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Demographic history of a complex polymorphism in populations of the Oriental Dwarf Kingfisher (*Ceyx erithaca/rufidorsa*) of Southeast Asia

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The Oriental Dwarf Kingfisher species-complex of South and Southeast Asia comprises two forms, the dark-backed *Ceyx erithaca* of India and Indochina and the rufous-backed *Ceyx rufidorsa* of Java and the Lesser Sunda Islands. Between these two extremes, the large area of Sundaland and the Philippines is occupied by individuals that have a rufous back, characteristic of C. *rufidorsa*, but exhibit a range of phenotypes that are intermediate between C. *erithaca* and C. *rufidorsa*. These potential intermediates have intrigued avian taxonomists for generations. To investigate the species dynamics of the two forms and understand the demographic history of the intermediates, we generated a genome-scale dataset (ddRAD) representing multiple individuals across the entire range of the complex. Our findings support the distinctiveness of the two forms based on back colour. Demographic analysis suggests the two populations were isolated *c.* 820 000 years ago followed by secondary contact *c.* 140 000 years ago, with asymmetrical dispersal of C. *rufidorsa* into C. *erithaca*. Although some limited introgression appears to have occurred more recently between the two taxa in the northern parts of their range, we were unable to find any association of recent hybridization with the intermediate plumages of C. *rufidorsa*. We also found no support for the commonly recognized Borneo subspecies *motleyi*.

Keywords: Alcedinidae, Borneo, colour morph, population genomics, RAD-seq.

Birds showcase a large variety of plumage polymorphisms, with more than 3.5% of described species exhibiting substantial variation in form (Galeotti *et al.* 2003). These species are spread throughout the avian phylogeny, with some families, such as owls (Strigidae) and cuckoos (Cuculidae), comprising a particularly large number of polymorphic taxa. Polymorphism may have a strong selective role, as in ratio-clines that facilitate adaptation to different environmental conditions (Galeotti *et al.* 2003), or it may simply exist as a hangover from old hybridization events (Lim *et al.* 2010). The genetic forces underlying polymorphic phenotypes may stem from variation at single loci (Theron *et al.* 2001, Pryke & Griffith 2006, Uy *et al.* 2009,

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Kim *et al.* 2019), multiple loci (Poelstra *et al.* 2014, Toews *et al.* 2016, Irwin *et al.* 2018) or supergenes (Küpper *et al.* 2015, Lamichhaney *et al.* 2015, Tuttle *et al.* 2016, Sanchez-Donoso *et al.* 2022). As a result of prevalent polymorphism in birds, understanding its genetic basis, as well as how it persists in bird populations, is an especially active area of research.

The *Ceyx erithaca/rufidorsa* species-complex of South and Southeast Asia consists of small, colourful, forest-dwelling kingfishers, currently called Oriental Dwarf Kingfishers (Gill & Donsker 2021). This group consists of two basic forms: C. *erithaca*, the Black-backed Dwarf Kingfisher, which has dark blue/black plumage on the mantle, wings and behind the ear coverts and a breeding range from India and Sri Lanka to Indochina and the northern Thai-Malay Peninsula; and C. *rufidorsa*, the Rufous-backed Dwarf Kingfisher, which is lilac-rufous on its back and wings and occurs on Java and the Lesser Sunda Islands (Fig. 1). Between these two geographical extremes is a large area, including the Malay Peninsula, Sumatra, Borneo, Palawan, Mindoro and associated smaller islands, that is occupied by what appear to be rufous individuals with varying degrees of intermediate plumage between rufous-backed and black-backed forms. Not surprisingly, these intermediate birds have intrigued taxonomists for generations (Ripley 1942, Voous 1951, Sims 1959, Ripley & Beehler 1987).

Global and local bird classifications regard C. *erithaca* and C. *rufidorsa* either as two distinct species (Smythies 1999, Rasmussen & Anderton 2005, Eaton *et al.* 2016, Clements *et al.* 2021) or as subspecies or colour morphs of a single species (Wells 1999, Dickinson & Remsen 2013, Gill & Donsker 2021). Uncertainty over the species' status stems not only from the unusually large range and variability of intermediate forms but also from the occurrence of non-breeding wintering black-backed (C. *erithaca*) migrants in Sundaland and the Philippines. On the Malay Peninsula and Sumatra, black-backed migrants are common in autumn and winter, but pure black-backed individuals appear to breed only above Ranong on the Peninsula, *c.* 9.9°N (Wells 1999). Rufous individuals exhibiting

