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The role of the Ord Arid Intrusion in the historical and contemporary genetic division of long-tailed finch subspecies in northern Australia

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Abstract

The effect of separation by biogeographic features followed by secondary contact can blur taxonomic boundaries and produce complex genetic signatures. We analyzed population structure and gene flow across the range of the long-tailed finch (*Poephila acuticauda*) in northern Australia (1) to test the hypothesis that Ord Arid Intrusion acted as the causative barrier that led to divergence of *P. acuticauda* subspecies, (2) to determine whether genetic data support the presence of a gradual cline across the range or a sudden shift, both of which have been suggested based on morphological data, and (3) to estimate levels of contemporary gene flow within this species complex. We collected samples from 302 individuals from 10 localities. Analyses of 12 microsatellite loci and sequence data from 333 base pairs of the mitochondrial control region were used to estimate population structure and gene flow, using analysis of molecular variance (AMOVA), haplotype network analysis, frequency statistics, and clustering methods. Mitochondrial sequence data indicated the presence of three genetic groups (regions) across the range of *P. acuticauda*. Genetic diversity was highest in the east and lowest in the west. The Ord Arid Intrusion appears to have functioned as a biogeographic barrier in the past, according to mtDNA evidence presented here and evidence from previous studies. The absence of isolation by distance between adjacent regions and the lack of population genetic structure of mtDNA within regions indicates that genetic changes across the range of *P. acuticauda* subspecies are characterized by discrete breaks between regions. While microsatellite data indicate a complete absence of genetic structure across this species' range, it appears unlikely that this results from high levels of gene flow. Mitochondrial data do not support the presence of contemporary gene flow across the range of this species.

Introduction

Australia's northern tropics harbor a wide range of species associated with distinct biogeographic areas and the range boundaries of many of these species are often concordant, suggesting vicariance (Keast 1961; Ford 1978; Bowman et al. 2010). However, current species distributions are the result of a multitude of processes operating over various time scales, impeding a clear understanding of their history. This is especially true in cases where a biogeographic barrier separated a taxonomic group for a period of time and then subsided, allowing secondary contact. Analysis of genetic data in the context of biogeography offers an approach that can elucidate the history of these species (Bowman et al. 2010), especially if genetic markers are chosen that reflect population history over multiple time scales (York et al. 2008; Taylor et al. 2011; Zarza et al. 2011).

The genetics of many extant species reflect events that occurred during the most recent glacial maximum, when advancing ice sheets resulted in the formation of refugia (Hewitt 2004). However, in Australia, species' range contractions into refugia were in response to aridity (Keast 1961), which was extreme during this period (De Deckker 2001). In northern Australia, two refugia were proposed by Keast (1961), the

Figure 1. Pair of long-tailed finches (*Poephila acuticauda*), Western Australia. Photo by Sarah Pryke.

"northwest" and the "northeast." The northwest refugium was bounded by the Great Sandy Desert to the west and an arid zone at the head of the Gulf of Carpentaria to the east. Further east of Carpentarian barrier was the northeast refugium in Cape York. Phylogenetic analyses across many taxa support the Carpentarian barrier (Macdonald 1969; Cracraft 1991; Lee and Edwards 2008; Toon et al. 2010), although exceptions exist (Kearns et al. 2010). Ford (1978) proposed a "minor" biogeographic barrier splitting Arnhem Land and the Kimberley (Bonaparte barrier), more recently termed the "Ord Arid Intrusion" (Bowman et al. 2010). Many vicariant pairs of bird species and subspecies are located to either side of this barrier (Ford 1978). However, the precise boundaries of this barrier have not been defined.

One species complex that demonstrates divergence across the Carpentarian barrier and the Ord Arid Intrusion is found in the Australian grass finch genus *Poephila*. *Poephila cincta* (black-throated finch) are found to the east of the Carpentarian barrier and *P. acuticauda* (long-tailed finch, Fig. 1) are found to the west, ranging from Barkly Tableland in western Queensland, throughout the Northern Territory and the Kimberley region of western Australia (Higgins et al. 2006). Two subspecies of *P. acuticauda* are recognized: these are phenotypically similar except in regard to bill color, those in the east of the range (*P. a. hecki*) have red bills and those to the west (*P. a. acuticauda*) have yellow bills. Divergence between *P. cincta* and *P. acuticauda* has been dated at approx-

imately 0.6 million years ago, while the divergence between *P. a. acuticauda* and *P. a. hecki* has been estimated at 0.3 million years ago based on multiple nuclear gene trees (Jennings and Edwards 2005).

The subspecies designation of *P. acuticauda* has been debated by Keast (1958) and many others since, primarily based on bill coloration. Mayr et al. (1968) and Immelmann (1982) suggested that bill coloration was clinal, possibly signifying a single taxonomic group whereas others indicate that coloration changes abruptly near the eastern edge of the Kimberley (Harrison 1974; Boles 1988; Schodde and Mason 1999), which coincides with location of the Ord Arid Intrusion. Additionally, intergradation between yellow and red forms (expressed intermediate phenotypes) occurs in the vicinity of the Ord Arid Intrusion (Schodde and Mason 1999) and, in captivity, crosses between the two forms produce orange-billed offspring (Higgins et al. 2006) suggesting that coloration is genetically based. In addition to differences in bill-color, distinct differences in song structure between the two forms (sampled from either side of the Ord Arid Intrusion) have been identified (Zann 1976).

