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Characterizing divergence through three adjacent Australian avian transition zones

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Abstract

Aim The diversification of the Australian avifauna has been greatly influenced by prominent historical and modern barriers to dispersal. The aims of this study are to characterize the patterns of divergence in population pairs of meliphagoid birds across adjacent transition zones and characterize how well morphometric divergence, habitat association and taxonomic or species ranking can predict genetic divergence.

Location Northern Queensland, Australia

Methods Genetic divergence between parental populations on either side of the three biogeographical barriers corresponding to three clusters of hybrid zones was characterized in 27 species complexes of meliphagoid birds using one mitochondrial, 23 autosomal, 12 Z chromosome loci collected from a sequence capture system. Within each species we characterized morphometric divergence using wing, bill and tail measurements from museum samples. Lastly, we evaluated the predictive power of these morphometric measurements on genetic divergence.

Results Population pairs on either side of a transition zone depict a wide range of genomic and morphometric divergence. For some systems, species exhibiting morphometric divergence show little to no genomic divergence while, conversely, other species exhibiting little to no morphometric divergence may show clear genomic divergence. Species rank is shown to be the strongest predictor for genetic divergence, habitat is the next strongest predictor and morphometric divergence is the weakest predictor.

Main Conclusions The variation in divergence levels of population pairs affirms that transition zones are ideal natural experiments to study the speciation process. In particular, transition zones allow understanding of how genomic divergence accumulates during speciation. Additionally, standing species-rank classifications mostly prove to be robust after genetic characterization. Lastly, the discordance between morphometric and genetic divergence suggests other non-morphometric phenotypic traits used to designate species rank, such as song or plumage, may play a more important role in predicting genetic divergence.

Key Words

Contact zone, suture zone, speciation, birds, Meliphagides, Australia, transition zone

Introduction

Understanding the accumulation of genomic divergence throughout the speciation process is a growing endeavour in evolutionary biology (Wu, 2001; Nosil & Feder, 2012). Hybrid zones have proven to be a rich field for speciation questions as they provide a test for the degree of reproductive isolation in nature by allowing the study of patterns of introgression among different genomic regions as influenced by selection and stochastic processes (Payseur, 2010; Abbott *et al.*, 2013; Kronforst *et al.*, 2013; Larson, Andrés, Bogdanowicz & Harrison, 2013). Additionally, variation in divergence and degree of introgression among the different DNA classes (autosome, sex chromosome and mitochondria) has provided insight into the role of different types of selection in specific cases of speciation (Huang & Rabosky, 2015; Lavretsky *et al.*, 2015). Hybrid zones provide great utility in studying the landscape of genomic divergence and its potential implications in phenotypic divergence and, ultimately, speciation (Ellegren *et al.*, 2012; Poelstra *et al.*, 2014).

If hybrid zones provide a rich field in understanding the mechanisms underpinning speciation and evolution, then "suture zones" provide an even stronger arena for comparative research to study these mechanisms. "Suture zones" are defined as geographic clustering of multiple hybrid zones, contact zones and phylogeographic breaks (Remington, 1968; Swenson & Howard, 2004, 2005). Suture zones are often formed when populations of multiple unrelated species experience periods of subdivision and isolation due to a shared barrier which eventually breaks down, resulting in multiple cases of secondary contact (Remington, 1968; Swenson, 2006). Comparative studies of multiple hybrid zones, as they relate to speciation, often focus on related taxa with spatially separated hybrid zones (Hendry, Bolnick, Berner, & Peichel, 2009; Kronforst *et al.*, 2013; Supple, Papa, Hines, McMillan, & Counterman, 2015). By contrast, suture zones provide a unique, natural experiment to study the accumulation of divergence and onset of reproductive isolation in a shared environmental setting (Moritz *et al.*, 2009; Dasmahapatra, Elias, Hill, Hoffman, & Mallet., 2010; Singhal & Moritz, 2013; Weir, Faccio, Pulido-Santacruz, Barrera-Guzmán & Aleixo, 2015).

Studies characterizing genomic divergence between populations on either side of

a suture zone have shown that divergence times tend to vary between these co-distributed population pairs (Dolman & Joseph, 2012; Singhal & Moritz, 2013; Winger & Bates, 2015). This variation leads to the hypothesis that although these populations experience broadly similar climatic histories resulting in geographically co-distributed breaks, the timing and degree of divergence tends to be unique to each population pair. In turn, the variation in genomic divergence between population pairs has also resulted in variation in outcomes of hybridization and resulting reproductive isolation in secondary contact (Singhal & Moritz, 2013).

Phenotypic characterization within suture zones has also shown a wide variety in divergence from cryptically diverging lineages to morphologically distinct populations (Winger & Bates, 2015). Additionally, degree of phenotypic divergence does not always coincide with genomic divergence. Two opposing examples of this are an Andean suture zone in the Marañon Valley, Peru, where plumage divergence in birds correlates with genomic divergence (Winger & Bates, 2015) whereas in a suture zone in the Wet Tropics, Australia, skink population pairs have variable levels of genetic divergence with no morphological divergence (Moritz *et al.*, 2009). Comparative studies of the relationship between genetic and phenotypic divergence help elucidate how these measures are either independently or jointly influenced by selection, drift and evolutionary history.

