Climate niche differentiation between two passerines despite ongoing gene flow

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Summary

1. Niche evolution underpins the generation and maintenance of biological diversity, but niche conservatism, in which niches remain little changed over time in closely related taxa and the role of ecology in niche evolution are continually debated.

2. To test whether climate niches are conserved in two closely related passerines in East Asia – the vinous-throated (*Paradoxornis webbianus*) and ashy-throated (*P. alphonsianus*) parrotbills – we established their potential allopatric and sympatric regions using ecological niche models and compared differences in their climate niches using niche overlap indices in background tests and multivariate statistical analyses. We also used polymorphism data on 44 nuclear genes to infer their divergence demography.

3. We found that these two parrotbills occupy different climate niches, in both their allopatric and potential sympatric regions. Because the potential sympatric region is the area predicted to be suitable for both parrotbills based on the ecological niche models, it can serve as a natural common garden. Therefore, their observed niche differences in this potential sympatry were not simply rendered by phenotypic plasticity, and probably had a genetic basis.

4. Our genetic analyses revealed that the two parrotbills are not evolutionarily independent for the most recent part of their divergence history. The two parrotbills diverged *c.* 856,000 years ago, and have had substantial gene flow since a presumed secondary contact *c.* 290,000 years ago.

5. This study provides an empirical case demonstrating that climate niches may not be homogenized in nascent species in spite of substantial, ongoing gene flow, which in turn

suggests a role for ecology in promoting and maintaining diversification among incipient species.

Introduction

Niche lability underpins a wide range of theories that depict how biological diversity is created and maintained (e.g. Hutchinson 1959; Holt 2009). However, the rate of niche evolution (niche shift, expansion or specialization) has been the subject of continual debate. Some argue that local adaptation and environmental heterogeneity should lead to frequent and rapid niche evolution (Holt & Gaines 1992), because it enables a species to adapt to harsh environments (Holt, Barfield & Gomulkiewicz 2004) and to expand its range into novel habitats (Sexton *et al*. 2009). Niche evolution accelerates ecological speciation (Nosil 2012), so the idea of frequent and rapid niche evolution challenges the view that allopatry, a physical barrier to gene flow, is a prerequisite for species split (e.g. Gavrilets, Hai & Vose 1998; Wu 2001). However, some propose that ecological niches, especially Grinnellian niches (e.g. temperature, precipitation), remain little changed within a species or among closely related species (Peterson, Soberón & Sánchez-Cordero 1999). This phenomenon is referred to as niche conservatism, which tends to confine species' range expansion (Wiens & Graham 2005) and prohibit gene flow between populations isolated by a habitat barrier, leading to allopatric speciation (Wiens 2004).

The degree of niche differentiation among different evolutionary lineages such as populations or species can be used to gauge the strength of niche conservatism (e.g.

Peterson *et al*. 1999). In a recent review, Peterson (2011) found that Grinnellian niches are conserved in short-to-moderate evolutionary time-spans but have a tendency to weaken at the transition from closely to distantly related taxa. Because niche conservatism operates at different time-scales to influence ecological and evolutionary processes, such as invasion, adaptation to climate change and speciation (Wiens *et al*. 2010), it is important to understand how it breaks down over time. Therefore, studies on niche differentiation between recently diverged species can provide useful insights into the tempo of niche evolution.

Climate niche differentiation between allopatric taxa that are exposed to different environments could be rendered by phenotypic plasticity (in which a single genotype produces different phenotypes in different environments). To account for the role of phenotypic plasticity, niche differences among taxa are frequently studied in common garden experiments. However, when the phenotype under consideration involves multidimensional niche use, it can be difficult to measure phenotypic values realistically in a laboratory setting. Alternatively, if we can delineate a potential sympatric region that is accessible to and suitable for two or more taxa, this potential sympatry (sympatry thereafter) could serve as their natural common garden. If niche use is largely driven by phenotypic plasticity, any niche difference among taxa should only be observed in allopatry but not in sympatry. By contrast, if niche use is largely genetically based, any niche difference among taxa should persist in both allopatry and sympatry.

Both divergent selection and random processes (e.g. genetic drift) can drive niche differentiation between two evolutionary lineages, especially when they have diverged in allopatry. However, if gene flow is present, the neutral traits in the two lineages should introgress at the same rate between them, whereas the traits under divergent selection should not (Pinho & Hey 2010). Therefore, the maintenance of niche differentiation between

two lineages with gene flow indicates ecologically divergent selection. This is why divergence history and the level of gene flow are critical information in assessing the role of divergent selection in niche evolution. Here, we report climate niche differentiation between a passerine, the ashy-throated parrotbill (*Paradoxornis alphonsianus*) and its sister species, the vinous-throated parrotbill (*P. webbianus*), in both their allopatric and sympatric regions, despite a significant level of recent and ongoing gene flow.