Genetic analyses of *P. acuticauda* are limited to Jennings and Edwards' (2005) phylogeographic study of 30 nuclear loci in a single individual of each form, taken from locations in western Queensland and the western Kimberley (near Chidna and Mt. House, respectively, Fig. 2) from the extreme ends of the species distribution. While their study provided well-supported evidence for historical divergence between the two forms, their approach was not intended to address contemporary population structure. Here, we use mitochondrial DNA (mtDNA) sequence and microsatellite genotyping of multiple individuals sampled across the range of *P. acuticauda* to address the following questions: (1) did the Ord Arid Intrusion act as an agent of vicariance in this species complex, (2) is there evidence to support a gradual genetic mixing of populations sampled across the range or do genetic data support the presence of a discrete break in population structure resulting in either two genetic groups separated by the geographical area upon which the Ord Arid Intrusion was situated or three groups comprised of localities from the vicinity of the Ord Arid Intrusion and those to the east and west of this area (Fig. 2), and (3) is there evidence of current gene flow across the range of this species complex? We place the genetic results of the current study, those from Jennings and Edwards (2005) and evidence of phenotypic difference, in the context of whether the Ord Arid Intrusion has shaped and continues to shape the history of this species.

Materials and Methods

Field sampling and laboratory procedures

Blood samples were collected from 302 long-tailed finches from 10 localities across the species' range (Fig. 2). This
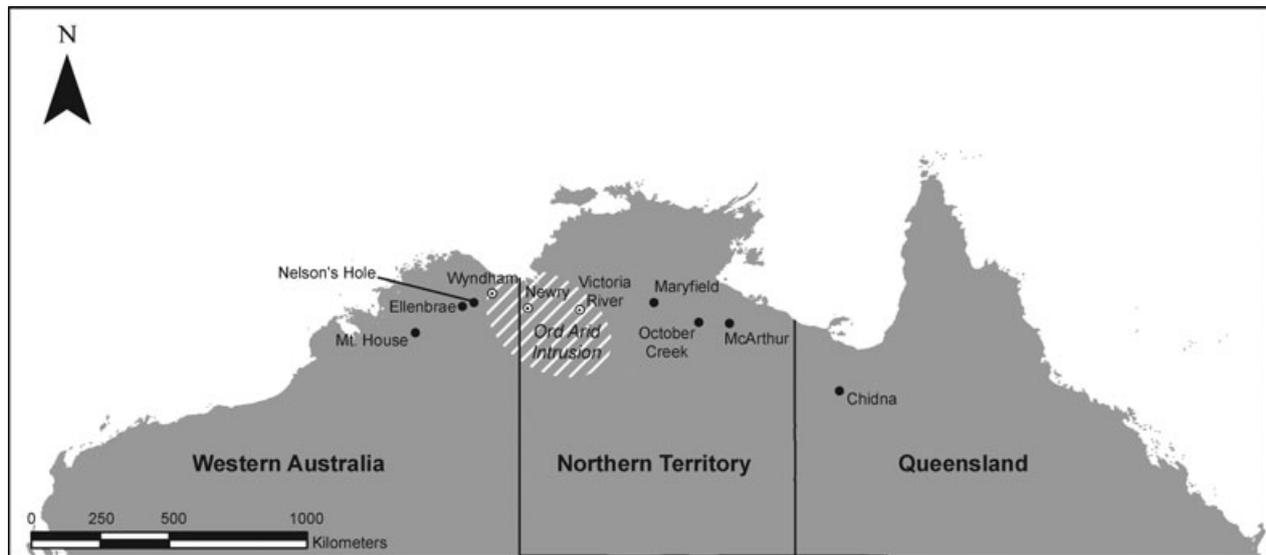
Figure 2. Map of sampling localities across the range of *P. acuticauda* (northern Australia). Open circles indicate localities in the vicinity of the Ord Arid Intrusion.

Table 1. Mitochondrial sequence diversity for each sampling locality including sample size (*N*), number of private haplotypes (N_{PH}), number of haplotypes (N_H), haplotype richness (H_R), and haplotype diversity (*h*) with standard errors (SE).

Locality	*N*	N_{PH}	N_H	H_R	*h* (±SE)
Western					
Mt. House	32	1	4	2.1	0.33 (0.10)
Ellenbrae	18	0	1	0	0
Nelson's Hole	30	1	5	3.1	0.46 (0.11)
Central					
Wyndham	30	1	6	4.0	0.73 (0.05)
Newry	24	1	6	4.2	0.73 (0.06)
Victoria River	20	0	3	2.0	0.62 (0.08)
Eastern					
Maryfield	30	6	12	8.2	0.89 (0.04)
October Creek	30	1	6	4.5	0.83 (0.03)
McArthur	30	4	9	5.6	0.81 (0.04)
Chidna	30	4	8	5.4	0.75 (0.07)

included three localities from the vicinity of the historical Ord River Intrusion (Bowman et al. 2010) hereafter referred to as the "central" region (Wyndham, Newry, Victoria River), three to the west of this putative barrier ("western"; Mt. House, Ellenbrae, Nelson's Hole), and four to the east ("eastern"; Maryfield, October Creek, McArthur, Chidna). Global Positioning System readings were taken at each sampling locality. DNA was extracted using a Gentra PureGene kit (Qiagen, Valencia, CA, USA) following manufacturer's instructions.

