *Allopatric differentiation with secondary hybridization*

The key prediction here is that Adelaide and Western Slopes should be genetic admixtures of Crimson and Yellow. mtDNA diversity in Western Slopes (figure 2) arguably is the admixture that this hypothesis predicts, but the microsatellite analyses show these birds to be genetically continuous with Crimson (figure 3), which is strongly counter to this prediction. Again, we suggest that further work should focus on the interplay of selection and drift in this region. In the west of the distribution, and as with the scenario of a continuous circular distribution we have just described, population expansion of the southern isolate (Fu's *Fs*; figure 2) could have been a mechanism that led to secondary contact of Crimson with Yellow, especially if population expansion equated with a range expansion not just an increase in population density. The genetic admixture of Crimson and Yellow that this hypothesis predicts in Adelaide is suggested more strongly in plumage than it is observed in mtDNA and microsatellite diversities, which clearly share more with Crimson than with Yellow (figures 2 and 3). This scenario differs from the circular distribution scenario we have just described, perhaps most importantly, in that the range of Adelaide

need only ever have been inhabited by Adelaide, never by Crimson or Yellow, and only after secondary contact of Crimson and Yellow established the Adelaide phenotype.

(c) *In situ vicariant differentiation*

This alternative predicts that today's various populations of Adelaide, Crimson, Yellow and Western Slopes each evolved in allopatry and so should be genetically independent from each other. By this scenario, Adelaide retains a strong mtDNA signal of evolution from a Crimson-like ancestor with occasional introgression from Yellow (figure 2; Joseph & Hope 1984). Its intermediate phenotype would have resulted only through drift in isolation; the development of its latitudinal cline in plumage and the origin of the Western Slopes birds would be as in other scenarios. This scenario does not readily explain key elements such as clustering of Adelaide with Kangaroo Island Crimson by microsatellites, or why Adelaide and Western Slopes so closely resemble each other phenotypically, or why Kangaroo Island Crimson mtDNA haplotypes cluster with Adelaide and Yellow in different parts of the mtDNA network (figure 2). Lastly and critically, and notwithstanding the relatively shallow temporal framework we have established (above), we did not recover the genetic independence of Crimson, Adelaide, Yellow and Western Slopes that this alternative predicts.

Four conclusions emerge from our discussion. First, we cannot reject that a geographically continuous circular distribution has been involved in the group's history or indeed that one was formed through secondary contact at the east and the west. Second, and in the light of the observed geographical and genetic discontinuities, we do reject that a ring speciation hypothesis with secondary contact only between Crimson and Yellow at the east of the distribution necessarily follows as an explanation of the group's history. This is regardless of whether a continuous circular distribution did in fact set the geographical stage for that process to occur. Third, one historical scenario probably cannot be chosen over another *in all details* when trying to unravel mechanisms behind the origins of the diversity and structure we have observed. Last, Pleistocene environmental dynamics (changes in sea level, vegetation and climate) in the western part of the putative ring would probably have caused this uncertainty by steadily eroding the geographical and genetic signals of the group's history. In the temporal snapshot of the group's history from which we have taken genetic data, the speciation process has been captured at a point where reproductive isolation and substantial divergence have not been attained in any of the southeastern Australian populations that are our focus, but the stage is set for their subsequent development, albeit now largely in allopatry.

Further work might assess whether the Crimson Rosella complex in southeastern Australia describes a situation with elements of an alloparapatric model of speciation *sensu* Endler (1977): selection-driven divergence initiated in allopatry (Yellow and Crimson) followed by range expansion (southern Crimson) and geographical contact in parapatry or sympatry (Adelaide and Western Slopes; Endler 1977; Coyne & Orr 2004; Price 2008). By this view, red–yellow plumage diversity that attracted interest in the evolution of this group and that gave rise to the ring-species hypothesis probably retains relatively more signal of recent, post-isolation selective regimes (Adelaide),

and, possibly as well, genetic drift (e.g. Kangaroo Island Crimson, Yellow), than of longer term population history. As we have identified areas of likely secondary contact, it is appropriate to reinforce Brumfield *et al.*'s (2001) call to see zones of secondary contact in speciation studies not as black holes from which genes cannot leave but as evolutionary conduits through which adaptive and neutral markers can differentially move.

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