Paradoxornis webbianus is widely distributed in open wooded habitats from northern Indochina to southern Siberia and from the eastern edge of the Tibetan Plateau to coastal China (Robson 2007). The habitat use of *P. alphonsianus* is similar to that of *P. webbianus*, but its range is restricted to the highlands of southwestern China and northern Vietnam (Robson 2007). Museum collections and field observations indicate that *P. alphonsianus* and a subspecies of *P. webbianus*, *P. w. suffusus* (widely distributed in central and southern China and Indochina), have a narrow contact zone along the western edge of the Chengdu Plain and the eastern edge of the Yunnan-Guizhou Plateau (Wu *et al*. 1986; Han 1991; Robson 2007; Fig. 1). Sporadic records of individuals from their contact zone with intermediate plumage coloration suggest their hybridization (Wu *et al*. 1986; Han 1991; Robson 2007). Molecular phylogenetic studies based on both mitochondrial and nuclear loci suggest that *P. alphonsianus* is likely to have split from *P. w. suffusus* (Crottini *et al*. 2010; Yeung *et al*. 2011). Their close phylogenetic relationship, distribution along altitudinal gradients from the Yunnan-Guizhou Plateau to the central and south-eastern China lowlands and hybridization potential make the *P. w. suffusus*/*P. alphonsianus* complex an ideal system to investigate climate niche evolution in nascent species.

Materials and methods

ECOLOGICAL NICHE MODELS

We generated ecological niche models (ENMs) for *P. w. suffusus* and *P. alphonsianus* using

Maxent (version 3.3.3) (Phillips & Dudik 2008) at a spatial resolution of *c.* 4 km. A total of

677 occurrence data points for *P. w. suffusus* and 139 for *P. alphonsianus* were initially

obtained from museum archives (Institute of Zoology, Chines Academy of Sciences; Kunming

Institute of Zoology, Chinese Academy of Science; Sichuan Agriculture University), bird

watching records (http://birdtalker.net/), literature (Wu *et al*. 1986; Yang 2004; Lu *et al*.

2006) and personal observations. The *P. alphonsianus* occurrences included two subspecies

(*P. a. alphonsianus* with 110 occurrences and *P. a. yunnenesis* with 29 occurrences).

However, because the climate niches of *P. a. alphonsianus* and *P. a. yunnenesis* are highly

conserved (Fig. S1, Supporting Information), we combined them to represent *P.

alphonsianus*. The study area included all ecoregions (Olson *et al*. 2001) that overlay the

minimum convex polygon surrounding the combined occurrences of the two parrotbills with

a buffer of 4 decimal degrees. To avoid over-prediction of suitability at intensely sampled

locations (Hortal *et al*. 2008), we randomly chose points that were at least 0.3 decimal

degrees apart from one another from among the original data points, which resulted in 268

occurrences for *P. w. suffusus* and 57 for *P. alphonsianus* (Fig. 1). We used nine of the 19

bioclimatic variables from WorldClim (Hijmans *et al*. 2005) as the environmental data layers

for the ENMs. The 10 bioclimatic variables that were excluded are highly correlated with the

nine variables used for the ENMs ($R > 0.9$; ENMTools, Warren, Glor & Turelli 2010). To

delineate the potential distributions of the two parrotbills, we applied the minimum training

presence threshold that allows 0% omission error to the suitability scores from the Maxent

output, which classifies each cell as either suitable or unsuitable for either parrotbill. We

then overlaid the distributions of the two parrotbills to generate their potential sympatric

region, the area that is suitable for both parrotbills.

CLIMATE NICHE COMPARISONS

We estimated the niche overlap between *P. w. suffusus* and *P. alphonsianus* based on their ENMs (ENMTools) (Warren, Glor & Turelli 2008; Warren, Glor & Turelli 2010) using Schoener's *D* and similarity index *I*. We compared this niche overlap value to a distribution of 200 background niche overlap values, which were generated using the ENM of one parrotbill and an ENM created with random points drawn from the geographic range of the other parrotbill. We carried this process out twice, using the geographic range of either *P. w. suffusus* and *P. alphonsianus* as background. A niche overlap value that is smaller than the background distribution would imply niche divergence between the two parrotbills, whereas a larger niche overlap value would imply niche conservatism. To avoid drawing background values from areas without species occurrence, we used the minimum convex polygons surrounding the original occurrence records of *P. w. suffusus* or *P. alphonsianus* as their respective geographic ranges.

Where two different species' geographic ranges are used in ENM background tests, inconsistent results can occur, such as niche divergence against one species' background and niche conservatism against the other (e.g. Nakazato, Warren & Moyle 2010). Therefore, we also used discriminant function analyses to examine climate niche differentiation between *P. w. suffusus* and *P. alphonsianus*. To do this, we extracted raw values of the nine bioclimate variables from the cells with species occurrence records (same dataset as in the construction of their ENMs: N = 268 for *P. w. suffusus* and N = 57 for *P. alphonsianus*), and tested whether any of the canonical functions could significantly differentiate the two parrotbills. We then compared the scores of the canonical functions with sufficient discriminating power between the two parrotbills, in their allopatric and sympatric regions respectively.