The Australian avifauna provides an excellent resource for suture zone studies. Many zones of contact and hybridization have been characterized using phenotypic traits such as morphology, plumage and song characters (Cracraft, 1986; Ford, 1987; Schodde & Mason, 1999). Based on geographic clustering of 79 - 87 hybrid zones, Ford (1987) identified 11 important biogeographic barriers where multiple hybrid zones seem to cluster. Though the clustering of hybrid zones suggests putative suture zones, we will refer to these clusters broadly as “transition zones” since the classification as “suture zones” has not been formalized by Ford or others. In this study, we focus on the environmentally heterogeneous region of northern Queensland where multiple barriers and clusters of hybrid zones have been proposed. This region’s hybrid zones are situated on three adjacent biogeographic barriers: the Torresian Barrier (“Normanby Barrier” of Ford 1986, 1987 or “Laura Basin” of Bryant & Krosch, 2016), the Burdekin Gap (Keast,

1961; Galbraith, 1969) and the Einasleigh Uplands (also referred to as the Burdekin-Lynd Divide; Fig. 1; Keast, 1961; Schodde & Mason, 1999). The Torresian Barrier (TB) and the Burdekin Gap (BG) are lowland barriers of dry country habitats that influence species associated with more mesic rain forest habitats. The TB separates Cape York Peninsula's (CYP) rain forest elements from those of the Wet Tropics region (see Bryant & Krosch, 2016 for discussion). The BG separates the rain forests of the Wet Tropics region from those of the subtropical rain forests further south. Both barriers have been argued to have been more prominent in drier periods during the Pleistocene (Ford, 1987). The Einasleigh Uplands (EU) is an upland barrier located between TB and BG. Part of the Great Dividing Range, the EU separates the lowlands of Cape York Peninsula and central coastal Queensland. Collectively, the transition region encompassing these three barriers occupies an intermediate climatic space between the northern and southern ranges, as a bioclimatic examination shows (Fig. 1). Ford (1986, 1987) identified 91, 69 and 20 isolates and discontinuities for bird fauna across the TB, BG and EU, respectively, and noted that the TB and BG are among a handful of areas in Australia where the greatest numbers of contacts coincide.

Of the bird species influenced by these barriers, we focus on species from the Australasian endemic superfamily Meliphagoidea (Gardner, Trueman, Ebert, Joseph & Magrath, 2010 = Infraorder Meliphagides *sensu* Cracraft 2014). Most are wholly or partially insectivorous, small passerine birds. Their ecology, shared climatic history and geographical ranges are the main benefits of focusing on a particular clade instead of a broader sampling of all bird species. Additionally, they span variable habitat types from mangroves and rain forests, to drier eucalypt woodlands (Schodde & Mason, 1999).

In this study we sample multiple contacts at three taxonomic levels, within subspecies, between subspecies and between species (hereafter "species rank" for brevity) across this heterogeneous region. This region is occupied by 30 meliphagoid taxon pairs comprising 25 species whose ranges extend through the barriers and five species pairs whose ranges abut these barriers (Table 1). Our main aim is to characterize divergence among the DNA classes in relation to species rank (itself based on phenotypic divergence, *sensu* Schodde & Mason, 1999), morphometric divergence and habitat. We hypothesize that levels of divergence would also be variable for these transition zones.

We address the following questions (1) how do genomic and morphometric divergence vary through a transition zone? and (2) how well do morphometric divergence, habitat and species ranking predict genetic divergence? Lastly, we also discuss the significance of our findings with respect to the systematics of the birds themselves and how levels of divergence across these transition zones compare with any similar studies.

Methods

Sampling

Taxonomic sampling consisted of 27 out of the 30 meliphagoid species complexes (total of 32 out of 35 species) that occur in the transition zone (Table 1). *Cissomela pectoralis*, *Melithreptus gularis* and *Meliphaga lewinii* were omitted due to insufficient geographic sampling. For each complex, we sampled five individuals from each of the parental populations and putative hybrid zone for mitochondrial sequencing, two individuals per population for nuclear DNA sequencing and a variable number of individuals for morphometric measurements. Care was taken to sample broadly through the area of interest. Twelve taxa that were not proposed to have hybrid zones were still sampled across the area of the transition zone so that we could test for cryptic divergence. Populations on either side of the transition zone are mostly sister taxa; the two exceptions are noted in Table 1 (Christidis & Schodde, 1993; Joseph & Moritz, 1993; Nyári & Joseph, 2011; Andersen, Naikatini & Moyle, 2014).

Genetic sequencing and analysis

We collected mitochondrial and nuclear sequence data to assess levels of genomic divergence. We sequenced the mitochondrial ND2 gene using Sanger sequencing on an ABI 3100. The primers used for these species were L5204 (5' TAACTAAGCTATCGGGCGCAT 3') and H6312 (5' CTTATTTAAGGCTTTGAAGGCC 3') (Sorenson, Ast, Dimcheff, Yuri & Mindell, 1999). We used the protein-coding region of ND2 as it is proposed to be one of the fastest evolving genes in the mitochondrial genome and is commonly used in avian studies. A comparative study by Pacheco *et al.* (2011) designates ND2 as the mitochondrial gene with the highest substitution rate and a study with even more mitochondrial genes by Lerner *et al.* (2011) designates ND2 as the gene with the second highest substitution rate.

Both studies agree on the utility of ND2 for shallow levels of divergence. For the nuclear genome, we used an array of loci from the autosomes (n = 23) and the Z chromosome (n = 12; see Table S1.1; see Appendix S1 in Supporting Information). The loci were chosen based on their predicted location on the chicken genome and with the aim of sampling many different chromosomes and broadly sampling the Z chromosome (Kimball *et al.*, 2009; Backström *et al.*, 2010; Wang, Braun, & Kimball, 2012). We prepared genomic DNA libraries for six individuals per species complex (two from each parental population and two from the contact zone) following Meyer and Kircher (2011). We captured the autosomal loci and Z chromosome loci using the target enrichment method sequence capture using PCR-generated probes (SCPP; Peñalba *et al.*, 2014) and sequenced the samples on a single lane of the Illumina HiSeq 2500.