A segment of the mtDNA control region was sequenced in 30 individuals per population where available (Table 1) using primers *paCRL1* (5′-TGT-AGG-ATA-GCC-AAT-GTC-ATA-CG) and *paCRH1* (5′-CGC-CTG-AAG-CCA-ATA-ACC-TA). These primers were designed in PRIMER3 (Rozen and Skaletsky 2000) using consensus sequence between the zebra finch (*Taeniopygia guttata*, GenBank #DQ453512-5) and the black-throated finch (GenBank #AY324294). PCR reactions for the mtDNA sequence contained approximately 30 ng of genomic DNA, 2 pmoles of each primer, 1× buffer, 3.5 mM MgCl, 200 μM dNTPs, and 0.2 units of AmpliTaq Red (Applied Biosystems, Foster City, CA, USA). Cycling conditions included a five-min step at 94°C; 30 cycles of 94°C for 30 sec, 53°C for 15 sec, and 72°C for 30 sec; and a final extension step for 10 min at 72°C. PCR products were cleaned using ExoSAP-IT (USB Corporation, Cleveland, OH, USA). Big-Dye chemistry (Applied Biosystems) was used in sequencing reactions and products were ethanol precipitated and run on an Applied Biosystems 3730. A subset of samples was sequenced in the reverse direction to assure accuracy of nucleotide identification. All individuals assigned singleton haplotypes were sequenced twice to rule out sequencing error. Sequences were edited in CHROMAS LITE version 2.0 and any samples having unclear electropherograms were sequenced again. Accepted sequences were aligned using CLUSTALX2 version 2.0.5 (Thompson et al. 1997) and trimmed to a uniform length of 333 base pairs (bp). We considered this sequence to be genuine mitochondrial DNA because double peaks were not identified in any position and because of the close match between the central haplotype in our network (Hap A, see below and Fig. 3) and to previously identified control region sequence of the congener *P. cincta* (95% match, GenBank #AY324294).
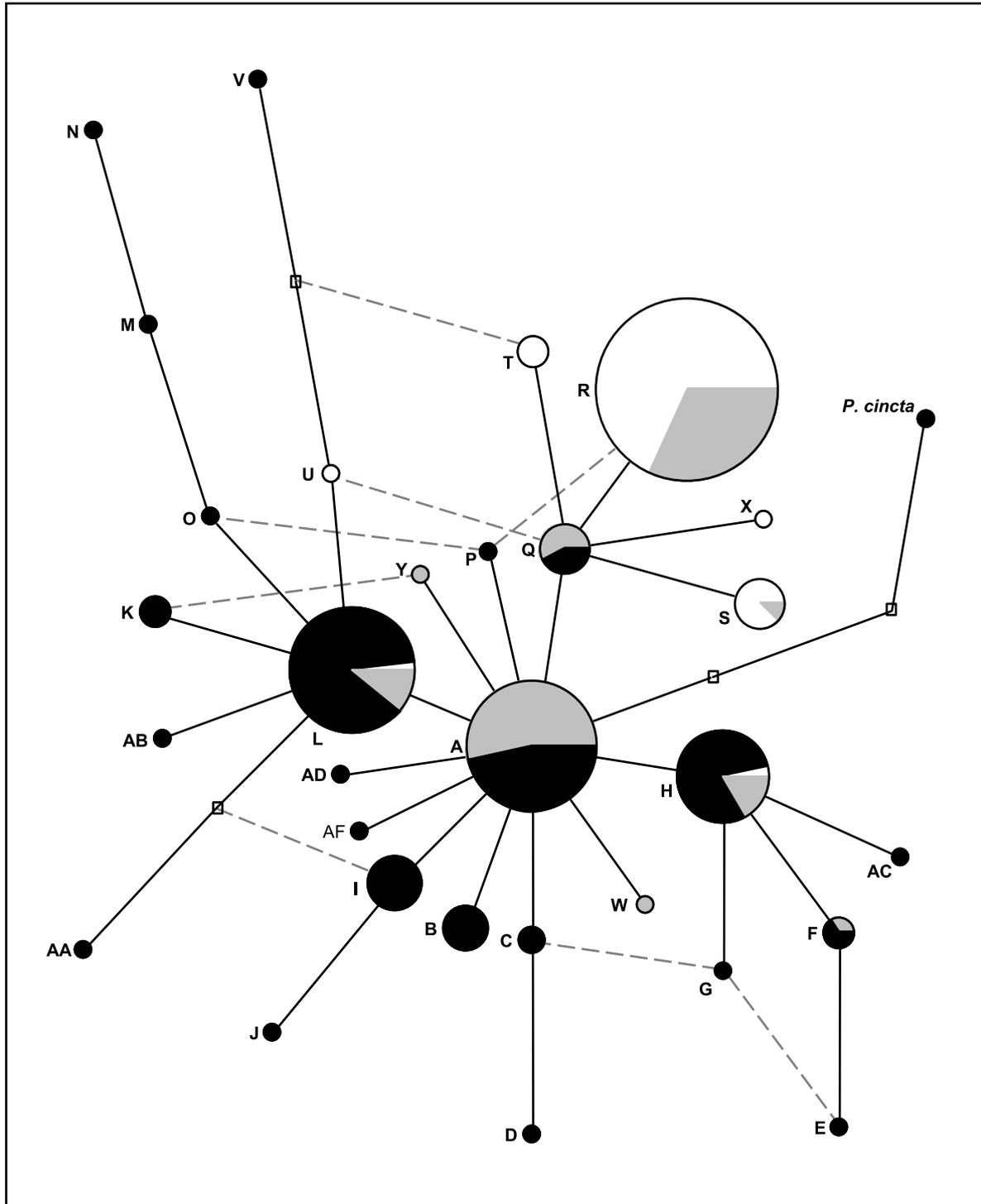
Figure 3. Network depicting evolutionary relationships between mitochondrial DNA haplotypes of *P. acuticauda* samples from northern Australia. Circle size indicates proportion of individuals assigned that haplotype. Solid lines connecting haplotypes are proportional to the number of mutations separating them. Dotted lines represent resolved network ambiguities, each representing a single mutational step. Open squares denote positions of unsampled intermediate haplotypes. Black shading represents proportions from the eastern localities, gray represents proportions in central localities, and white represents the proportions in the west. The network is rooted to the congener *P. cincta*.