A difference in the canonical function score between the two parrotbills might reflect the underlying climate gradient, rather than a niche difference. In particular, because *P. w.*

suffusus tends to occur at lower elevation than *P. alphonsianus* across their entire geographic range (Fig. S2, Supporting Information), an underlying climate gradient driven by elevation is very likely. To address this issue, we performed the analysis of covariance (ANCOVA) to test if niche differentiation in the allopatric and sympatric regions remains after elevation is controlled for. In addition, we tested niche differences between the two parrotbills at various spatial scales within the sympatric region, i.e., < 50 Km, < 100 Km, < 150 Km of the contact zone. The contact zone is delineated by connecting the cells that contain occurrences of both parrotbills, which divides the sympatric region into two parts (Fig. 1, the solid black line in the top-left panel). The relatively small number of co-occurrences (14 cells out of 268 occurrences for *P. w. suffusus* and 57 occurrences for *P. alphonsianus*) resulted in a narrow contact zone that is *c.* 4% the area size of their potential sympatry based on the niche models (a 5-Km buffer was added to the contact line prior to calculating the area size of the contact zone, which reflects the mobility of the parrotbills; dispersal ability for female *P. webbianus* is up to 4.6 km per generation as suggested by banding records; Hsu Y-C, unpublished). As the two parrotbills approach the contact zone, we expect that the influence of any underlying environmental gradient, such as elevation, to gradually weaken, allowing us to examine the robustness of their niche differentiation.

GENETIC ANALYSES

We collected tissue samples from 48 *P. w. suffusus* individuals in the Henan (N = 3), Shaanxi (N = 10), Hunan (N = 9), Guizhou (N = 4) and Guangxi (N = 22) provinces of China, from 29 *P. alphonsianus* individuals in the Guizhou (*P. a. alphonsianus*; N = 16) and Yunnan (*P. a. yunnenesis*; N = 13) provinces of China and from one individual of golden parrotbill *P. verreauxi* in Vietnam as the outgroup. All samples of *P. w. suffusus* and *P. alphonsianus* were

from localities outside their known contact zone (Fig. 1). Gross DNA was extracted following Gemmell & Akiyama (1996), and was suspended in ddH$_2$O for later use.

We amplified 44 autosomal exonic loci by polymerase chain reaction (PCR; sequences of PCR primers, and detailed PCR protocols are listed in Table S1, Supporting Information). We mapped the short read sequences of each library to the reference sequence from a single individual of *P. w. suffusus* generated by traditional Sanger sequencing (LifeScope™ Genomic Analysis Software from Life Technologies). To estimate substitution rates of autosomal loci, we sequenced mitochondrial cytochrome *b* (Cytb) genes from 17 *P. w. suffusus* and 10 *P. alphonsianus* individuals (randomly picked from our samples) following the protocol in Li *et al*. (2010).

Only sequences with average sequence coverage greater than 100 were retained for the subsequent analysis. The Quality-Based Variant Detection tool (CLC Genomic Workbench ver 5.5 beta2, CLC Biolab) was used to detect heterozygous sites, defined as those with a minor base frequency of more than 35%. Genotype data were phased (Li *et al*. 2010), but all phased haplotypes were retained because the accuracy of phasing would not affect the estimations of polymorphism used in subsequent analyses. Ten intra- and inter-specific DNA polymorphism statistics for the two parrotbills (Table S2, Supporting Information) were used in Approximate Bayesian Computation analysis (ABC) (e.g. Beaumont, Zhang & Balding 2002). The Hudson-Kreitman-Aguadé (HKA) test (Hudson, Kreitman & Aguadé 1987) was performed to test the neutrality of loci (HKA program by Jody Hey, available at http://lifesci.rutgers.edu/~heylab/). Statistical significances of χ^2 values were determined by comparing a distribution from 10,000 coalescent simulations. We followed the method in Li *et al*. (2010) to estimate the substitution rate of each nuclear gene and the generation time of the parrotbills was set to 2.5 years.

HISTORICAL DEMOGRAPHIC MODELS

To infer the divergence history, we used an ABC approach to fit the observed polymorphic pattern in the two parrotbills to four demographic models (Fig. 2). The isolation model assumes no gene flow between the populations since their divergence. The isolation-with-migration model allows gene flow through the entire history of their divergence. The early gene flow model allows gene flow to be present only in the early stages of divergence. By contrast, the secondary contact model assumes no gene flow between two parrotbills in their early divergence history, but does allow for gene flow later on. The most recent common ancestor of the two parrotbills was assumed to be an admixed population in all four models. We estimated the population split time (T_1), long-term effective population sizes for both parrotbills (N_{Pw} and N_{Pa} for *P. w. suffusus* and *P. alphonsianus* respectively) and the effective population size of their most recent common ancestor (N_A) in all models. For models allowing gene flow, we also estimated the number of immigrants per generation, M_{Pw} and M_{Pa}, for *P. w. suffusus* and *P. alphonsianus* respectively ($M_{Pw} = 4\,N_{Pa}m_{Pa}$ and $M_{Pa} = 4\,N_{Pw}m_{Pw}$, where m_{Pa} and m_{Pw} indicate the proportions of immigrants within *P. w. suffusus* and *P. alphonsianus* in each generation). For the secondary contact model, gene flow was set to zero before a time point, T_2. In contrast, gene flow was only allowed before T_2 in the early gene flow model.