For the ND2 data, we used GENEIOUS 7.18 to and double-check the sequences by eye using the reverse compliment sequence. For the nuclear data, we mainly used the existing data processing pipeline that accompanied the SCPP method (http://www.github.com/MVZSEQ/SCPP). From this pipeline the script for recovering homologous sequences was rewritten to use a clustering method instead of the previous method described by Peñalba *et al.* (2014). First, we downloaded a single reference sequence per target locus from GenBank. Then, for each individual we used BLAST 2.2.29+ to determine which assembled contig is a best reciprocal match for that reference – these contigs were given an arbitrary score of three. We then used BLAST, again, to match all assembled contigs between individuals. If a contig (ex. contig 1A) from sample A was given a score of three from the first iteration, the contig from sample B that best matches contig 1A is given an additional score of one. In other words, a contig that is one degree separated from the GenBank reference is weighted even higher. The contig with the highest score for a corresponding locus is then chosen as a reference for that locus for that individual. (see Fig. S1.2). The alignment of the sequence data was also assessed by eye and spurious flanking or the entire locus were excluded from the analyses if the alignment shows evidence of paralogous sequences. Uncorrected raw genetic divergence estimates were calculated using the 'ape' package in R (Paradis, Claude & Strimmer, 2004). Average pairwise distances were calculated between the parental populations

using the ND2 mitochondrial gene and a separate concatenated sequence of autosomal and Z-linked loci.

Morphometrics

The raw morphometric data used for this study are those used by Schodde & Mason (1999; obtained from the authors) who consistently measured wing, tail and exposed bill lengths using vernier callipers in most individuals included in the study (Appendix S3). Re-analyses here involved log-transforming their measurements and plotting principal components. Only individuals from populations north of the Torresian Barrier and south of the Burdekin Gap and so outside of the putative hybrid zones were used. The samples were chosen so that morphometric divergence will reflect differences between allopatric populations rather than variation in introgression and morphological clines. We used a multivariate analysis of variance (MANOVA) to test for sexual dimorphism and found that some species exhibited significant sexual dimorphism. In those cases, only measurements from males were used to prevent sex-driven morphometric divergence confounding results. The Mahalanobis distances were calculated using the principal component variables of PC1 and PC2 between population clusters and served as a proxies for morphometric divergence.

Statistical Analyses

We carried out separate pairwise comparisons of the genetic divergence of the different DNA classes with the morphometric divergence, habitat and species rank. Nonparametric tests were used, as genetic divergence did not have a normal distribution (Shapiro-Wilk test - ND2: W = 0.713, P = 5.915e-06; autosomal: W = 0.911, P = 0.0247; Z chromosome: W = 0.914, P = 0.0282). We used Spearman's correlation test to compare genetic divergence with phenotypic divergence. For comparisons of genetic divergence with habitat and species rank, we used a Kruskal-Wallis rank sum test. We then used an analysis of variance (ANOVA) to compare morphometric divergence with species rank and habitat. Finally, to assess the contribution of each of the variables (morphometric divergence, habitat and species rank) to genetic divergence we carried out separate generalized linear models for each DNA class. We ran a generalized linear model (GLM) where we systematically added one explanatory variable at a time to see how it changed the corrected Akaike information criterion (AICc) value of the model. For proper model

comparisons of AICc values, species complexes with missing data were removed to ensure comparability of data points. We ran a GLM for each explanatory variable separately and used model selection within the 'MuMin' (Burnham & Anderson 2002) package to compare predictive power of the three variables. All statistical analyses were carried out in the R 3.2.2 statistical program (R Core Team 2015).

Results

Genetic divergence through the transition zones

Population pairs on either side of the transition zones show a wide range of genetic divergences (Table 1; Fig. 2). Among the loci tested, the mitochondrial ND2 gene had the highest genetic divergence between populations (0.1% to 7.47%; Table 1, Fig. 3). Unsurprisingly, genetic divergence in the nuclear genome varied less than that of the mitochondrial genome and rarely exceeded 1% in average pair-wise divergence. Overall, pairwise divergences were correlated for all pairwise comparisons of DNA class (see Fig. S1.1). Associations in 11 of 18 species complexes showed consistency between the location of the mitochondrial break and the location of the taxonomic break in Schodde & Mason (1999; see Appendix S2). Genetic breaks in species without a corresponding taxonomic break also correspond to one of the three barriers (i.e. *Myzomela obscura* in the TB). Most of the incongruence is due to species having phenotypic but not a corresponding genetic break (i.e. *Pardalotus rubricatus*, *P. striatus*, *Smicrornis brevirostris* and *Stomiopera flava*). These cases aside, *E. cyanotis* and *P. citreogularis* are the only complexes where the phenotypic and genetic breaks do not co-occur in geographic space (Appendix S2). Pairwise genetic divergence increases with increasing species rank, despite ranking having been established prior to genetic classification (Fig. 3) especially for mitochondrial DNA (mtDNA) and for Z-linked loci (Kruskal-Wallis rank sum test - df = 2: mtDNA Chi-squared = 12.428, P = 0.002; Z-linked Chi-squared = 9.852, P = 0.007). *Post hoc* tests reveal that the trend in increasing divergence is driven by the difference between the "within-species" (both "within subspecies" and "between subspecies") and "between-species" classification (Figure 3).