We analyzed 12 cross-specific microsatellites including: *Indigo41* (Sefc et al. 2001); *TG01–000, TG02–088, TG03–031, TG04–012, TG05–046, TG11–011, TG22–001* (Dawson et al. 2010); *Tgu1, Tgu3, Tgu12* (Forstmeier et al. 2007); and *Titgata02* (Wang et al. 2005). Microsatellites were amplified in three multiplex PCR reactions using a Qiagen Multiplex Kit. One multiplex contained Dawson et al. (2010) primers and followed their protocol. The second multiplex contained *Indigo41*, *Tgu1*, *Tgu3*, and *Tgu12* and was run using a step-down PCR program consisting eight cycles each at the following annealing temperatures: 70°C, 64°C, 58°C, 54°C, and 50°C. A third PCR contained *Titgata02* and was run using a step-down program containing nine cycles each at the following annealing temperatures: 64°C, 60°C, 56°C, 53°C, and 50°C. The second and third reactions were combined prior to genotyping. Samples were genotyped on an ABI 3730 (Applied Biosystems) using GS-500 (Liz) in each capillary as a size standard. Allele sizes were estimated on GENEMAPPER version 3.7 (Applied Biosystems). A subset of individuals ($N = 112$) from Mt. House, Nelson's Hole, and October Creek (Fig. 2) were sexed using multiplexed sex-linked markers 3007F, 2987F, and 3112R (Fridolfsson and Ellegren 1999) following Gilby et al. (2011).

Statistical analyses

Mitochondrial DNA sequence

We examined genetic diversity by calculating for each sample the number of haplotypes (N_H) and haplotype diversity (h) (the probability that two haplotypes randomly chosen from the sample are different) in ARLEQUIN version 3.5 (Excoffier et al. 2005). We also calculated haplotype richness (H_R) in CONTRIB version 1.02 (Petit et al. 1998), which uses a rarefaction procedure to adjust for unequal sample sizes. The number of private haplotypes (N_{PH}) (i.e., those found in only one locality) was calculated manually. We used NETWORK version 4.6.0.0 (Bandelt et al. 1999) to construct a median-joining network to investigate evolutionary relationships among haplotypes. This method combines a minimum spanning approach representing all possible shortest trees with a maximum parsimony algorithm that removes superfluous links. Network ambiguities were solved following Pfenninger and Posada (2002).

Pairwise F_{ST} values between sampling localities were calculated in ARLEQUIN using the Kimura 2-parameter model and *P*-values were Bonferroni corrected for multiple testing. Due to the presence of significant F_{ST} values between localities (see below), we used analysis of molecular variance (AMOVA) (Excoffier et al. 1992) to determine which of the following hypotheses our data best fit: (1) sampling localities would represent two genetic groups divided by the historical Ord Arid Intrusion or (2) sampling localities would be divided into three genetic groups represented by one to the

eastern, central, and western regions defined above. For the former hypothesis, we separated localities to the east and west of the estimated geographic center of the Ord Arid Intrusion (i.e., a division between Victoria River and Newry, Fig. 2). The AMOVA having the lowest amount of variance among regions would indicate which hypothesis was most likely. Mantel tests implemented in ARLEQUIN were used to determine if there was evidence for a relationship between genetic (Slatkin's linear F_{ST}) and geographic distance (kilometers).