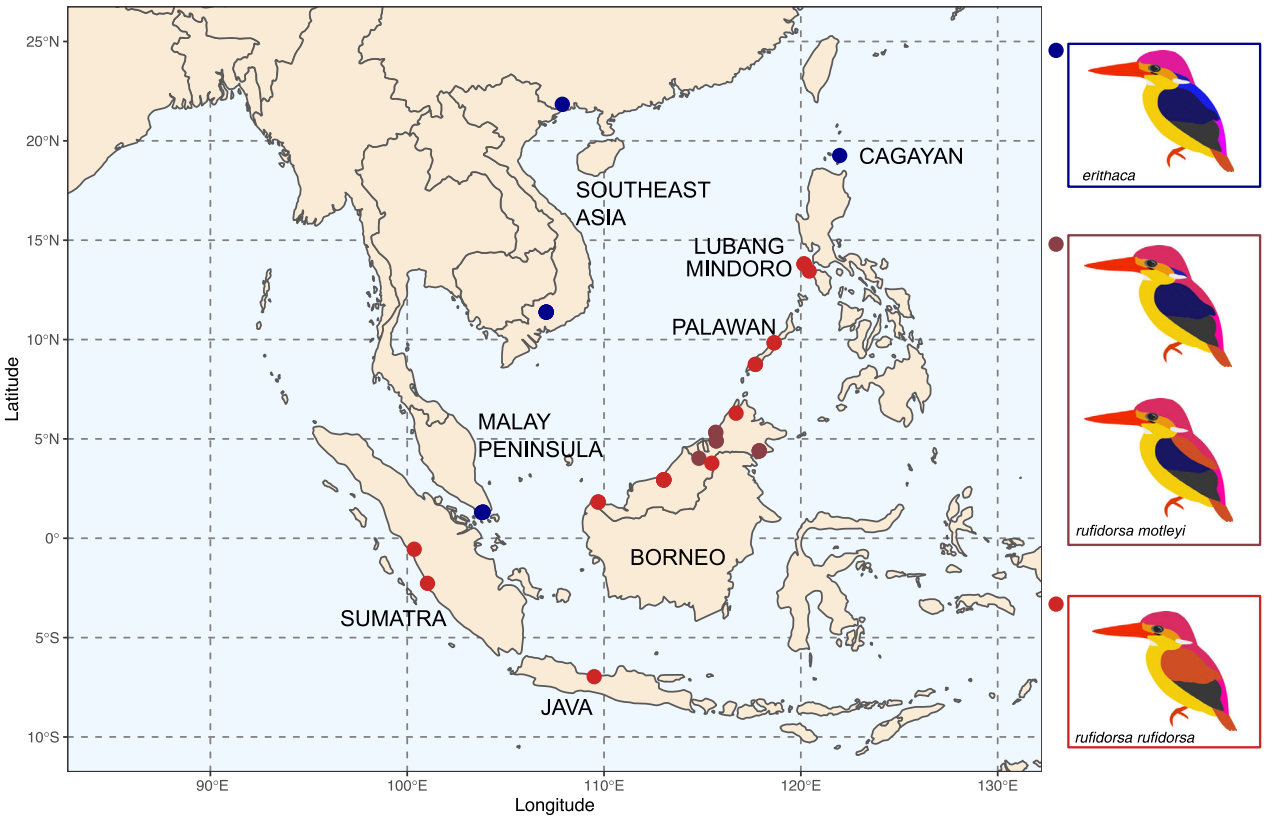

Figure 1. Map of Southeast Asia showing sampling locations of *Ceyx erithaca* (blue), *Ceyx rufidorsa motleyi* (brown) and *Ceyx rufidorsa rufidorsa* (red), along with representative illustrations of each group (drawings by S. B. Shakya).

a range of black-back plumage colours predominate on both the Malay Peninsula and Sumatra (Voous 1951, Ripley & Beehler 1987, van Marle & Voous 1988, Wells 1999). On the west Sumatran island of Nias, the population has distinctively dark wings, causing it to be treated generally as a separate subspecies, C. *rufidorsa captus* (Ripley 1944, Voous 1951, Dickinson *et al.* 1991, Rheindt *et al.* 2020). On Borneo, the situation is much the same as on Sumatra and the Malay Peninsula, except there are many fewer migrants. In northeastern Borneo (Sabah), the extensive blue feathering on the wings of some individuals has resulted in the recognition of a separate subspecies in that area, *motleyi* (Chasen & Kloss 1929, Ripley & Beehler 1987, Gill & Donsker 2021). In the Philippines from Palawan to Mindoro (and smaller islands), the resident birds are also fundamentally rufous-backed (Dickinson *et al.* 1991), but individuals with mixed dark plumage occur on Mindoro and have previously been considered a separate subspecies, *vargasi* (Manuel 1939).

To obtain a quantitative assessment of colour distribution in the C. *erithaca/rufidorsa* complex, and thus insight into its evolution and appropriate classification, Sims (1959) and Ripley and Beehler (1987) conducted hybrid-index analyses of the group's plumage variation. Although their sampling was admirable, they appear not to have accounted for migrant individuals in their studies. Also, they failed to include the single most important plumage character in their data, mantle colour. Subsequently, Lim *et al.* (2010) conducted plumage and multilocus nuclear and mitochondrial DNA analyses of the kingfishers on the Malay Peninsula and Greater Sunda Islands and shed substantial light on their population relationships and history. They found that all mixed-plumage and pure rufous individuals were members of a single clade, C. *rufidorsa*. Moreover, this clade was defined by possession of a rufous, as opposed to blue, mantle. They also found that the variable plumage in breeding birds on the Malay Peninsula, Sumatra and Borneo was the result of past (not current) introgressive hybridization between black-backed and rufous-backed populations. This conclusion largely agrees with that of Ripley and Beehler (1987). The findings of Lim *et al.* (2010) did not support the designation of *motleyi* as a subspecies distinct from other Bornean residents. The dark-winged populations on Nias (*captus*) and Mindoro (*vargasi*) were not examined.

In this study, we revisit the C. *erithaca/rufidorsa* system using genome-wide data produced by restriction site-associated DNA sequencing (RAD-seq). To determine more extensively the patterns of introgression among the forms, we infer the level of genetic admixture present in black-backed, rufous-backed and intermediate individuals, including specimens from a wider range on mainland Asia and, for the first time, from the Philippines. We also apply demographic methods to investigate models of isolation and divergence, along with the rates of past and present dispersal. Finally, we use phylogenetic methods to infer genealogical relationships of the taxa.

METHODS

Sampling and colour classification

We selected 38 individuals representing the geographical range and plumage variation across C. *erithaca/rufidorsa* (Table S1). In addition, we included two individuals of Dimorphic Dwarf Kingfisher *Ceyx margarethae*, and one of Philippine Dwarf Kingfisher *Ceyx melanurus*, as outgroups (Table S1). The C. *erithaca* samples were collected outside of the migratory season to avoid confusing migrant and breeding populations (Lim *et al.* 2010). We characterized the colouring of four characters among individuals (mantle, flight coverts/scapulars, neck patch and forehead) that are known to vary among individuals (Ripley 1942, Voous 1951, Sims 1959, Ripley & Beehler 1987, Fry *et al.* 1992, Lim *et al.* 2010). For each character (Table S1), individuals were scored as rufous-type if no blue colouring was present, intermediate (indicated by blue in any one category in Table S1) if limited or barely perceptible blue was present, or as blue-type. With respect to *motleyi*, it is difficult to identify the subspecies with confidence due to substantial colour variation in its range. Therefore, we refer to an individual as *motleyi* only if the specimen was recorded as such in its respective museum database. We limited the name *motleyi* to birds from NE Borneo even though similar-coloured forms occur in the Philippines.

Laboratory methods

We extracted genomic DNA from using a QIAGEN DNeasy blood and tissue kit, quantified the concentration of the DNA extracts using Qubit

Fluorometric Quantification (Life Technologies) and standardized the concentration of the extracts to 5 ng/μL. We then followed a single-digest modified RAD-seq protocol (Miller *et al.* 2007), as described by Manthey *et al.* (2016). For each sample, we digested 10 μL of the DNA extract with 2 μL NEB buffer 4, 0.15 μL of *Nde*I and ddH_2O to a total reaction volume of 20 μL. We incubated these reactions at 37 °C for 3 h, followed by 65 °C for 20 min to inactivate the enzyme. We then ligated sample-specific barcoded adaptors to our digested genomic DNA by adding 5 μL of ligase buffer, 0.1 μL high-concentration (400 U) T4 ligase, 0.5 μL sample-specific adapter oligos (at 10 μM) and 24.4 μL ddH_2O. We incubated these reactions at 16 °C for 3 h, followed by 65 °C for 10 min to inactivate the ligase.