We used msABC (Pavlidis, Laurent & Stephan 2010) to perform coalescent simulations for all neutral loci. Demographic parameters were sampled from the uniform random distribution within their prior ranges. The ranges of the priors for demographic parameters in all models, and the command line for each model, are listed in Tables S3 & S4, Supporting Information, respectively. One million simulations were performed for each model, assuming the same sample size as the empirical data for each locus. We assumed no intra-

locus recombination in coalescent simulations because of the short length of the loci (all loci were less than 1.5 kilobases) and the slow rate for the decay of linkage disequillibrium in birds (e.g. Backström *et al*. 2006; Li & Merilä 2009; Stapley *et al*. 2010). Even if there were intra-locus recombination, all summary statistics used in the ABC analysis are SNP (single nucleotide polymorphism)-frequency-based, which are more robust for demographic inferences than those that are based on haplotype frequencies (Ramírez-Soriano *et al*. 2008). All time estimates were scaled to the unit of 4 N_{Pw} generations. The 10 summary statistics mentioned above were calculated for each coalescent simulation in subsequent analyses.

We used the program abc (Csilléry, François & Blum 2011) to perform model selection and infer the population parameters of the preferred model. The tolerance was set to 0.005. We used the multinomial logistic regression method (Beaumont 2008) to infer the posterior probability of each demographic model. We used the neural network method (Blum & François 2010) with four million additional simulations to estimate the population parameters of the selected models. The number of neutral networks was set to 50. The demographic parameters were logit-transformed, except for T_1, which was log-transformed. These transformations ensured that the posterior densities fell within the ranges of the prior distributions (Blum & François 2010). The Epanechnikov kernel was used to calculate the posterior densities of the parameters. We used the medians as the point estimates of the parameters (e.g. Li *et al*. 2010), and report the median, mean and mode of each parameter and the range of its 95% highest probability distribution (HPD) interval.

Results

THE ECOLOGICAL NICHE MODELS OF THE TWO PARROTBILLS AND THEIR CLIMATE NICHE DIFFERENTIATION

The predicted distributions of *P. w. suffusus* and *P. alphonsianus* based on the ENMs

matched their actual occurrences closely (Fig. 1). The ENM of one parrotbill generally did not

predict the occurrences of the other parrotbill well. The omission error when using the ENM

of *P. w. suffusus* to predict occurrences of *P. alphonsianus* was 0.16; the omission error

when using the ENM of *P. alphonsianus* to predict occurrences of *P. w. suffusus* was 0.83.

The mean value of the area under the operating receiver curve (AUC) was similar between

the two parrotbills (0.86 for *P. w. suffusus* and 0.96 for *P. alphonsianus*). The niche overlap

values between *P. w. suffusus* and *P. alphonsianus* were 0.32 (Schoeners' *D*) and 0.60

(similarity statistic *I*) (Fig. 3). The background tests indicated that the two parrotbills are

more divergent in climate niche than expected from the available climate conditions in the

geographic range of *P. w. suffusus* (99% confidence intervals: *D* = 0.33-0.39, *I* = 0.63-0.70; *P* <

0.01; Fig. 3), but neither more divergent nor more conservative than expected from the

available climate conditions in the geographic range of *P. alphonsianus* (99% confidence

intervals: *D* = 0.24-0.33, *I* = 0.51-0.62; *n.s.*; Fig. 3). The background tests, therefore, do not

provide decisive support for niche divergence between the two parrotbills.

Discriminant function analysis revealed one canonical function that contributed

significantly to the differentiation between the two parrotbills (canonical function 1, R^2 =

0.45, $F_{9,315}$ = 29.1, *P* < 0.0001). This canonical function was negatively correlated with

temperature seasonality, mean temperature of the warmest quarter and precipitation in the

driest quarter, and positively correlated with isothermality, precipitation seasonality and

precipitation in the warmest quarter (Table 1). The canonical function score was higher for *P.

alphonsianus* than for *P. w. suffuses*, in both allopatry and sympatry (Fig. 4). The difference

in the canonical function score in either allopatric or sympatric region persisted even after

elevation was controlled for (allopatry: species effect: $F_{1,229}$ = 144.89, *P* < 0.0001; elevation

effect: $F_{1,229}$ = 40.59, *P* < 0.0001; sympatry: species effect: $F_{1,90}$ = 12.33, *P* = 0.0007; elevation

effect: $F_{1,90} = 30.49$, $P < 0.0001$). The difference in the canonical function score also persisted at various spatial scales, from < 50 Km to < 100 Km and < 150 Km of the contact zone (Fig. 4).

GENETIC POLYMORPHISM, DIVERGENCE, NEUTRALITY AND MUTATION RATES IN THE TWO PARROTBILLS

We sequenced 32,127 base pairs of 44 exonic loci, successfully mapped 76,995,521 short reads (75-bp) to reference sequences, and used them to reconstruct 3,301 sequences with sufficient coverage (mean of sequence coverage = 2291.1) for further analyses. The multilocus HKA test showed that none of the polymorphisms observed in the 44 nuclear loci deviated from a neutral expectation ($\chi^2 = 13.97$, d.f. = 90, $P = 1$).

The estimated substitution rates ranged from 7.34×10^{-11} to 1.84×10^{-9} substitutions/site/year (mean = 8.39×10^{-10} substitutions/site/year). The numbers of substitutions per locus per generation are shown in Table S5 (Supporting Information) with other variants of substitution rates for all 44 loci.

HISTORICAL DEMOGRAPHY OF THE TWO PARROTBILLS

Polymorphism patterns in *P. w. suffusus* and *P. alphonsianus* fit best with the secondary contact model (posterior probability = 0.96, Fig. 2d), in which the two parrotbills were first geographically or ecologically isolated, then diverged, and then gene flow resumed after secondary contact.