Microsatellite analyses

Microsatellite data were checked for departures from Hardy–Weinberg in ARLEQUIN and for linkage disequilibrium using GENEPOP version 4.0 (Rousset 2008). FSTAT version 2.9.3.2 (Goudet 1995, 2001) was used to calculate the total number of alleles (N_A), the number of private alleles (N_{PA}), allelic richness (R), gene diversity (GD), and allele frequencies. The method used to calculate R incorporates a rarefaction method (Mousadik and Petit 1996) to compensate for unequal sample sizes. Population pairwise R_{ST} values (Slatkin 1995) were calculated in ARLEQUIN and *P*-values were Bonferroni corrected for multiple comparisons. As a second method of assessing population structure, we used the Bayesian clustering approach implemented in STRUCTURE version 2.3.3 (Pritchard et al. 2000; Falush et al. 2003), which determines the number of genetic groups present (K). We analyzed STRUCTURE results using the ΔK method (Evanno et al. 2005) to infer the number of genetic groups sampled. Because ΔK is a measure of the rate of change, this method cannot calculate a ΔK value for the smallest and the largest K included in the run. Therefore, in order to determine if our data had a true $K = 1$, we examined plots of the log posterior probability of the data ($Ln\ P(D)$) for each value of K to determine if the $Ln\ P(D)$ value was maximum for $K = 1$. If alpha plots are unstable across all values of K and if *Q*-plots show all individuals as equally admixed, true $K = 1$ can also be inferred (Pritchard et al. 2007). In STRUCTURE, we chose the admixture model with correlated allele frequencies and ran 10 replicates of each run ($K = 1$ though 6). Each run consisted of a burn-in period of 100,000 Markov chain Monte Carlo steps followed by 10^6 iterations. We examined a subset of individuals for the presence of sex-biased dispersal using a test based on the variance of the assignment index (Goudet et al. 2002) in FSTAT and generated *P*-values based on 10,000 randomizations.

Results

Mitochondrial DNA sequence

Across all samples, a total of 30 haplotypes (Fig. 3; GenBank JQ255398–JQ255427) were identified containing 20 polymorphic sites. Genetic diversity was highly variable across
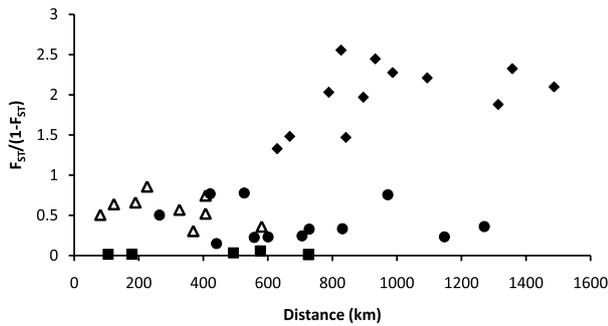
Figure 4. Graph illustrating the relationship between geographic and genetic distance using mitochondrial DNA sequence data for each pair of sampling localities (all pairs: $r = 0.65$, $P = 0.001$). This relationship is not significant between adjacent regions (eastern/central = filled circles, $r = 0.37$, $P = 0.08$; western/central = open triangles, $r = 0.22$, $P = 0.12$) but is significant between eastern and western sampling localities (filled diamonds, $r = 0.83$, $P = 0.01$). Within-region comparisons (filled squares) had the lowest values of genetic distance, despite having geographic distances overlapping those of all other across-region comparisons.

localities with the number of haplotypes per locality ranging from one (Ellenbrae) to 12 (Maryfield) (Table 1). Only four haplotypes (A, H, L, and R) were common across all sampling localities and 78% of all individuals carried one of these haplotypes. However, the frequencies of these haplotypes within regions were skewed: haplotype R was absent in the east although it had a frequency of 0.35 across all individuals sampled, haplotype A was absent in the west although it had a frequency of 0.18 across all individuals sampled, and haplotypes H and L had frequencies of 0.09 and 0.16, respectively, but each was only found in a single individual in the west. Eighteen haplotypes were found in only one individual. In general, genetic diversity was greatest in the east (Table 1).

Pairwise F_{ST} values ranged from zero to 0.72 and clearly indicated genetic breaks between eastern (Chidna, McArthur, October Creek, Maryfield), central (Victoria River, Newry, Wyndham), and western (Nelson's Hole, Ellenbrae, Mt. House) sampling localities: all comparisons of localities within these regions had low and nonsignificant F_{ST} values (0–0.07) whereas all comparisons of localities between regions had high and significant F_{ST} values (0.13–0.72). Average F_{ST} values between eastern and western localities were high (0.66), whereas those between the central localities and those to the east (0.36) or to the west (0.28) were intermediate. AMOVA results for the two-region hypothesis (separated by the geographic center of the historical Ord Arid Intrusion) indicated there was significant structuring at all levels: 35.7% of the variation was among regions ($P = 0.03$), 14.3% of the variation was among populations within regions ($P < 0.001$), 50% of the variation was within populations ($P <$

0.001). However, for the three-region hypothesis, the variation among regions increased to 47.4% of the total variation ($P < 0.001$), the variation among populations within regions was no longer significant and only accounted for 1% of the total variation ($P = 0.07$), and the within-population variation accounted for 51.6% of the total variation. These results indicate the three-region hypothesis is more likely. Although there was a significant positive relationship between geographic and genetic distance when all pairwise comparisons were included ($r = 0.65$, $P = 0.001$), this relationship was not consistent across the range of *P. acuticauda* (Fig. 4). Post-hoc Mantel tests including combinations of two of the three regions supported by AMOVA (three tests: western/central, eastern/central, western/eastern) indicated that no relationship could be detected between geographic and genetic distance between adjacent regions: for eastern and central samples, the correlation coefficient was 0.37 ($P = 0.08$) and for central and western samples the correlation coefficient was 0.22 ($P = 0.12$). However, a significant relationship was detected between geographic and genetic distance when eastern and western samples were tested (correlation coefficient 0.83, $P = 0.01$). Within-region pairwise comparisons (Fig. 4, filled squares) had the lowest genetic distances, although these comparisons had geographic distances overlapping all across-range comparisons.