Following ligation, we purified the samples through ethanol precipitation. To the ligated restricted genomic DNA of each sample, we added 5 μL of 3 M sodium acetate pH 5.2 and 50 μL isopropanol. We then pooled the samples into a single tube before adding 1 μL glycogen and chilling them for 8 h. We pelleted the precipitate by centrifuging at 4000 *g* for 30 min at 4 °C. After discarding the supernatant, we carried out a wash with 1 mL of 70% ethanol, centrifuging the tube at 4000 *g* for 5 min. We air-dried the pellet, and then resuspended in 100 μL TE pH 8.0. We further purified our samples using 150 μL Agencourt AMPure beads, followed by two washes with 200 μL of 70% ethanol, before resuspending in 30 μL TE. We carried out size selection of fragments between 500 and 600 bp using PippinPrep (Sage Science, Beverly, MA, USA), followed by an enrichment PCR consisting of 98 °C for 30 s, and then by 14 cycles of 98 °C for 10 s, 64 °C for 20 s and 72 °C for 20 s. This was followed by a final extension at 72 °C for 7 min. The enrichment PCR was followed by two rounds of purification using the previously described Agencourt AMPure bead protocol before eluting in 22 μL Qiagen EB buffer. The pooled library was submitted to the KU Genome Sequencing Core, where it was quantified by quantitative (q)PCR and Agilent Tapestation, before being sequenced on a partial lane of an Illumina Hiseq 2500 single-end 100-bp high-output run.

RAD loci processing

Demultiplexed libraries were processed using STACKS v2 (Rochette *et al.* 2019). We used the *de novo* pipeline because a reference genome for the species was not available. We adopted the workflow from Paris *et al.* (2017) in choosing values for the parameters M, m and n in the *denovo_map.pl* program in STACKS. After optimization, we used parameter values of 4, 3 and 4 for M, m and n, respectively, in running *denovo_map.pl*. We then used the program *populations* in STACKS to filter the SNP dataset to contain only one random SNP in those loci containing a maximum of 20% missing data. As different levels of filtering of minor alleles can influence subsequent analyses (Linck & Battey 2019), we applied the most relevant parameters in different analyses. For population genetic analysis, including MOMENTS analysis, we ran the STACKS *populations* program without outgroup taxa. For STRUCTURE and principal components analysis (PCA), we also applied a minimum minor allele frequency filter of 0.05.

Population genetics and demographic inference

We conducted a PCA using the *glPCA* function in the R-package *adegenet* v2.0.1 (Jombart 2008). We assessed population structure with STRUCTURE v2.3.4 (Pritchard *et al.* 2000) implemented through PARALLELSTRUCTURE (Besnier & Glover 2013) in the CIPRES Science Gateway (Miller *et al.* 2010). STRUCTURE was initiated for 50 000 generations with a burn-in of 5000 for *k*-values ranging from 1 to 5. For each value, we carried out five independent runs. We used the program STRUCTUREHARVESTER (Earl & vonHoldt 2012), which makes use of the Evanno *et al.* (2005) method, to identify the best value of *k*. For this value ($k = 2$) we ran STRUCTURE 10 times for 500 000 generations each, with a burn-in of 50 000. In our initial analysis, we found a *k*-value of 3 was also meaningful in understanding variation in populations. Therefore, even though it was not the best supported value, $k = 3$ was applied in 10 runs of 500 000 generations each with a burn-in of 50 000 to examine its effect.

To ascertain the admixture history of the three male *erithaca* from mainland Asia that showed introgression of *rufidorsa* alleles based on the glPCA and STRUCTURE analyses, we first estimated the number of single nucleotide polymorphisms (SNPs) that were perfectly segregated between the other *erithaca* and Sundaic *rufidorsa*

individuals, i.e. not including the three introgressed birds. We then characterized the number of SNPs that were associated with *erithaca* and *rufidorsa* among the three admixed birds. For SNPs that were found to be heterozygous only in the admixed birds, we checked whether those loci were congregated in specific parts in the genome. For this, we generated a consensus fasta file of all the RAD loci using STACKS. We then used BLAST to match the loci to the genome of *C. cyanopectus* (assembly ASM1340135v1; Feng *et al.* 2020). We then tabulated the scaffolds that matched the heterozygous SNPs to check the distribution of the SNPs across the genome to see whether the majority of the SNPs matched only a few scaffolds. Although these were between different species, birds are known to have high levels of synteny (Lovell *et al.* 2014), allowing comparison between closely related species. To identify potentially admixed birds, we also calculated a hybrid index and degree of interspecific heterozygosity using the R-package *introgress* (Gompert & Alex Buerkle 2010).

To infer demographic history between *rufidorsa* and *erithaca*, we used the program MOMENTS (Jouganous *et al.* 2017). We divided the individuals into two populations, blue-backed (n = 9) and rufous-backed (n = 29). The vcf file was projected into a 2D site-frequency spectrum (SFS) using easySFS (https://github.com/isaacovercast/easySFS). The number of segregating sites in each population was projected downwards to determine the maximum number of segregating sites for each population (13 for mainland Asia and 49 for Sundaland) using easySFS. The resulting SFS was fitted to seven different two-population models using MOMENTS_PIPELINE v3.1.4 (Portik *et al.* 2017). For each model, we first optimized the demographic parameters of each model. Each optimization step included four rounds of increasingly focused optimization routines repeated 10, 10, 20 and 50 times, each round with a maximum of 5, 10, 10 and 15 iterations, respectively. For every model, initial parameters were selected randomly with the population size parameter bound between 10^{-3} and 100, and divergence time and migration rate parameters bound between 0 and 100. The parameters of the model with the highest likelihood were used to calculate uncertainty in parameter estimates using a Godambe Information Matrix (GIM) with the GIM uncerts function in MOMENTS. A total of 100 bootstrap replicates of the frequency spectra were generated using the *sample* function in MOMENTS and applied in the GIM matrix to calculate range.

To convert parameter values to biologically relevant units, we used the metrics of Linck *et al.* (2020) in their study of the population dynamics of *Syma* kingfishers, namely mutation rate (μ), 2.3×10^{-9} per bp per year; generation time, 2 years; and effective sequence length (L) of the pruned dataset. Effective population size (N_{ref}) was calculated using the equation $\theta = 4\mu N_{ref}L$. Split time parameters are reported in 2 N_{ref} generations and subsequently converted to years using the calculated value of N_{ref} and the generation time. The number of migrants from population b to population a was estimated using the formula $(m_{ab}*2*N_{ref})*nu_a$.

Phylogenetic analysis

We used two methods to infer phylogenetic relationships. The three outgroup taxa were included in the SNP matrix for phylogenetic analyses. In IQ-TREE v1.6.12 (Nguyen *et al.* 2014) we used a GTR model and ascertainment bias flag (ASC) to account for the SNP-only data when building the maximum-likelihood tree. We ran 500 bootstrap replicates to infer node support. We used snapper (Stoltz *et al.* 2021) for phylogenetic estimation in a Bayesian framework. Due to the computational demand of such coalescent analyses, we ran snapper by grouping individuals by islands. We then ran two independent runs of snapper using the default settings for five million generations. We used TRACER v1.7.1 to check for convergence of the two runs. As the two runs did not converge, we reran snapper using the updated scaling parameters suggested by the initial run of snapper. We also omitted the three hybrid birds in this run, as they might affect the tree estimation. We ran snapper twice for 5 million generations and checked for convergence. Although some parameters still did not converge, the resulting trees from the two runs differed only in height and not in the assignment of clades.