The long-term effective population size of *P. w. suffusus* is *c.* 3.6 times that of *P. alphonsianus* (median $N_{Pw} = 2.31 \times 10^5$ and median $N_{Pa} = 0.65 \times 10^5$; Table 2; Fig. 5a). The long-term effective population size of the most recent common ancestor, N_A, is much greater than

N_{Pw} (median = 1.02×10^6). However, we found the distribution of its probability density function to be relatively flat (Fig. 5b). The split time of *P. w. suffusus* and *P. alphonsianus* was *c.* 853,000 years ago. Secondary contact was initiated *c.* 290,000 years ago (Table 2; Fig. 5c). Gene flow between the two incipient species was asymmetrical: *c.*181 individuals per generation immigrated from *P. w. suffusus* into *P. alphonsianus* during secondary contact, but only *c.* 47 individuals per generation from *P. alphonsianus* into *P. w. suffusus* (Table 2; Fig. 5d). The peaks of the probability density functions for both M_{Pw} and M_{Pa} are quite broad (Fig. 5d). The point estimates of M_{Pw} and M_{Pa} can be converted into the proportions of immigrants in the two parrotbills per generation (2.5×10^{-3} and 5.0×10^{-4} for m_{Pa} and $m_{Pw,}$ respectively).

Discussion

The climate niches of *P. w. suffusus* and *P. alphonsianus* are differentiated in both allopatry and potential sympatry, suggesting that their niche difference is likely to have a genetic basis. Within the sympatry, their climate niches remain differentiated even after a linear elevation effect was accounted for (Fig. 4). Although the effect of elevation may not be linear, and there may be other environmental variables at play, their niche difference appeared to be robust to linear elevational gradients, as well as to the spatial scale of the buffer applied to the contact zone. Furthermore, our genetic analyses support a speciation scenario in which their gene flow was re-established since the late Pleistocene, after their ancestors had been genetically isolated for most of their divergence history (Table 2; Fig. 5). This implies that, while the niche differentiation between the two parrotbills could have originated in allopatry early in their divergence history through a combination of genetic drift and divergent selection, the continued niche differentiation in the face of the recent gene flow has probably been maintained by ecologically divergent selection in the sympatric region.

The continued niche differentiation between the two parrotbills is not likely

maintained entirely through a neutral process, such as genetic drift. Their F_{ST} values of the

44 nuclear loci that span the entire parrotbill genome are extremely low (mean = 0.01,

standard deviation = 0.01; Table S3), which is comparable to or even smaller than that

observed among nearby avian populations (e.g. Nicholls et al. 2006). Such low F_{ST} values

imply that the substantial gene flow in their secondary contact is likely to erase the neutral

genetic divergences between them (Wright 1978). The homogenization effect of

interspecific gene flow on neutral genomic regions has also been reported in a recent study

of the carrion crow *Corvus corone* and the hooded crow *C. cornix* that hybrid in Europe

(Poelstra et. al. 2014). The genomic data from one population of the carrion crow and two

populations of the hooded crows that are near their hybridization zone indicate that the F_{ST}

values of their autosomes are merely 0.0172 and 0.026. It is similar to the level of F_{ST} value

between the two hooded crow populations near the hybridization zone (0.0153), and much

smaller than that between two carrion crow populations, for which one is near and the other

is far from the hybridization zone (0.1013). Furthermore, based on classic population

genetic theory, it only takes very few immigrants per generation ($Nm> 1$ for the island model)

to homogenize neutral genetic variations between populations (Wright 1940). Taken

together, the low F_{ST} (Table S3), the high numbers of immigrants, and the long period of gene

flow (Table 2) suggest that the neutral genomic regions of the two parrotbills should be

highly homogenized, making it unlikely that their continued niche differentiation could be

maintained by genetic drift alone.

A recent study of another pair of closely related passerines, the common nightingale

(*Luscinia megarhynchos*) and the thrush nightingale (*L. luscinia*), reported a similar pattern

of niche differentiation in the face of gene flow (Reifová *et al*. 2011). The two nightingales

diverged in relative bill size in sympatry but not in allopatry, a fact which is believed to

reflect partitioning of their feeding niches in sympatry (i.e., character displacement).

Although ecological niche models have been widely applied in the past decade in combination with genetic data to infer species' glacial refugia and range expansion (Carstens & Richards 2007; Zink *et al*. 2013), this study is to our knowledge the first to combine ecological niche models and historical demography to infer the important ecological process of niche evolution.