Microsatellite analyses

No departures from Hardy–Weinberg or linkage disequilibrium were detected in any of our microsatellite markers. Microsatellites were highly polymorphic with a mean of 16.4 alleles per locus (range 6–42) but showed no differences in genetic diversity across sampling locations (Table 2). Population pairwise R_{ST} values were generally low (mean $R_{ST} = 0.03$, range 0–0.10) and few had significant P-values (three

Table 2. Microsatellite diversity for each sampling locality including sample size (N), average number of alleles (N_A) per locus, number of private alleles (N_{PA}), allelic richness (R), and gene diversity (GD).

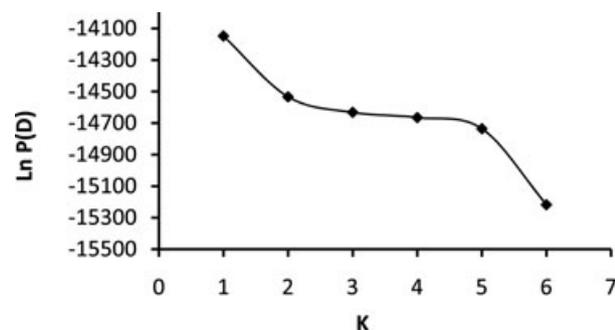| Locality	N	N_A	N_{PA}	R	GD
Western					
Mt. House	40	8.4	6	7.1	0.69
Ellenbrae	18	8.0	6	8.0	0.76
Nelson's Hole	40	9.1	7	7.3	0.68
Central					
Wyndham	30	8.1	1	7.2	0.70
Newry	24	9.0	5	8.1	0.70
Victoria River	22	7.5	3	7.1	0.72
Eastern					
Maryfield	30	8.7	2	7.5	0.71
October Creek	32	8.6	4	7.4	0.72
McArthur	36	9.3	7	7.7	0.73
Chidna	30	8.3	6	7.1	0.70

Figure 5. Graph illustrating the log posterior probabilities of the microsatellite data (*Ln P(D)*) for each number of genetic groups (*K*) tested. Maximum value of *Ln P(D)* corresponds to *K* = 1 indicating data represent a single population.

out of 45: Nelson's Hole/Wyndham; Nelson's Hole/Victoria River; Newry/Victoria River). The general lack of population differentiation indicated by pairwise R_{ST} comparisons from microsatellite data was mirrored in the results of STRUCTURE analyses. The maximum value of *Ln P(D)* corresponded to $K = 1$ (Fig. 5), alpha plots were unstable across runs and Q-plots indicated all individuals were equally admixed for all values of *K*. In combination, this evidence suggests the presence of a single genetic group across northern Australia. The variance assignment test showed no evidence of sex-biased dispersal ($P = 0.82$).

Discussion

Our results support previous morphological and phylogenetic evidence suggesting that the subspecies of *P. acuticauda* were separated by the Ord Arid Intrusion. However, mtDNA analyses presented here indicate the presence of three (not two) distinct genetic groups of *P. acuticauda* in Australia: one to either side of the Ord Arid Intrusion and a third group located in the vicinity of this biogeographic barrier. In contrast, microsatellite data show no evidence of population structure across the range of this species. Because this collection of evidence addresses different time spans, we are able to construct a more complete history of this species with respect to the effect of the Ord Arid Intrusion than previous studies.

Phylogenetic data from nuclear markers provide information regarding a species' history in the more distant past than mitochondrial markers. Using nuclear markers, Jennings and Edwards (2005) concluded that *P. a. hecki* and *P. a. acuticauda* diverged approximately 0.3 million years before present, which is consistent with the time scale over which the Ord Arid Intrusion was present. Incomplete lineage sorting, common in recently isolated taxa, was found in *P. acuticauda* subspecies and explained by a large population size relative to divergence time (Jennings and Edwards 2005). However, mtDNA has one-quarter the effective population size of the nuclear genome (Birky et al. 1989), resulting