RESULTS

After optimizing the *de novo* pipeline, we recovered 6817 loci encompassing 25 610 SNPs. For phylogenetic analysis, the data were filtered to one SNP per locus, with a maximum of 20% missing data per site, leaving 5112 SNPs. After rerunning the program *populations* for demographic analysis, with outgroups excluded, we had a dataset of

5065 SNPs. For STRUCTURE and PCA, using a minor allele frequency of 0.05, we were left with 3611 SNPs.

PC1 of the PCA accounted for 26.4% of the variation and clearly separated C. *erithaca* from C. *rufidorsa* (Fig. 2a). PC2 accounted for 7.1% of the variation and separated Philippine *rufidorsa* (Palawan, Lubang and Mindoro) from *rufidorsa* on the Malay Peninsula and Greater Sunda Islands. PC3 and PC4 accounted for 3.5% and 3.1% of the variation, respectively. The STRUCTURE plot (Fig. 2b) showed a similar pattern, given an optimal *k*-value of 2 determined by the Evanno method. An obvious split occurred between *erithaca* and *rufidorsa*. Three of the individuals identified as *erithaca* by plumage, however, lay between *erithaca* and *rufidorsa* in the PCA and exhibited mixed ancestry in STRUCTURE. Two of these birds were from Vietnam, and one (presumably a migrant) was from Singapore (Wells 1999). We also ran STRUCTURE with a *k*-value of 3, which separated individuals from Mindoro, Lubang and Palawan from Greater Sunda and Malay Peninsula populations. Individuals from Palawan exhibited a mixture of Mindoro/Lubang and Bornean clusters, plus some *erithaca*. Neither PCA nor STRUCTURE indicated a difference between *rufidorsa* and subspecies *motleyi*.

Of the final 3611 SNPs, 237 were perfectly associated between individuals that were designated *a priori* as *erithaca* and *rufidorsa* based on plumage, excluding the three individuals of *erithaca* that were intermediate in the PCA and STRUCTURE plots. Of the 237 SNPs, 153 and 158 were heterozygous in the two individuals from Vietnam that were intermediate in PCA and STRUCTURE plots, respectively (Table 1). Only 12 SNPs were heterozygous in the admixed Singapore bird but that individual had a substantial proportion of missing data (205 of the 224 SNPs were unaccounted for). Altogether, 118 heterozygous SNPs were shared between the two admixed Vietnamese birds, and these mapped to 101 scaffolds in the C. *cyanopectus* genome. We did not find any consistent blocks of introgression of SNPs within scaffolds. A hybrid triangle plot supports the designation of the three hybrid individuals as backcrossed with *erithaca* parentals (Fig. 3). We also note that the three intermediate *erithaca* individuals were all males. The only other *erithaca* male in our dataset was from Singapore and it was not admixed.

Analysis of the demographic history of *erithaca* and *rufidorsa* using MOMENTS indicated that the best supported model was secondary contact with asymmetrical dispersal (log likelihood: −903.51; Akaike information criterion (AIC): 1819.02; Table 2). Parameter estimates (Fig. 4) suggested the two species split around 820 000 years ago (se

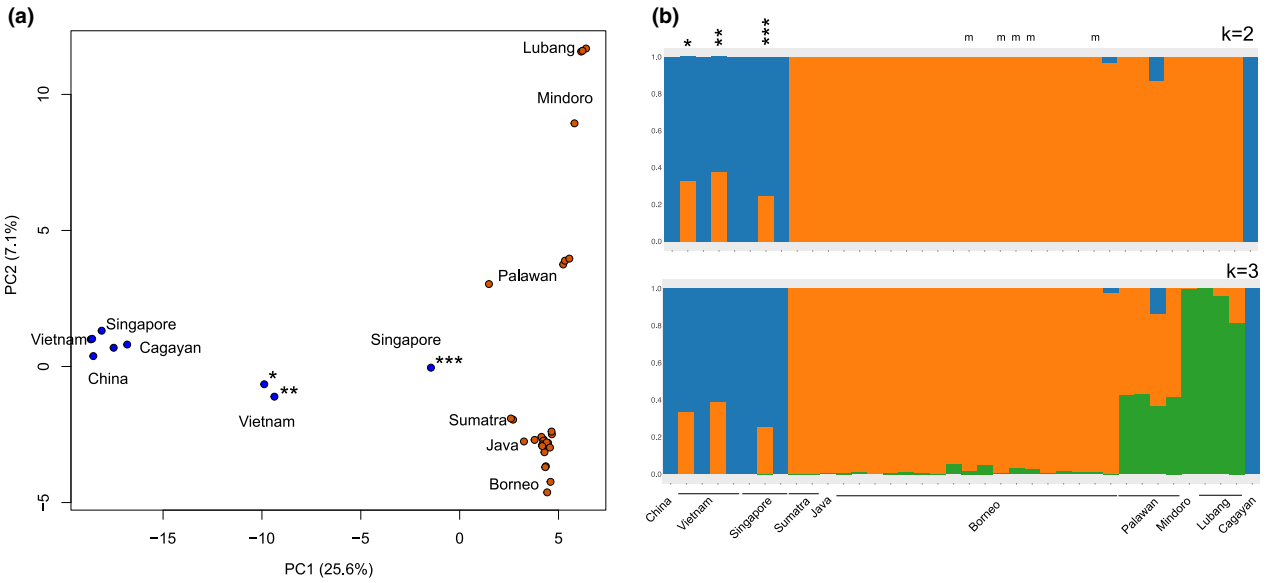

Figure 2. (a) PCA showing the first two principal component axes for 5112 SNPs. (b) STRUCTURE plot showing the assignment of individuals with *k*-values of 2 and 3. *, ** and *** indicate the three individuals that are admixed. Individuals marked 'm' belong to subspecies *motleyi*.

Table 1. Distribution of alleles in the three admixed individuals for 237 SNPs that are perfectly segregated between pure *Ceyx erithaca* and *Ceyx rufidorsa*. These three individuals were determined *a priori* by plumage to be *C. erithaca*.

	Homozygous for typical *erithaca*	Heterozygous	Homozygous for typical *rufidorsa*	Unknown
KU 116750 (Vietnam)	67	153	2	15
KU 116751 (Vietnam)	57	158	0	22
UWBM 67542 (Singapore)	24	12	3	198

170 000) and then came back into secondary contact *c.* 140 000 years ago (se 40 000). The effective population size of *erithaca* was estimated as 110 000 (se 10 000) and *rufidorsa* as 530 000 (se 60 000). The number of individuals dispersing from *rufidorsa* to *erithaca* was 0.60 individuals per generation (se 0.07) and from *erithaca* to *rufidorsa* 0.21 individuals per generation (se 0.05).