Species associated with rain forest and mangrove show similar variation in genetic divergence but the woodland species tend to have less variation in divergence levels as well as a lower absolute divergence (Fig. 4). However, differences in divergence

among habitat classes were significant only for mtDNA (Kruskal-Wallis rank sum test; P = 0.0451, df = 2, Chi-squared = 6.20), and are driven by higher mtDNA divergence across species from rain forests and mangroves than those from woodland habitats. There is no significant difference between rain forest and mangrove-associated populations.

Morphometric divergence through the transition zones

Similar to genetic divergence, the extent of morphometric divergence is variable through the transition zones. Spearman correlation tests between divergence for different DNA classes and morphometric divergences within males show no significant correlations (ND2: ρ = 0.404, P = 0.137; autosomal: ρ = -0.125, P = 0.656; Z chromosome: ρ = -3.57e-2, P = 0.995; Fig. 5). Change in morphometric divergence shows a gradual increase with increasing species rank (see Fig. S1.3). Associations between morphometric divergence and the three taxonomic levels are non-significant but male morphometric divergence is significantly associated with habitat (ANOVA, P < 0.05, df = 2). Variation in morphometric divergence when compared to habitat preference is mostly driven by the lower differences between birds of woodlands relative to that for rain forest or mangrove birds (Fig. S1.4). Morphometric divergence has varied associations with genetic divergence, species ranking and habitat preference.

Predictors of genetic divergence

Results of the generalized linear model are consistent with the above pairwise comparisons of the divergence in the different DNA classes with morphometric divergence, species rank and habitat association. All three DNA classes were compared separately but each yields the same trend. The trends remain when non-sister species are excluded from the analyses. Genetic divergence is best predicted by species rank, followed by habitat and morphometric divergence (Table 2). Adding habitat as an additional explanatory variable to the model results in a poorer fit than a model with species rank alone (see Table S1.2).

Discussion

Using three biogeographical barriers with corresponding transition zones in north-eastern Australia and 32 bird species from the Meliphagoidea, we explored the relative roles of species ranking, morphometric divergence and habitat preference as predictors of

genetic divergence across mtDNA, autosomal and Z-linked nuclear loci. A key finding was that current species rank (Schodde & Mason, 1999) was the best predictor of genetic divergence. A second key finding was the lack of correspondence between molecular and morphological divergence as well as the wide range of variability in divergence of the parental populations. We also found that the levels of divergence differed among the three DNA classes: autosomal, Z chromosome and mtDNA; as expected based on differences in effective population sizes. Between the two nuclear DNA classes, the Z chromosome loci were the most divergent. This lends support to the role of sex chromosomes in the study of speciation and the utility of using different marker sources for reconstructing evolutionary history (Sætre *et al.*, 2003; Claramunt, 2010; Dhami, Joseph, Roshier, & Peters, 2015; Lavretsky *et al.*, 2015).

Habitat association was a weaker predictor of genetic divergence. In the context of transition zones, which may have formed due to changes in connectivity of habitat and persistence of lineages in refugia, longer-term residents of particular habitats would have likely experienced shared population histories. The greater mitochondrial divergence of taxa in rain forest and mangrove habitats likely reflects the more effective isolation of taxa by unsuitable habitat relative to the woodland species. Since the association between neutral genetic divergence and habitat is only significant in the mitochondrial DNA, the DNA class with the lowest effective population size, the correlation would likely be due to population demography and genetic drift. The effects of selection at these early stages of speciation would likely be localized to certain nuclear genes that underlie the phenotype under divergent selection, which may not yet influence neutral divergence throughout the nuclear genome (Via, 2012; Poelstra *et al.*, 2014).

Next, morphometric divergence was the poorest predictor of neutral genetic divergence. Although an unexpected result, there are many reasons as to why morphometric and genetic divergence may be weakly correlated. Firstly, phenotypic divergence can manifest in different morphometric and non-morphometric traits in varying degrees. Overall phenotypic divergence, as used for species rank, has varied contributions from morphology, song, plumage, physiology and other traits depending on the species. It then follows that morphometric distance alone should be expected to be a poor predictor of species rank. Other traits, such as plumage, may be more likely to be

influenced by diversifying selection and therefore may be better at predicting genetic divergence (Greene *et al.*, 2000; *cf.* Lank, 2002). Secondly, hybridization and introgression can also decouple the two divergence measures. If the phenotypic trait of interest is under selection in one but not the other population, the rate at which that particular phenotypic character and neutral genomic variants introgress into the population will vary (Baldassarre, White, Karubian & Webster *et al.*, 2014). Lastly, genomic and phenotypic characters are under the influence of different selective and stochastic forces and so will evolve at different rates. It is increasingly evident that genomic divergence underlying certain major phenotypic divergence, particularly in plumage, may be localized to certain parts of the genome while other genomic regions move more freely between populations (Poelstra *et al.*, 2014; Küpper *et al.*, 2015; Lamichhaney *et al.*, 2015). In contrast, long-term isolation between populations can lead to elevated neutral genomic divergence from drift yet the lack of diversifying selection on phenotypes in such populations might result in cryptic lineages.