in more rapid lineage sorting following separation (Avise 2000). For this reason, Edwards et al. (2005) have criticized the sole use of mtDNA as a measure of separation, suggesting that the rapidity with which it segregates may prematurely indicate divergence. However, in this study, mtDNA haplotypes were not private to putative subspecies, which may be the result of introgression following secondary contact, but may also be due to incomplete lineage sorting. This may indicate that the divergence identified by Jennings and Edwards (2005) using nuclear DNA analyses has also been too recent for reciprocal monophyly to form in the mitochondrial genome. If the Ord Arid Intrusion is the biogeographic barrier responsible for Pleistocene separation of these subspecies and secondary contact/interbreeding has not occurred, then we would expect to see two genetic groups represented in mtDNA. Instead, we identified three groups, with the central group being geographically centered on the historical Ord Arid Intrusion and genetically intermediate to those to either side. This may suggest that individuals from the central region are the result of secondary contact and interbreeding between eastern and western regions. However, an alternate explanation for the mtDNA patterns we have identified exists. It is possible that individuals from our eastern and central regions are representative of two groups that were separated at one point in time by the Ord Arid Intrusion and that have not had sufficient time to become reciprocally monophyletic. The population in the western region may have been subsequently founded from a small number of individuals from the central region expanding the range westward when arid conditions abated. Levels of genetic diversity support this concept: samples from the western region had the lowest level of genetic diversity (Table 1; Fig. 3). The highest levels of genetic diversity were found within mtDNA from the eastern region, consistent with larger postseparation population sizes in this area. Additionally, the topology of the haplotype network (Fig. 3) supports this alternative explanation. Haplotypes found in the western region were mostly terminal, suggestive of a more recent origin whereas the most common haplotypes found in the central and eastern regions (A, H, L) appear to be ancestral as they located at the center of a star-shaped radiations typical of evolutionarily recent population expansion (Slatkin and Hudson 1991). Furthermore, only in the western region are haplotype proportions highly skewed toward a single haplotype (85% of individuals carry haplotype R), which is indicative of a small founding population and subsequent genetic drift.

Evidence of gene flow

Nuclear microsatellite data can provide different information about population structure than sequence data from either the nuclear or mitochondrial genome. This class of genetic

markers will coalesce at the same rate as nuclear sequence data, but due to high rates of mutation and homoplasy, data from microsatellites may obscure population history, making them less than ideal for phylogenetic studies (Zink and Barrowclough 2008). However, the high mutation rates of microsatellites may result in greater statistical power to identify even small genetic differences between populations (Hedrick 1999). If population differentiation can be identified, it then can be used for estimating contemporary gene flow through several methods that detect recent dispersal (Pritchard et al. 2000; Wilson and Rannala 2003; Piry et al. 2004). These methods can be highly useful for detecting gene flow following secondary contact (e.g., Jones et al. 2005). However, both our study and that of Vallender et al. (2007) failed to find any evidence of population structure across species contact zones using microsatellite loci, despite evidence of structure from mtDNA. Many other avian studies have found substantially less structure at microsatellites than mtDNA (Haavie et al. 2000; Tiedemann et al. 2004; Wright et al. 2005). The absence of substantial structure in microsatellite data between populations prevents the use of the methods mentioned above to assess contemporary gene flow.

The studies discussed above indicate that contrasting patterns of population structure from microsatellite and mtDNA loci are not unusual, and cases where mtDNA show greater population structure have often been explained as resulting from male-biased dispersal (reviewed in Zink and Barrowclough 2008). However, in most species of birds, dispersal is female-biased (Greenwood 1980). We found no evidence of sex-biased dispersal in *P. acuticauda* in concordance with recapture data from birds banded as nestlings (van Rooij and Griffith 2011). Zink and Barrowclough (2008) suggested these differences are more parsimoniously explained by differences in coalescent times, such that nuclear markers reflect structure in the more distant past than those from mtDNA. While the pattern we have observed could be the result of recent divergence, which is only evident in mtDNA, this seems unlikely based on the findings of Jennings and Edwards (2005) that divergence was evident using nuclear markers. It is more likely that the inconsistency of our results across marker classes is related to issues specific to microsatellites, such as homoplasy, which can obscure signals of structure. The use of microsatellite markers in studies attempting to separate historical from contemporary processes has been the subject of recent debate (Zink 2010; Zink et al. 2010, 2011; Barr et al. 2011) and will continue to require careful interpretation.

Two alternate explanations suggest the presence of current gene flow with differential introgression of marker classes. First, Haldane's rule states that when hybrid offspring have differential fertility or viability according to their sex, the heterogametic sex is more affected (Haldane 1922). If Haldane's rule applies to hybrids of *P. a. acuticauda* and *P. a. hecki*,

then we would expect to see introgression of nuclear DNA (biparental) in the absence of introgression of mtDNA (maternal) because females are the heterogametic sex in birds. Second, Petit and Excoffier (2009) suggest that markers associated with the more dispersive sex (i.e., mtDNA in our case) should provide better discrimination between species across hybrid zones than those associated with both sexes. While this at first appears counterintuitive, it is explained by higher levels of within-species gene flow at markers associated with the more dispersive sex counteracting the effects of genetic drift. Petit and Excoffier (2009) cite numerous examples of species having female-biased dispersal showing higher levels of introgression across hybrid zones in nuclear markers than those from the mitochondrial genome, suggesting that mtDNA is more useful in identifying subspecies. It is clear that if either of these explanations is correct, only a small number of individuals need to be exchanged in order to prevent drift from creating population divergence (Wright 1969; Mills and Allendorf 1996).