The maximum-likelihood (ML) tree indicates that *erithaca* and *rufidorsa* are members of a single well-supported clade relative to the outgroup, but their reciprocal monophyly is poorly supported (Fig. 5a). In addition, the branching pattern between Sundaic and Philippine *rufidorsa* is not well resolved (Fig. 5a). In contrast, the Bayesian snapper tree (Fig. 5b) supports the distinction between *erithaca* and *rufidorsa* with 100% support and monophyly of all island populations, except *erithaca* from Singapore.

Table 2. Parameters, likelihood values and AIC scores for the best replicates of seven demographic models compared in MOMENTS. The model with the highest likelihood and lowest AIC is shown in bold.

Model	Parameters	Likelihood	AIC
Secondary contact followed by asymmetric migration	**6**	**−903.51**	**1819.02**
Split with asymmetric migration	5	−988.94	1987.88
Ancestral asymmetric migration followed by split	5	−1020.40	2052.80
Secondary contact followed by symmetric migration	5	−1156.54	2323.08
Split with symmetric migration	4	−1179.08	2366.18
Ancestral symmetric migration followed by split	4	−1185.61	2381.22
No migration	3	−1410.95	2827.90

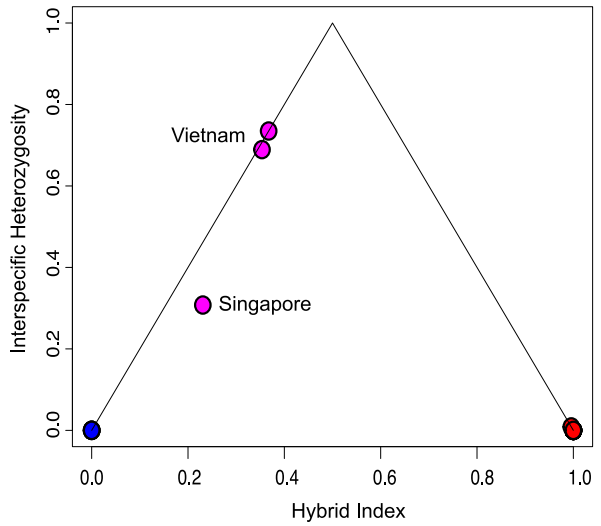

Figure 3. Hybrid triangle plot showing interspecific heterozygosity plotted against the hybrid index of *erithaca* (blue) versus *rufidorsa* (red). Purple dots represent the three admixed individuals.

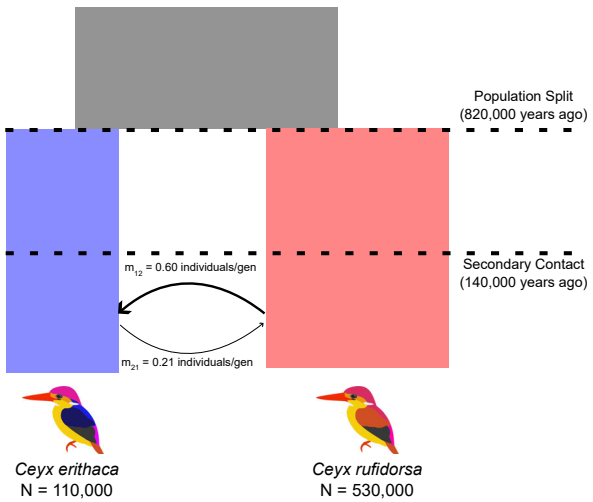

Figure 4. Visual representation of the demographic parameter estimates from MOMENTS analysis of the ancestral split and secondary contact between *Ceyx erithaca* and *Ceyx rufidorsa* (bird illustrations by S. B. Shakya).

DISCUSSION

Using genome-wide RAD-seq data, we recovered population patterns supporting the distinction between C. *erithaca* and C. *rufidorsa*, consistent with those found by Lim *et al.* (2010). The Black-backed Dwarf Kingfisher is a bird of mainland South and Southeast Asia. The Rufous-backed