The discordance we have observed between genomic and phenotypic divergence is useful for exploring their individual contributions to promoting speciation. This discordance is also consistent with other studies of morphometric divergence in transition zones where plumage may have stronger concordance with genetic divergence (Winger & Bates, 2015). Although we did not quantify plumage divergence in these systems we acknowledge that they may play an important role in shaping genomic divergence, especially in the context of sexual selection. Certain subspecies breaks that have been defined by plumage differentiation often do not have corresponding genetic breaks. In particular, *Pardalotus striatus*, *P. rubricatus*, *Malurus melanocephalus* (see also Lee & Edwards, 2008) and *Smicrornis brevirostris* have been described as having plumage breaks in these regions (Schodde & Mason, 1999) but have not shown genetic breaks in our data. Alternatively, plumage-based taxonomic breaks in *Gerygone palpebrosa*, *Entomyzon cyanotis* and *Melithreptus albogularis* have corresponding genetic breaks according to our data. Systematic quantification of plumage divergence between the populations through these transition zones would be valuable in describing its contribution to genomic divergence and speciation.

Cryptic lineages

Cryptic lineages are defined as genetically distinct lineages that show little or no obvious phenotypic divergence (Bickford *et al.*, 2007). For example, *M. obscura* in eastern Australia is recognized as a single subspecies, *M. o. harterti*, yet it shows relatively deep divergence and reciprocal monophyly for mtDNA at the Torresian Barrier (Fig. 2). This species would benefit from a broader biogeographic study, including all of its populations in Australia and New Guinea, so that the diversity we have observed can be placed in a more complete phylogeographic context. In *M. albogularis*, our data affirm Toon Hughes, & Joseph's (2010) earlier finding that mitochondrial and nuclear genetic divergences are far deeper than expected in our study region. Clearly, the possibility of cryptic speciation in this case needs to be examined more closely.

Other notable substructuring is present within the CYP populations in all "between-species" comparisons (Fig. 2). These breaks are not geographically congruent with one another. The substructuring in the mitochondrial haplotypes may be an outcome of immigrants from populations beyond the present study zone (see Roshier, Heinsohn, Adcock, Beerli & Joseph, 2012). Alternatively, localized population structuring may have formed within the Cape York Peninsula between different habitat patches. Closer examinations of these cases are also warranted in light of the multitude of cryptic lineages revealed in recent work (Bickford *et al.*, 2007; Voda, Dapporto, Dinca & Vila, 2015; Potter, Bragg, Peter, Bi & Moritz, 2016). Most importantly, these lineages provide the context for studying other factors that may influence genetic divergence but not phenotypic divergence in the characters measured here.

Barrier comparisons

The proximity of the three geographic barriers studied here is not only unusual in the literature of speciation and suture zone studies but it also begs the question of whether the different barriers differentially affect patterns of genomic and phenotypic divergence. We reiterate that the TB and BG are patches of drier lowland habitat affecting the rain forests whereas the EU is an upland barrier affecting the woodland habitat. The different barrier types and geographic proximity may result in variable influences on molecular and phenotypic divergence between the three different barriers, the populations being influenced by more than one barrier, or a combination of both (Bryant & Krosch, 2016).

401 The following cases show the idiosyncratic nature of each barrier's effects. The Torresian
402 Barrier has influenced divergence in the rain forest species *Meliphaga notata* and
403 *Microptilotis gracilis* but not *Malurus amabilis*. The Einasleigh Uplands/Burdekin-Lynd
404 Divide demarcates divergent phenotypic forms of *Pardalotus rubricatus*, *Smicrornis*
405 *brevirostris*, *Gerygone olivacea* and *Melithreptus albogularis* but only the latter two have
406 corresponding genetic divergence. Lastly, the Burdekin Gap influences populations in
407 *Malurus melanocephalus*, *Entomyzon cyanotis* and *Sericornis magnirostra* but not in
408 *Stomiopera flava*, *Myzomela obscura* or *Ramsayornis fasciatus*.
409 Differences in the degree of similarity of the habitats on either side of each of the
410 barriers could also play a role in facilitating genetic and morphometric divergence.
411 Although genetic divergence is not significantly correlated with morphometric
412 divergence, mitochondrial and morphometric divergences are significantly correlated
413 with habitat. Differentiation of the habitat on either side of the barriers would have a
414 direct ecological selective pressure on morphometric divergence while genetic
415 divergence would be influenced by both selection and gene flow. Different types of rain
416 forest elements are discontinuous along the TB and BG. The northern rain forests are
417 mostly composed of lowland to mid-elevation rain forest elements while the southern rain
418 forests are a broad mix of subtropical montane rain forest elements (Webb, Tracey &
419 Williams, 1984). The mangrove elements on either side of the TB are not very
420 differentiated and are often lumped together though they are considered fairly
421 differentiated south of the BG (Saenger *et al.*, 1977). On the other hand, woodland habitat
422 in CYP area is the most compositionally divergent, being classified as a monsoonal
423 group, while the other two areas are classified into an eastern/south coastal group
424 (Cracraft, 1991; Crisp, Linder & Weston, 1995). It is important to note that differences
425 habitat structure, rather than differences in plant community composition, may play a key
426 role in driving divergence in phenotype and genotype (Patten, Rottenberry & Zuk, 2004,
427 Seddon, 2005). How habitat structure varies on either side of transition zones and its
428 influence in divergence would be an important avenue for future studies.
429 Population pairs across other transition zones often have different levels of
430 genetic divergence (Moritz *et al.*, 2009; Dasmahapatra *et al.*, 2010; Singhal & Moritz,
431 2013; Weir *et al.*, 2015; Bryant & Krosch, 2016; Edwards, Potter, Schmitt, Bragg, &

Moritz, 2016). Edwards *et al.* (2016) and Singhal & Moritz (2013), in particular, emphasize idiosyncrasies in sequence divergence levels in nearby suture zones situated in the Carpentarian Barrier and the Wet Tropics respectively. Despite limiting the study to closely related bird species, this study shows that the northern Queensland transition zone reflect the same idiosyncratic nature as other suture zone studies comparing different levels of taxonomic distinctness, from within genera or between orders. The resulting variation in genomic and phenotypic divergence, despite sharing biogeographical barriers, likely result from a combination of lineage-specific selection, variation in effective population sizes, gene flow, dispersal capabilities and timing of initial population split (Coyne & Orr, 2004; Seehausen, Butlin, & Keller, 2014). The "messy" nature of suture zones lends itself to studying the influence of these different drivers of genomic and phenotypic divergence while controlling for biogeographical context.