While the effect of a low level of exchange between populations is predicted to result in genetic homogeneity, this may not be representative of demographic connectivity. The relative contributions of effective population size and dispersal rate to estimates of genetic differentiation have recently been examined by Chapuis et al. (2011). Using genetic data from Australia plague locust (*Chortoicetes terminifera*) populations, they demonstrated that when effective population sizes are very large, a nonsignificant result from genotype frequency comparisons cannot be interpreted as evidence of demographic connectivity. Demographic connectivity is driven by migration rate, whereas genetic connectivity is driven by the product of migration rate and effective population size (Waples and Gaggiotti 2006). Therefore, very large effective population sizes combined with low levels of migration may homogenize populations genetically while they remain demographically independent. Historical population sizes for *P. acuticauda* are estimated to have been in the hundreds of thousands (Jennings and Edwards 2005) and currently this species is described as "common" (Clement 1999). Therefore, it is possible that our microsatellite data are not useful in drawing conclusions regarding contemporary connectivity between subspecies of *P. acuticauda*.

Little is known about dispersal in *P. acuticauda*. High levels of adult site fidelity and natal philopatry have been reported for this species (van Rooij and Griffith 2011), suggesting restricted dispersal. This is also supported by previous conclusions regarding levels of gene flow in this species (Jennings and Edwards 2005). Further, observations regarding bill color support restricted dispersal. Crosses between red- and yellow-billed forms produce offspring having orange bills (Higgins et al. 2006). Therefore, it would be expected that high levels of long-distance dispersal would result in an eventual loss of differential coloration, in the absence of assortative

mating or selection, yet strong color divergence persists across the range. However, in the presence of either assortative mating or selection, differential coloration may be maintained in the face of high levels of long-distance dispersal. Breeding experiments between the two morphs are required to better understand assortative mating and selection on bill color in this species complex. We did identify several individuals carrying mtDNA haplotypes that were uncommon in the region where they were sampled. For instance, a single western individual was assigned haplotype L and a single western individual was assigned haplotype H. These haplotypes are common in the east and are also found in the central region. Similarly, two individuals sampled in the central region carried haplotypes unique to other regions (one carried S, unique to the western region barring this individual; one carried F, unique to the eastern region barring this individual). It is possible that these individuals are dispersers, although we cannot rule out the possibility that this is simply the result of incomplete lineage sorting. Even low levels of dispersal should result in genetic homogenization of populations, which is not supported by mitochondrial or bill color data from this species complex. Therefore, if these individuals are genuine dispersers, they do not appear to be reproductively successful. We found no evidence of genetic differentiation when analyzing mtDNA within regions, which does support gene flow at these scales.

Conclusions

In this study, we aimed to provide genetic evidence regarding the role of the Ord Arid Intrusion in shaping populations of *P. acuticauda*. First, we asked if this geographical feature functioned as an agent of vicariance between the subspecies *P. a. acuticauda* and *P. a. hecki*. Our mtDNA data support the presence of three genetically distinct regions (Fig. 2): one to the east of the Ord Arid Intrusion, one in the vicinity of this historical feature, and one to the west. The division we identified between the eastern and central regions using genetic data coincides with morphological data regarding bill color changes (Higgins et al. 2006) and song variation (Zann 1976), suggesting that the Ord Arid Intrusion has functioned as a biogeographic barrier in this species complex resulting in vicariance. The population in the western region appears to have been founded by individuals from the central region. Not only were western haplotypes terminal to network branches (Fig. 3), but genetic diversity was reduced in this region (Table 1). Further, western individuals only shared haplotypes with those from the central region, barring two individuals that may be dispersers.

Second, we asked which of two hypotheses based on morphological observations was better supported by genetic data: that differences in bill coloration represent a gradual genetic cline across the range of *P. acuticauda* or an abrupt mor-

phological shift in the vicinity of the historical Ord Arid Intrusion (Harrison 1974; Boles 1988; Schodde and Mason 1999). The relationship between geographic and genetic distance (mtDNA) was not significant across adjacent regions or within regions (Fig. 4). These results support a complete absence of population genetic structure within regions (up to 726 km between localities), as do results of AMOVA (1% of the variation within regions). Additionally, comparisons between the central/eastern regions (up to 1271 km between localities) and the central/western regions (up to 581 km between localities) do not support the presence of isolation by distance, although genetic distances between these regions were greater than genetic distances within them. We conclude that mtDNA data show no evidence of a gradual cline, but are more indicative of three discrete groups with individuals from the central region being genetically intermediate to those from the eastern and western regions.

Third, we looked for evidence of contemporary gene flow across the Ord Arid Intrusion. Given the extreme phenotypes found at the range extremities (bill color) and the short dispersal distances reported by banding studies, we expected to detect population structure using microsatellites. However, 12 polymorphic microsatellite loci showed no evidence of population structuring (Fig. 5), nor did these markers display reduced diversity in populations from the western region (Table 2). We were, therefore, unable to quantify gene flow because methods that use individual microsatellite genotypes to infer contemporary dispersal are inappropriate in the absence of population structure. Although microsatellite results such as those we present here are often interpreted as evidence of high levels of gene flow (e.g., Feijoo et al. 2011; Finnegan et al. 2011; Reudink et al. 2011), this seems an extremely unlikely explanation in this study given what is known about the biology and history of the species. It is possible that the contrasting genetic patterns we have identified result from the effects of Haldane's rule or from differential introgression of the marker classes we utilized. Alternatively, large population sizes may obscure our ability to detect biologically relevant differentiation using microsatellites. In any case, it does seem plausible that the Ord Arid Intrusion has served as biogeographic barrier historically, but now acts as a zone of secondary contact between these subspecies.

Acknowledgments

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