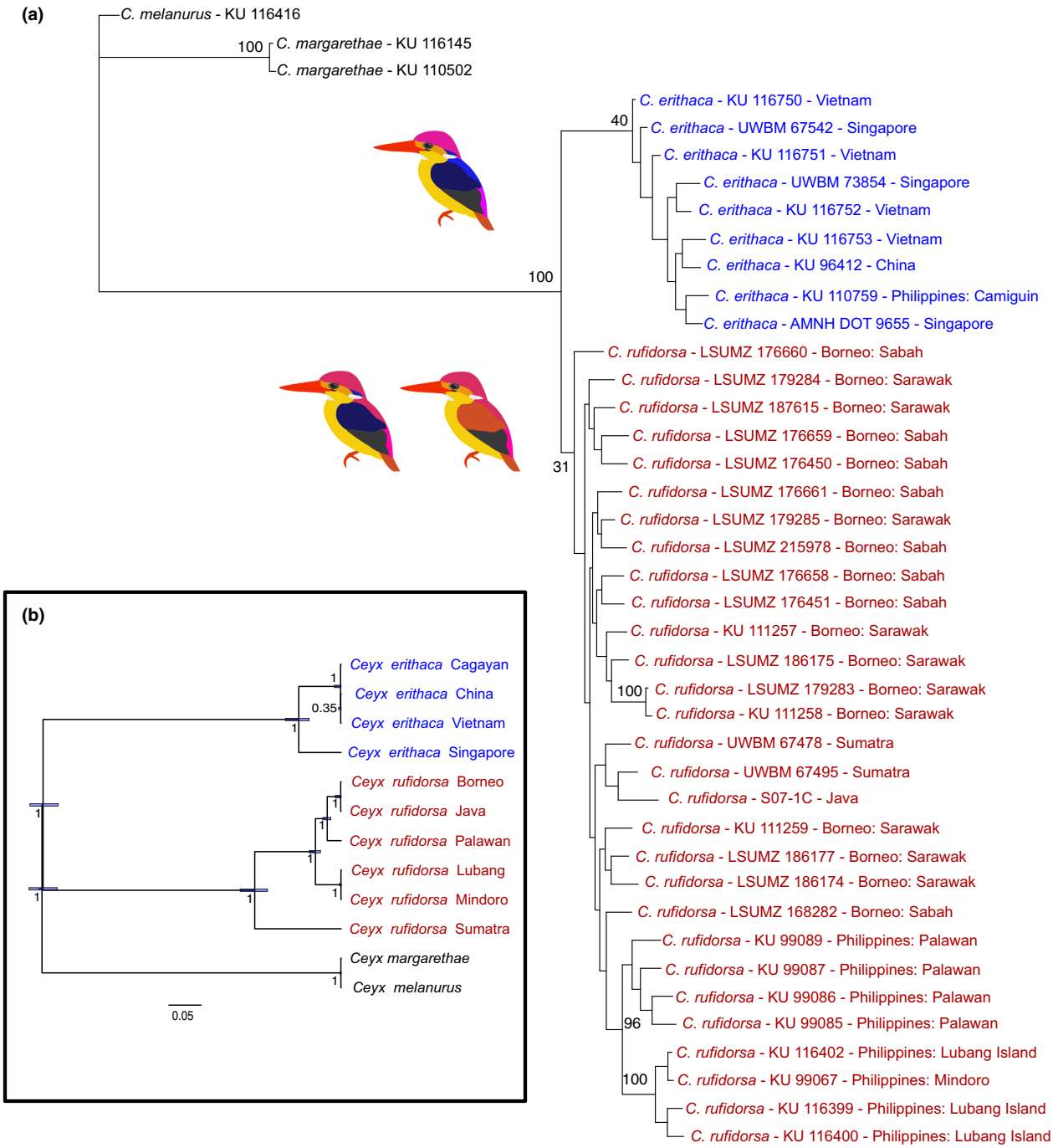

Figure 5. (a) Maximum-likelihood phylogenetic tree of *Ceyx erithaca/rufidorsa*. Bootstrap values of major and well-supported clades are indicated (bird illustrations by S. B. Shakya). (b) Phylogenetic tree implemented in the program snapper of *Ceyx erithaca/rufidorsa* using a Bayesian framework with posterior probabilities of major nodes. Scale bars represent uncertainty in node length.

Dwarf Kingfisher occurs from the Malay Peninsula and Sumatra across Borneo to Palawan and Mindoro in the Philippines and south to Java and the Lesser Sundas. All C. *rufidorsa* individuals possess a rufous mantle, regardless of the degree of blue and black elsewhere in their plumage. We did not find any evidence that the subspecies *motleyi* in eastern Borneo differs genetically from other Bornean individuals. Even with the large amount of data brought to bear, we were not able to detect a substantial signal of introgression of *erithaca* loci into intermediate-plumaged variants of *rufidorsa*, suggesting the origin of plumage variation in *rufidorsa* is not due to contemporary introgression. However, we did find evidence of recent hybridization in three individuals with *erithaca* plumage on the Asian mainland.

Sims (1959) and Ripley and Beehler (1987) have suggested that C. *erithaca* and C. *rufidorsa* were isolated from one another and evolved in allopatry. Subsequently, through dispersal of C. *erithaca*, the two taxa came into contact in Borneo, Sumatra, the Malay Peninsula and the Philippines. Demographic analysis using MOMENTS supports this idea. The optimal historical model indicates secondary contact with asymmetrical dispersal in favour of C. *erithaca*. Specifically, the two populations appear to have diverged *c.* 820 000 years ago and then came into secondary contact *c.* 140 000 years ago. This divergence date is more recent than that reported by Lim *et al.* (2010), namely 1.9–2.9 Ma, but this may be because a simpler model was used in the earlier study. The short span of separation between the taxa does not appear to have been enough for complete reproductive isolation, allowing some level of hybridization upon secondary contact. Our data also support a higher effective population size in C. *rufidorsa* than in C. *erithaca*, as implied by Sims (1959).

There is little evidence supporting current introgression of *erithaca* genes into *rufidorsa*, and there is no evidence that the subspecies *motleyi* in northeastern Borneo results from recent hybridization. Ripley and Beehler (1987) have suggested that this dark-winged population was isolated in eastern Borneo and then came into secondary contact and hybridized with *rufidorsa* during a time of lower sea level when land bridges connected the Sunda landmasses. Certainly, *motleyi* has extensive blue wing colouration reminiscent of *erithaca*. However, inheritance of colour can be accomplished through introgression of only a small portion of the genome that contains genes responsible for colour polymorphism (Giska *et al.* 2019, Gazda *et al.* 2020). The blue colouration could also result from an ancient polymorphism that was lost over time due to effects of genetic drift or selection. The prevalence of blue colouration in some *rufidorsa* individuals on Borneo (and perhaps Nias and Mindoro), therefore, may result from inheritance of a very a small region of the genome left over from ancient hybridization events or ancient polymorphism. Reduced representation analysis methods, such as ours, that employ a random assortment of SNPs from across the genome can miss minor genetic components responsible for colour variation (Lowry *et al.* 2017). To find evidence of such introgression would require whole-genome sequencing.

An unexpected result in our study is the occurrence of three highly admixed individuals of C. *erithaca*. These birds were identified *a priori* as C. *erithaca* by plumage. Indeed, they look like typical *erithaca* and not like Sundaic birds with varying levels of intermediate plumage. All three were collected on the mainland (two in Vietnam and one in Singapore – the latter a probable migrant; Wells 1999) and all three are males. The presence of these recent hybrids that look exactly like *erithaca* indicates that introgression of C. *rufidorsa* into C. *erithaca* occurs. Although the Singapore individual is missing quite a bit of data, the two Vietnamese birds share extensive heterozygosity; 153 and 158 of the 237 *erithaca–rufidorsa* specific alleles are heterozygous in the two individuals, respectively (Table 1). The frequency of heterozygous 'diagnostic' sites suggests very recent hybridization. We also found that the variable sites were spread throughout the genome, eliminating the idea that variation was maintained only in specific chromosomes or genomic regions.

Because C. *erithaca* is migratory, all three admixed individuals are potential offspring of migrants that came into contact with *rufidorsa* in the southern part of their range, where the two forms overlap. The only current source of contact between the admixed individuals and C. *rufidorsa* would be through migration and hybridization. No breeding black-backed birds are known from Singapore (Wells 1999), so the mixture may have occurred farther north. Where this would beis uncertain. No C. *rufidorsa* have been recorded in Vietnam but one individual (probably a vagrant) is known from the Himalayan foothills (Ripley & Beehler 1987). Further complicating the issue,

however, is that during times of low sea level, as recently as 21 000 years ago, southern Vietnam and Sunda landmasses were in direct contact (Sheldon *et al.* 2015), thus providing substantial opportunity for admixture.