NICHE EVOLUTION IN RECENTLY DIVERGED POPULATIONS AND SPECIES

The rate of niche evolution is continually debated (rapid niche evolution e.g. Holt & Gaines 1992; Holt, Barfield & Gomulkiewicz 2004; Sexton *et al.* 2009; Guisan *et al.* 2014; niche conservatism e.g. Peterson, Soberón & Sánchez-Cordero 1999; Peterson 2011). On the one hand, a recent review of 180 cases of biological invasions at species level reported climate niche shift in 50% of them (Guisan *et al.* 2014). On the other hand, another review of 299 taxa (species or higher, ranging from microbes to plants, invertebrates and vertebrates) concluded that Grinnellian niches such as climate niches are generally conserved (Peterson 2011). In fact, while climate niches are conserved in short-to-moderate evolutionary time-spans, this tendency can weaken at the transition from sister taxa to closely related species (*c.* 10^5-10^7 years; Peterson 2011). Our genetic analyses indicate that the two parrotbills split *c.* 8.53×10^5 years ago, at the point when niche conservatism tends to break down. By contrast, the two subspecies of *P. alphonsianus* were found to have highly conserved climate niches (*P. a. alphonsianus* versus *P. a. yunnenesis*; Fig. S1, Supporting Information). Although estimating the divergence history of these two subspecies of *P. alphonsianus* is beyond the scope of the current study, their divergence is probably much more recent than that of the *P. alphonsianus—P. w. suffuses* pair, such that they might not have had sufficient time to evolve different niches. Therefore, our findings are in general agreement with the tempo of the niche evolution reported in Peterson (2011).

The majority of the previous studies used phylogenies to infer the relationships between taxa, an approach which assumes evolutionary independence of the taxa. Such an approach is likely to underestimate the role of ecology in niche evolution by overestimating the time or amount of genetic isolation required to generate niche differentiation. By measuring the post-divergence gene flow between the two parrotbills, we were able to show that these two taxa were not evolutionarily independent lineages for one third of their post-divergence history (*c.* 2.9×10^5 years). In spite of their evolutionary dependence, the niche differentiation between the two parrotbills is maintained even in their sympatry, which presumably offers suitable and accessible environments to both parrotbills. (The dispersal ability for female *P. webbianus* is up to 4.6 km per generation as suggested by banding records; Hsu Y-C, unpublished.) These findings strongly suggest a role for ecology in the niche differentiation observed between these two closely related species.

MECHANISMS OF NICHE EVOLUTION WITH GENE FLOW

In this study, we found that *P. alphonsianus* is more strongly associated with stable thermal conditions (i.e., high isothermality, low temperature seasonality) than is *P. w. suffuses* (Table 1; Fig. 4a). Assuming the ancestral climate niche to be broad or highly plastic, *P. alphonsianus* could have undergone niche evolution characterised by specialised use of stable thermal conditions through a two-step process: (i) directional selection that shifted its niche and (ii) balancing selection that maintained this specialised, narrower niche. The ancestor of *P. alphonsianus* could have become restricted to regions of high thermal stability due to glaciation. Subsequently, ecologically divergent selection in this restricted region could have promoted their specialised niche use. Our results agree with those of Keller & Seehausen (2012), who identified 16 animal and plant systems in which divergent thermal adaptation might have been strong enough to maintain a bimodal genotype distribution

upon secondary contact, allowing reproductive isolation between populations or stable coexistence of sibling taxa.

Although genetic drift could also have caused a loss of genetic variation in an isolated population, and consequently narrowed its niche width, this is a less likely explanation in the case of *P. alphonsianus*. Unless neutral alleles or genes interact to cause a genetic problem in hybrids (e.g. Bateson–Dobzhansky–Müller incompatibility, reviewed in Cutter 2012), divergent neutral traits arising from genetic drift between the two parrotbills would probably have been homogenised by the substantial gene flow in their secondary contact, as discussed above.

Ecologically divergent selection can arise when two taxa experience differences in environment (e.g. habitat, climate, resources), when there is spatial variation in selection on sexual traits or when the two taxa directly interact with each other such as in the case of character displacement (reviewed by Rundle & Nosil 2005). Because the climate niches of the two parrotbills are not more different in sympatry than in allopatry (Fig. 4a), character displacement is unlikely in this case. Theoretical studies suggest that local adaptation driven by divergent selection can be maintained when the intensity of migration or proportion of immigrants (m) is less than s, a coefficient for the intensity of selection (Haldane 1930; Nagvlaki 1975). The s for a single population in the wild is estimated to be around 0.15 (Hoekstra *et al*. 2001). Although the estimated Nm between the two parrotbills are very high (Table 2), the large effective population sizes of the two parrotbills (Table 2) make their migration rate in either direction far less than 0.15. Therefore, although the number of immigrants for both parrotbills are high enough to retard the differentiation of neutral genetic variations (Wright 1940), local adaptation could still have been maintained given the level of their gene flow. Furthermore, the plumage differentiation between the two parrotbills (Wu *et al*. 1986; Han 1991; Roboson 2007; Crottini *et al*. 2010) in the face of

significant gene flow implies a role of sexual selection (e.g. plumage-based mate choice).

Therefore we suggest that environmental differences, working synergistically with

assortative mating, are the probable mechanism for the maintenance of the divergence

between the two parrotbills (Doebeli & Dieckmann 2003; van Doorn, Edelaar & Weissing

2009).

EFFECT OF PHYLOGENETIC ACCURACY

One of the assumptions in our inference of the divergence demography between *P. w.*

suffusus and *P. alphonsianus* is that the two parrotbills are sister species, as indicated in the

mtDNA-based phylogeny of parrotbills (Yeung *et al*. 2011). Although a single-locus gene tree

might not depict the "true" phylogenetic relationship (e.g. Degnan & Rosenberg 2009)

between all evolutionary lineages within the *P. webbianus*/*P. alphonsianus* complex, the

high substitution rate and short coalescent time of the mitochondrial genome still make

mitochondrial DNA a more powerful genetic marker to resolve recent population

diversification than the nuclear genome (Zink & Barrowclough 2008). This argument is

further supported by the low phylogenetic resolution among subspecies of the *P.*

webbianus/*P. alphonsianus* complex provided by three nuclear fragments (Yeung *et al*. 2011).