Differential responses to a shared climatic history likely coupled with population or lineage-specific habitat and life history traits result in varying levels of genomic and phenotypic divergence. This in turn could affect the propensity for hybridization or introgression when the population pairs are in secondary contact. This augurs well for the use of transition and suture zones in studies of speciation. Additionally, suture zones would also greatly contribute to empirical studies of "speciation-with-gene-flow." Recent genomic studies of hybrid zones have been rapidly accelerating our understanding of the speciation process (Teeter *et al.*, 2008; Ellegren *et al.*, 2012; Poelstra *et al.*, 2014). The next step is to explore parallel hybrid zone dynamics in a shared geographic region in order to start teasing apart general genomic patterns of divergence as opposed to lineage specific patterns. Studies exploring the genomic landscape of divergence and introgression using a comparative approach through a suture zone will be an invaluable contribution for further advancing our understanding of speciation and evolutionary theory.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Supplementary tables and figures.

Appendix S2 Geographic ranges and mitochondrial breaks

Appendix S3 Morphometric measurements

Data Accessibility

Sequence data has been deposited in GenBank (KY382831 - KY385189). Morphometric data has been included in the appendix (S3).

Biosketch

Joshua V. Peñalba is a PhD candidate at the Australian National University and Commonwealth Scientific and Industrial Research Organisation under the Centre of Biodiversity Analysis. He is particularly interested in genomic speciation of birds, focusing on Australian species.

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Author contributions

J.V.P, C.M. and L.J. designed and conceived ideas for this project. I.J.M and R.S. collected morphometric data and provided ideas for the manuscript. J.V.P. collected genomic data, performed analyses and led the writing of the manuscript.

Tables and Figures

Table 1. Meliphagoidea complexes in northern Queensland and their associated habitat, geographic barrier and raw genetic divergence measures. The predominant barriers listed here are derived from Schodde and Mason (1999). The delimitation of *S. beccarrii* as a full species diverged from *S. magnirostris* has fluctuated since Schodde and Mason (1999). For the purpose of this paper, they will be treated as separate species (Christidis, Schodde & Baverstock, 1988; Joseph & Moritz, 1993). * - species pair are not sister species (Nyári & Joseph, 2011).

Species rank	Eastern Queensland	Cape York	Habitat	Barrier	% Genetic divergence		
					mtDNA	Autosome	Z chromosome
Within subspecies							
Lovely Fairywren	*Malurus amabilis*		Rain forest	None	0.421	0.208	0.236
Large-billed Gerygone	*Gerygone magnirostris cairnsensis*		Mangrove	None	0.173	0.482	0.216
Helmeted Friarbird	*Philemon buceroides yorki*		Woodland	None	0.316	0.274	0.171
Noisy Friarbird	*Philemon corniculatus corniculatus*		Woodland	None	0.348	0.467	0.553
White-gaped Honeyeater	*Stomiopera unicolor*		Woodland	None	0.220	0.281	0.000
Brown Honeyeater	*Lichmera indistincta ocularis*		Woodland	None	0.285	0.456	0.146
Bar-breasted Honeyeater	*Ramsayornis fasciatus*		Woodland	None	0.272	0.345	0.259
Brown-backed Honeyeater	*Ramsayornis modestus*		Woodland	None	0.658	0.366	0.490
Dusky Honeyeater	*Myzomela obscura harterti*		Rain forest	None	2.404	0.372	0.763
Between subspecies							
Red-backed Fairywren	*Malurus melanocephalus melanocephalus*	*M. m. cruentatus*	Woodland	EBD	1.055	1.103	1.225
Red-browed Pardalote	*Pardalotus rubricatus rubricatus*	*P. r. yorki*	Woodland	EBD	0.181	0.432	0.219
Striated Pardalote	*Pardalotus striatus melanocephalus*	*P. s. melvillensis*	Woodland	EBD	0.336	0.258	0.370
Weebill	*Smicrornis brevirostris brevirostris*	*S. b. flavescens*	Woodland	EBD	0.295	0.705	0.533
White-throated Gerygone	*Gerygone olivacea olivacea*	*G. o. cinerascens*	Woodland	EBD	0.544	0.632	0.071
Fairy Gerygone	*Gerygone palpebrosa flavida*	*G. p. personata*	Rain forest	EBD	0.757	0.514	0.101
Mangrove Gerygone	*Gerygone levigaster cantator*	*G. l. levigaster*	Mangrove	ALL	0.914	0.884	0.802
Blue-faced Honeyeater	*Entomyzon cyanotis cyanotis*	*E. c. griseigularus*	Woodland	EBD	0.496	0.262	0.120
Little Friarbird	*Philemon citreogularis sordidus*	*P. c. citreogularis*	Woodland	BG	0.502	0.536	0.659