In the Philippines, C. *rufidorsa* occurs only on the islands of Mindoro, Tawi Tawi, Palawan and adjacent smaller islands. Some migratory individuals of C. *erithaca* also occasionally reach the Philippines, as highlighted by our single sample of a dark-backed migrant from Cagayan Island, north of Luzon. In the rest of the Philippines, C. *rufidorsa* is replaced by the similar-looking Philippines Dwarf Kingfisher C. *melanurus*. Molecular phylogenetic comparisons have shown that these two species, along with several other species from the Philippines and Wallacea, form a well-supported clade (Moyle *et al.* 2007, Andersen *et al.* 2013). Extensive variation exists among the populations of C. *rufidorsa* in the Philippines, including the formerly recognized, dark subspecies *vargasi* on Mindoro (Ripley & Beehler 1987). Although our comparisons support the conclusion that all resident Philippine populations are members of the C. *rufidorsa* clade, STRUCTURE, PCA and the phylogenetic tree distinguish individuals on Mindoro and Lubang from those in the rest of Sundaland (Figs 2 and 5). Birds on Palawan are of mixed ancestry between Mindoro/Lubang and Borneo populations and share some ancestry with *erithaca* (Fig. 2b). Three of the four birds on Palawan that we sampled are rufous with no blue plumage. However, the one individual with blue colouration in its wings does not have any *erithaca* ancestry. This mixed ancestry suggests a role for Philippines birds in the demographic history of populations in Sundaland, particularly Borneo.

The demography of C. *erithaca* also provides complementary data to help us further decipher the complex history of the current assemblage of avian communities in Sundaland. The patterns observed within this species appear to be older versions of more recent interactions among many Sundaic taxa (e.g. Lim *et al.* 2011, Sheldon *et al.* 2015). In particular, although not necessarily an example of colour polymorphism, variation in colouration and distribution of many Sundaic subspecies and species groups, such as *Copsychus* magpie-robins and shamas (Sheldon *et al.* 2009, Lim *et al.* 2011, 2020), results from periodic isolation, colonization and hybridization. Together, examination of older and newer events yields important insight into the dynamics of species movement and interaction across Sundaland.

In conclusion, our study indicates that C. *rufidorsa* and C. *erithaca* are distinct taxa. We found that the two species separated from one another *c.* 820 000 years ago and have subsequently come into secondary contact. Three individuals with C. *erithaca* plumage recently hybridized with C. *rufidorsa*, but we did not find evidence of recent C. *erithaca* introgression into C. *rufidorsa*. The highly variable plumage in C. *rufidorsa* (including C. *r. motleyi*) appears to result from ancient hybridization or maintenance of ancestral polymorphism. We also found that Philippines birds, especially those from Mindoro and Lubang, are genetically distinguishable from those in the rest of Sundaland but are still part of the *rufidorsa* clade.

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AUTHOR CONTRIBUTIONS

Subir B. Shakya: Formal analysis (lead); methodology (equal); visualization (lead); writing – original draft (lead); writing – review and editing (equal). **Alana Alexander:** Investigation (equal); methodology (equal); writing – review and editing (equal). **Haw Chuan Lim:** Conceptualization (equal); writing – review and editing (equal). **Joseph D. Manthey:** Investigation; methodology; writing – review and editing. **Dewi Prawiradilaga:** Resources (equal). **Kin Onn Chan:** Resources (equal); writing – review and editing (equal). **Frederick H.**

Sheldon: Conceptualization (equal); funding acquisition (equal); supervision (equal); writing – review and editing (equal). **Robert G. Moyle:** Conceptualization (equal); funding acquisition (equal); supervision (equal); writing – review and editing (equal).

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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ETHICAL NOTE

None.

Data Availability Statement

Raw reads from this study are available on GenBank (SRA Bioproject PRJNA916481).

REFERENCES

Andersen, M.J., Oliveros, C.H., Filardi, C.E. & Moyle, R.G. 2013. Phylogeography of the variable Dwarf-Kingfisher *Ceyx lepidus* (Aves: Alcedinidae) inferred from mitochondrial and nuclear DNA sequences. *Auk* **130**: 118–131.

Besnier, F. & Glover, K.A. 2013. ParallelStructure: a R package to distribute parallel runs of the population genetics program STRUCTURE on multi-core computers. *PLoS One* **8**: e70651.

Chasen, F.N. & Kloss, C.B. 1929. Some new birds from North Borneo. *J. Ornithol.* **77**: 106–121.

Clements, J.F., Schulenberg, T.S., Illiff, M.J., Billerman, S.M., Fredericks, T.A., Gerbracht, J.A., Lepage, D., Sullivan, B.L. & Wood, C.L. 2021. *The eBird/Clements checklist of Birds of the World: v 2021*. https://www.birds.cornell.edu/clementschecklist/download/

Dickinson, E.C. & Remsen, J.V. 2013. *The Howard and Moore Complete Checklist of the Birds of the World*, Vol. **1**, 4th edn. Non-passerines. Eastbourne, UK: Aves Press.

Dickinson, E.C., Kennedy, R.S. & Parkes, K.C. 1991. *The Birds of The Philippines*. Tring, Herts, UK: British Ornithologists' Union.

Earl, D.A. & vonHoldt, B.M. 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conserv. Genet. Resour.* **4**: 359–361.

Eaton, J.A., van Balen, S., Brickle, N.W. & Rheindt, F.E. 2016. *Birds of the Indonesian Archipelago: Greater Sundas and Wallacea*. Barcelona, Spain: Lynx Editions.

Evanno, G., Regnaut, S. & Goudet, J. 2005. Detecting the number of clusters of individuals using the software structure: a simulation study. *Mol. Ecol.* **14**: 2611–2620.

Feng, S., Stiller, J., Deng, Y., Armstrong, J., Fang, Q., Reeve, A.H., Xie, D., Chen, G., Guo, C., Faircloth, B.C., Petersen, B., Wang, Z., Zhou, Q., Diekhans, M., Chen, W., Andreu-Sánchez, S., Margaryan, A., Howard, J.T., Pacheco, G., Sinding, M.S., Puetz, L., Cavill, E., Ribeiro, Â.M., Fjeldså, J., Hosner, P.A., Brumfield, R.T., Christidis, L., Bertelsen, M.F., Tietze, D.T., Robertson, B.C., Song, G., Borgia, G., Claramunt, S., Lovette, I.J., Cowen, S.J., Njoroge, P., Dumbacher, J.P., Ryder, O.A., Fuchs, J., Bunce, M., Burt, D.W., Cracraft, J., Meng, G., Hackett, S.J., Ryan, P.G., Jønsson, K.A., Jamieson, I.G., da Fonseca, R.R., Braun, E.L., Houde, P., Mirarab, S., Suh, A., Stervander, M., Hansson, B., Sigeman, H., Ponnikas, S., Frandsen, P.B., van der Zwan, H., Balakrishnan, C.N., Clark, A.G., Fitzpatrick, J.W., Bowman, R., Chen, N., Cloutier, A., Sackton, T.B., Foote, D.J., Shakya, S.B., Sheldon, F.H., Vignal, A., Soares, A.E., Shapiro, B., González-Solís, J., Ferrer-Obiol, J., Rozas, J., Riutort, M., Tigano, A., Friesen, V., Dalén, L., Urrutia, A.O., Székely, T., Liu, Y., Campana, M.G., Corvelob, A., Fleischer, R.C., Rutherford, K.M., Gemmell, N.J., Dussex, N., Mouritsen, H., Thiele, N., Delmore, K., Liedvogel, M., Franke, A., Hoeppner, M.P., Krone, O., Fudickar, A.M., Milá, B., Ketterson, E.D., Fidler, A.E., Friis, G., Parody-Merino, Á.M., Battley, P.F., Cox, M.P., Barroso, N., Prosdocimi, F., Parchman, T.L., Schlinger, B.A., Loiselle, B.A., Blake, J.G., Lim, H.C., Day, L.B., Fuxjager, M.J., Baldwin, M.W., Braun, M.J., Wirthlin, M., Dikow, R.B., Ryder, T.B., Camenisch, G., Keller, L.F., DaCosta, J.M., Hauber, M.E., Louder, M.I., Witt, C.C., McGuire, J.A., Mudge, J., Megna, L.C., Carling, M.D., Wang, B., Taylor, S.A., Del-Rio, G., Aleixo, A., Vasconcelos, A.T., Mello, C.V., Weir, J.T., Haussler, D., Li, Q., Yang, H., Wang, J., Lei, F., Rahbek, C., Gilbert, M.T., Graves, G.R., Jarvis, E.D., Paten, B. & Zhang, G. 2020. Densely sampling genomes across the diversity of birds increases power of comparative genomics analyses. *Nature* **587**: 252–257.