In any case, our conclusion on the high level of recent and continuing gene flow between

these two parrotbills does not depend on the assumption that they are sister species.

INFERRING SPECIES' PAST DISTRIBUTION WITH ECOLOGICAL NICHE

MODELS

In the last decade, there have been growing interests in reconstructing species'

paleoistributions based on their modern-day occurrence (e.g. Hugall *et al*. 2002; Carstens &

Richards 2007). However, a key assumption in such extrapolations is that the species' niches

are conserved. Although we could not pinpoint the time when these two parrotbills began to differentiate in their niches, our results raise the question that climate niches may not be conserved in some cases. We caution that the assumption of niche conservatism should be verified whenever possible. For instance, one may examine the niche differentiation between a focal species or population and a close relative, such as sister species (e.g. *P. w. suffuses* versus *P. alphonsianus*; Fig. 3), another subspecies (*P. a. alphonsianus* versus *P. a. yunnenesis*; Fig. S1, Supporting Information) or a different geographic population, and a lack of niche differentiation would provide support for the assumption of niche conservatism.

Data accessibility

Sequences of all nuclear loci included in current study are deposited in GenBank (accession number KF153938-KF201868). Species presence data are available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.rs434 (Pei-Jen et al. 2014).

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Figure legends

Fig. 1. Sample locations for the genetic analyses, occurrences for the ecological niche models and predicted allopatric and sympatric regions for *Paradoxornis webbianus suffusus* and *P. alphonsianus*. The circles denote the occurrences of *P. alphonsianus* (Pa) and the triangles denote the occurrences of *P. w. suffusus* (Pws). The filled symbols are the sample locations for the genetic analyses. The potential sympatry is the overlapping area of the predicted distributions of Pws and Pa, based on their respective ENM with the minimum training presence threshold applied. The top-left panel shows the contact zone of Pws and Pa (solid black line), delineated with the line that connects the locations where Pws and Pa both occurred.

Fig. 2. Four demographic models for the divergence of *Paradoxornis webbianus suffusus* and *P. alphonsianus* and their corresponding posterior probabilities for model selection in ABC: (a) isolation (no gene flow between the two diverging taxa since their split), (b) isolation with migration (post-divergence flow throughout the entire divergence history), (c) early gene flow (post-divergence gene flow in the early stages of the divergence history) and (d) secondary contact (gene flow since secondary contact between the two diverging taxa). The posterior probability of each model estimated by model selection procedure based on one million simulations is labelled under each model. The tolerance was set to 0.005. N_{Pw} and N_{Pa} are long-term effective population sizes for *P. w. suffusus* and *P. alphonsianus* respectively; N_A is the effective population size for the most common recent ancestor of the two parrotbills; M_{Pw} and M_{Pa} denote the gene flows from *P. w. suffusus* to *P. alphonsianus* and from *P. alphonsianus* to *P. w. suffusus* respectively; T_1 is the population split time of the two parrotbills and T_2 is the time for cessation or initiation of post-divergence gene flow in the early divergence and secondary contact models.

Fig. 3. ENM background tests of niche divergence between *Paradoxornis webbianus suffusus* and *P. alphonsianus*. (a) The Schoeners' *D* and (b) the similarity statistic *I*. The niche overlap values are represented by the arrows; the distribution of background niche overlap values based on the niche model of *P. alphonsianus* and a niche model generated with random points drawn from the geographic range of *P. w. suffusus* is in black and the distribution based on the niche model of *P. w. suffusus* and a niche model generated with random points drawn from the geographic range of *P. alphonsianus* is in grey.

Fig. 4. Comparisons of the canonical function score between *Paradoxornis webbianus suffusus* (Pws) and *P. alphonsianus* (Pa). (a) The canonical function score of each occurrence of Pws (triangles) or Pa (circles) against its distance to the contact zone (vertical shaded line). (b) The mean canonical function score of Pws (unfilled bars) and Pa (filled bars) in allopatry and sympatry, as well as at various spatial scales within the sympatric region, from < 50 Km to < 100 Km and < 150 Km of the contact zone. The error bars denote 95% confidence limits. The asterisks indicate significant differences between the two parrotbills.

Fig. 5. Probability densities of seven demographic parameters for the secondary contact model for divergence of *Paradoxornis webbianus suffusus* and *P. alphonsianus*. (a) Long-term effective population size for *P. w. suffusus* (N_{Pw}) in black, and *P. alphonsianus* (N_{Pa}) in grey; (b) Long-term effective population size of the two parrotbills' most recent common ancestor (N_A); (c) Population split time T_1 (solid line) and time of initiation of secondary contact T_2 (dotted line); (d) Migration coefficient (4 $N_e m$) of gene flow from *P. w. suffusus* into *P. alphonsianus* M_{Pw} (solid line) and *P. alphonsianus* into *P. w. suffusus* M_{Pa} (dotted line). N_e is the long-term effective population size of the recipient species, and *m* is the proportion of immigrant genes in the focal species.
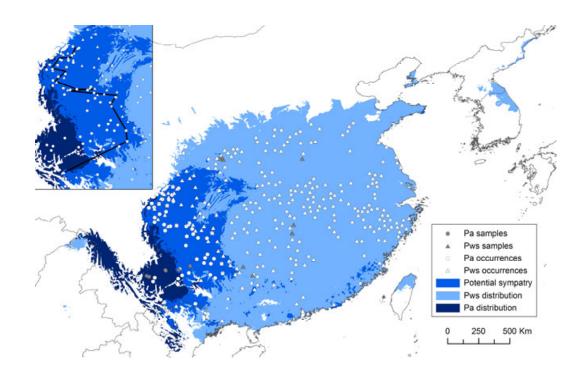
Tables