Yellow-spotted Honeyeater	*Meliphaga notata mixta*	*M. n notata*	Rain forest	TB	0.516	0.176	0.195
Graceful Honeyeater	*Microptilotis gracilis imitatrix*	*M. g gracilis*	Rain forest	TB	1.880	1.156	0.900
Yellow Honeyeater	*Stomiopera flava addenda*	*S. f. flava*	Woodland	EBD	0.197	0.184	0.000
White-throated Honeyeater	*Melithreptus albogularis inopinatus*	*M. a. albogularis*	Woodland	EBD	3.139	0.534	0.237
Between species							
Large-billed / Tropical Scrubwren	*Sericornis magnirostra*	*S. beccarii dubius/minimus*	Rain forest	TB + BG	3.675	1.041	1.000
Macleay's / Tawny-breasted Honeyeater	*Xanthotis macleayanus*	*X. flaviventer filiger*	Rain forest	TB	4.342	0.223	1.447
Mangrove / Varied Honeyeater	*Gavicalis fasciogularis**	*G. versicolor versicolor**	Mangrove	BG	3.562	0.810	1.065
Fuscous / Yellow-tinted Honeyeater	*Ptilotula fusca*	*P. flavescens flavescens*	Woodland	EBD	2.811	0.741	0.957
Scarlet / Red-headed Honeyeater	*Myzomela sanguinolenta**	*M. erythrocephala erythrocephala**	Mangrove	TB	7.472	0.590	0.847

Table 2. The results of the generalized linear models to estimate the explanatory power of species rank, habitat and morphometrics in predicting the divergence measure of each chromosomal type (mtDNA, autosome and Z chromosome). These models comprise all Queensland meliphagoid species in this study. Df – degrees of freedom, AICc – corrected Akaike information criterion, Δ – difference between the predictor's AICc value and lowest AICc value.

DNA class	Predictor	Intercept	df	Log likelihood	AICc	Δ	Weight

Mitochondria	Species rank	21.86	4	49.226	-86.5	0.00	0.860
	Habitat	13.38	4	47.392	-82.8	3.67	0.137
	Morphometrics	238.30	3	41.772	-75.1	11.31	0.003
Autosome	Species rank	154.10	4	69.722	-127.4	0.00	0.778
	Habitat	169.40	4	68.379	-124.8	2.69	0.203
	Morphometrics	-50.62	3	64.180	-120.0	7.48	0.018
Z chromosome	Species rank	94.16	4	67.077	-122.2	0.00	0.673
	Habitat	118.00	4	66.293	-120.6	1.57	0.307
	Morphometrics	148.20	3	61.779	-115.2	7.00	0.020

698

699 **Figures**

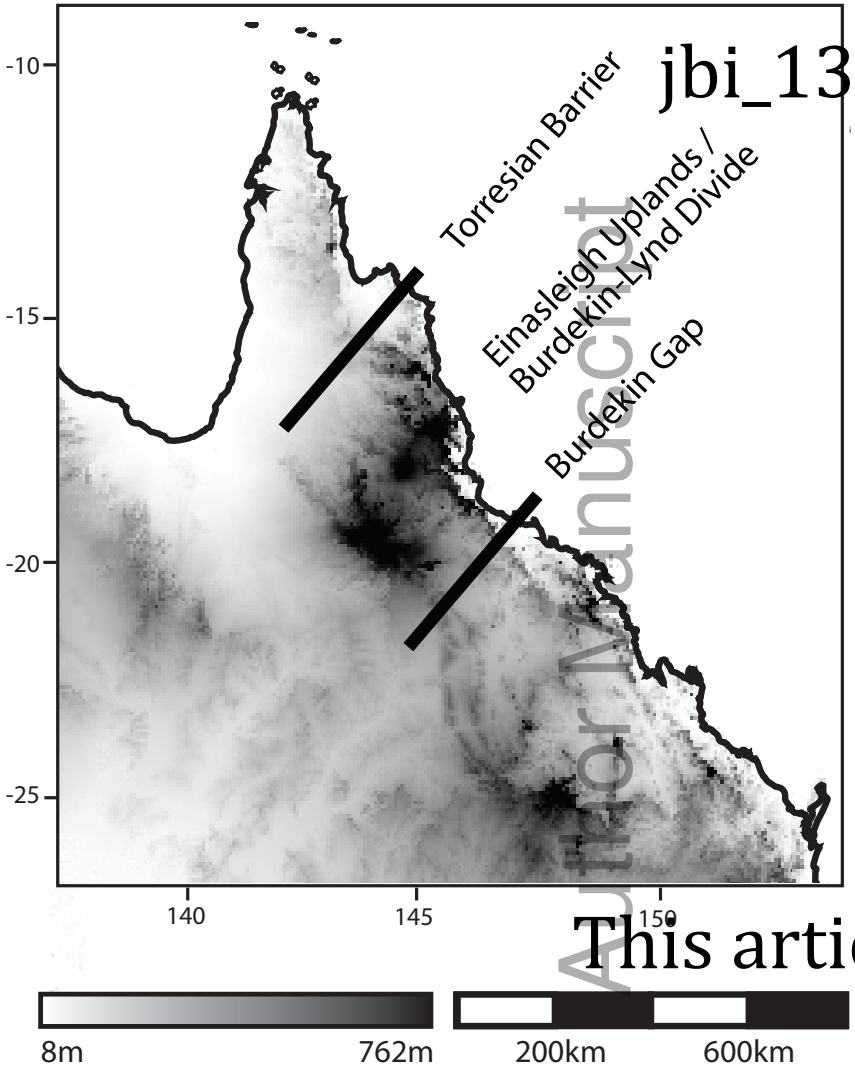
700 Figure 1. *Left*: The location of all three barriers within Queensland, Australia. The dark region depicts the mountainous Einasleigh
701 Uplands/Burdekin-Lynd Divide. *Right*: Principal components analysis using all 19 BIOCLIM (http://www.worldclim.org/bioclim)
702 variables. As expected, PC1 is loaded mainly with temperature and precipitation.