Table 1. Total canonical structure of the discriminant functional analyses on the nine

bioclimate variables extracted from the occurrences of *Paradoxornis webbianus suffusus* and

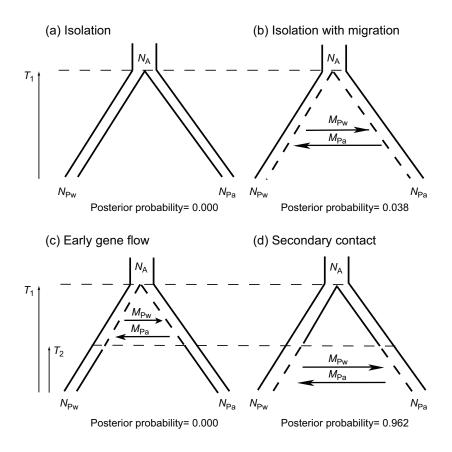*P. alphonsianus* in their entire geographic ranges.

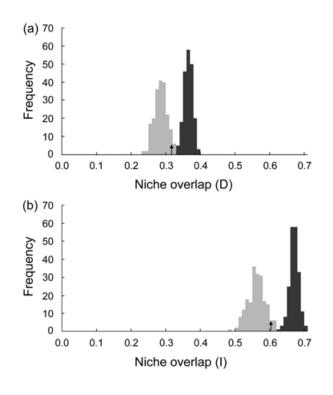| Bioclimate variable	Canonical function 1
Bio1 = Annual Mean Temperature	-0.0250
Bio2 = Mean Diurnal Range	-0.0481
Bio3 = Isothermality	0.7360
Bio4 = Temperature Seasonality	-0.8276
Bio10 = Mean Temperature of Warmest Quarter	-0.6127
Bio12 = Annual Precipitation	-0.0713
Bio15 = Precipitation Seasonality	0.5473
Bio17 = Precipitation of Driest Quarter	-0.4868
Bio18 = Precipitation of Warmest Quarter	0.4506

Table 2. Posterior median, mean, mode and range of 95% highest probability distribution (HPD) for seven demographic parameters in the secondary contact model for divergence of *Paradoxornis webbianus suffusus* and *P. alphonsianus.*
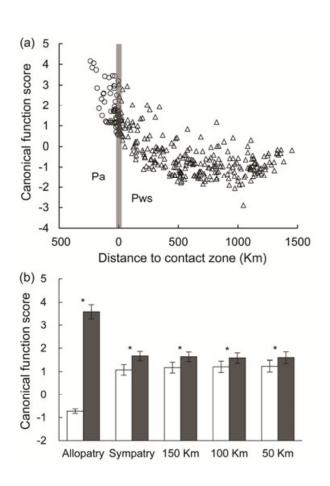
	N_{Pw}	N_{Pa}	N_A	T_1	T_2	M_1	M_2
2.5% HPD	0.37×10^5	0.26×10^5	2.81×10^5	4.57×10^5	0.57×10^5	21.68	278.90
Median	2.31×10^5	0.65×10^5	10.22×10^5	8.53×10^5	2.90×10^5	189.18	723.61
Mean	4.12×10^5	0.98×10^5	17.26×10^5	8.54×10^5	2.84×10^5	259.12	700.61
Mode	1.06×10^5	0.50×10^5	5.58×10^5	8.66×10^5	3.00×10^5	80.15	856.69
97.5 HPD	17.63×10^5	3.22×10^5	74.32×10^5	12.51×10^5	4.79×10^5	832.49	983.68

N_{Pw}: long-term effective population sizes of *P. w. suffusus*; N_{Pa}: long-term effective population sizes of *P. alphonsianus*; N_A: long-term effective population sizes of the most common ancestor of the two parrotbills; T_1: population split time (years ago); T_2: initiation of secondary contact (years ago). M_1: immigration rate per generation from *P. w. suffusus* into *P. alphonsianus* ($4N_em$); M_2: immigration rate per generation from *P. alphonsianus* into *P. w. suffusus* ($4N_em$). N_e is the long-term effective population size, and *m* is the proportion of immigrants.

(a) Isolation

T_1

N_A

N_{Pw} N_{Pa}

Posterior probability= 0.000

(b) Isolation with migration

N_A

M_{Pw}
M_{Pa}

N_{Pw} N_{Pa}

Posterior probability= 0.038

(c) Early gene flow

T_1

N_A

M_{Pw}
M_{Pa}

T_2

N_{Pw} N_{Pa}

Posterior probability= 0.000

(d) Secondary contact

N_A

M_{Pw}
M_{Pa}

N_{Pw} N_{Pa}

Posterior probability= 0.962