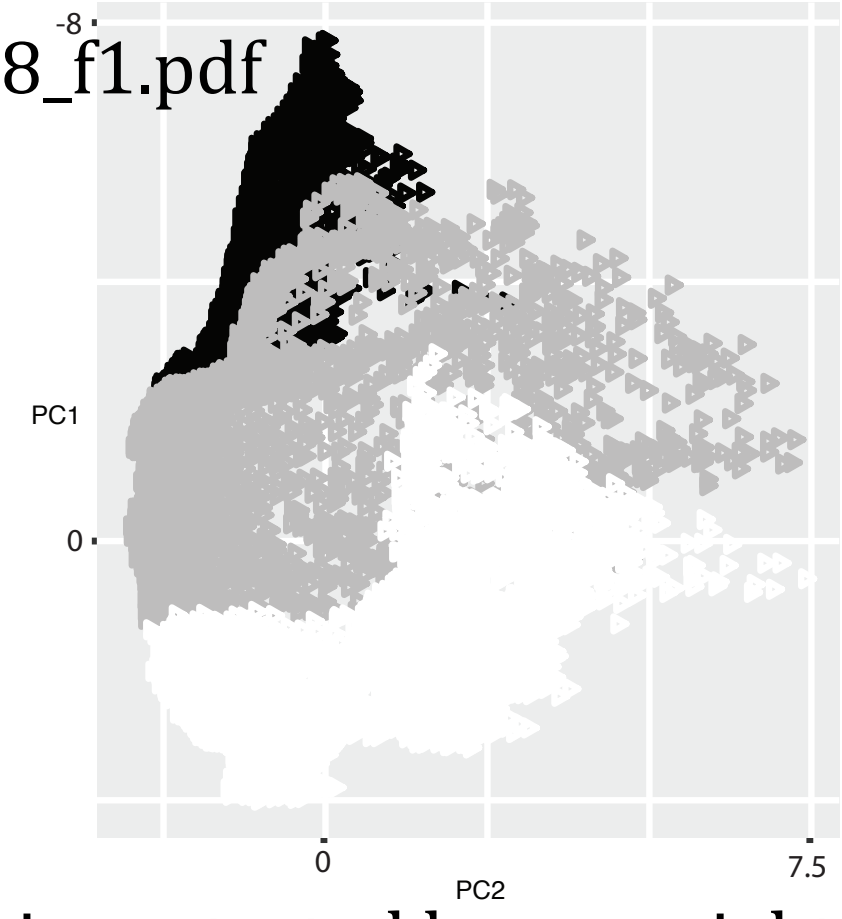
Figure 2. Networks of relationships among ND2 haplotypes for all study species, spanning all parental populations and those from each putative hybrid zone. The systems are divided based on the species ranking. Hash marks represent single mutations between samples and the size of the circle reflects number of samples that share that haplotype. Illustrations from del Hoyo, Elliott, Sargatal, Christie, & de Juana (2014).

Figure 3. Genetic divergence of the three chromosomal types (mitochondrial, autosomal and Z chromosome) were plotted against the species ranking. *Left*: Mitochondrial ND2 sequence divergences. *Right*: Nuclear sequence divergences divided between autosomal and Z-linked loci. The samples used were outside the putative hybrid zones to avoid lower divergence levels resulting from gene flow.

Figure 4. Genetic divergence plotted against habitat preference. The only significant association found is between rain forest and woodland as well as between mangrove and woodland.

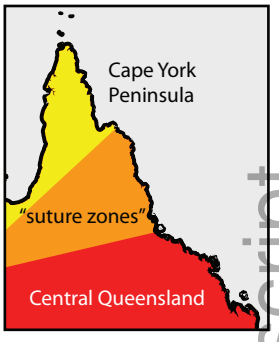
Figure 5. Genetic divergence was plotted against Mahalanobis distances (proxy for morphometric divergence). No significant associations were found for this comparison.

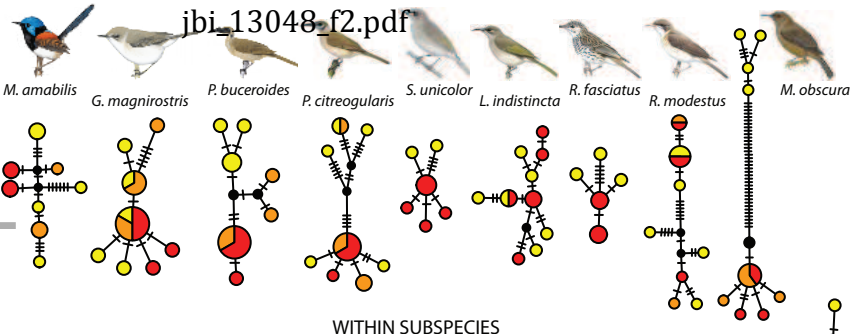




Elevation gradient

Environmental PCA





WITHIN SUBSPECIES

BETWEEN SUBSPECIES

BETWEEN SPECIES