Fry, C.H., Fry, K. & Harris, A. 1992. *Kingfishers, Bee-eaters and Rollers*. London, UK: Christopher Helm.

Galeotti, P., Rubolini, D., Dunn, P.O. & Fasola, M. 2003. Colour polymorphism in birds: causes and functions. *J. Evol. Biol.* **16**: 635–646.

Gazda, M.A., Araújo, P.M., Lopes, R.J., Toomey, M.B., Andrade, P., Afonso, S., Marques, C., Nunes, L., Pereira, P., Trigo, S., Hill, G.E., Corbo, J.C. & Carneiro, M. 2020. A genetic mechanism for sexual dichromatism in birds. *Science* **368**: 1270–1274.

Gill, F.B. & Donsker, D. 2021. *IOC World Bird List (v11.2)*. https://doi.org/10.14344/IOC.ML.10.1

Giska, I., Farelo, L., Pimenta, J., Seixas, F.A., Ferreira, M.S., Marques, J.P., Miranda, I., Letty, J., Jenny, H., Hackländer, K., Magnussen, E. & Melo-Ferreira, J. 2019. Introgression drives repeated evolution of winter coat color polymorphism in hares. *Proc. Natl Acad. Sci. USA* **116**: 24150–24156.

Gompert, Z. & Alex Buerkle, C. 2010. Introgress: a software package for mapping components of isolation in hybrids. *Mol. Ecol. Resour.* **10**: 378–384.

Irwin, D.E., Milá, B., Toews, D.P.L., Brelsford, A., Kenyon, H.L., Porter, A.N., Grossen, C., Delmore, K.E., Alcaide, M. & Irwin, J.H. 2018. A comparison of genomic islands of differentiation across three young avian species pairs. *Mol. Ecol.* **27**: 4839–4855.

Jombart, T. 2008. Adegenet: a R package for the multivariate analysis of genetic markers. *Bioinformatics* **24**: 1403–1405.

Jouganous, J., Long, W., Ragsdale, A.P. & Gravel, S. 2017. Inferring the joint demographic history of multiple populations: beyond the diffusion approximation. *Genetics* **206**: 1549–1567.

Kim, K.W., Jackson, B.C., Zhang, H., Toews, D.P.L., Taylor, S.A., Greig, E.I., Lovette, I.J., Liu, M.M., Davison, A., Griffith, S.C., Zeng, K. & Burke, T. 2019. Black or red: a sex-linked colour polymorphism in a songbird is maintained by balancing selection. *Nat. Commun.* **10**: 1852–1863.

Küpper, C., Stocks, M., Risse, J.E., Remedios, N., Farrell, L.L., Mcrae, B., Morgan, T.C., Karlionova, N., Pinchuk, P., Verkuil, Y.I., Kitaysky, A.S., Wingfield, J.C., Piersma, T., Zeng, K., Slate, J., Blaxter, M., Lank, D.B. & Burke, T. 2015. A supergene determines highly divergent male reproductive morphs in the ruff. *Nature* **48**: 79–83.

Lamichhaney, S., Fan, G., Widemo, F., Gunnarsson, U., Thalmann, D.S., Hoeppner, M.P., Kerje, S., Gustafson, U., Shi, C., Zhang, H., Chen, W., Liang, X., Huang, L., Wang, J., Liang, E., Wu, Q., Lee, S.M.-Y., Xu, X., Höglund, J., Liu, X. & Andersson, L. 2015. Structural genomic changes underlie alternative reproductive strategies in the ruff (*Philomachus pugnax*). *Nat. Genet.* **48**: 84–88.

Lim, H.C., Sheldon, F.H. & Moyle, R.G. 2010. Extensive color polymorphism in the southeast Asian oriental dwarf kingfisher *Ceyx erithaca*: a result of gene flow during population divergence? *J. Avian Biol.* **41**: 305–318.

Lim, H.C., Rahman, M.A., Lim, S.L.H., Moyle, R.G. & Sheldon, F.H. 2011. Revisiting Wallace's haunt: coalescent simulations and comparative niche modeling reveal historical mechanisms that promoted avian population divergence in the Malay Archipelago. *Evolution* **65**: 321–334.

Lim, H.C., Shakya, S.B., Harvey, M.G., Moyle, R.G., Fleischer, R.C., Braun, M.J. & Sheldon, F.H. 2020. Opening the door to greater phylogeographic inference in Southeast Asia: comparative genomic study of five codistributed rainforest bird species using target capture and historical DNA. *Ecol. Evol.* **10**: 3222–3247.

Linck, E. & Battey, C.J. 2019. Minor allele frequency thresholds strongly affect population structure inference with genomic data sets. *Mol. Ecol. Resour.* **19**: 639–647.

Linck, E., Freeman, B.G. & Dumbacher, J.P. 2020. Speciation and gene flow across an elevational gradient in New Guinea kingfishers. *J. Evol. Biol.* **33**: 1643–1652.

Lovell, P.V., Wirthlin, M., Wilhelm, L., Minx, P., Lazar, N.H., Carbone, L., Warren, W.C. & Mello, C.V. 2014. Conserved syntenic clusters of protein coding genes are missing in birds. *Genome Biol.* **15**: 1–27.

Lowry, D.B., Hoban, S., Kelley, J.L., Lotterhos, K.E., Reed, L.K., Antolin, M.F. & Storfer, A. 2017. Breaking RAD: an evaluation of the utility of restriction site-associated DNA sequencing for genome scans of adaptation. *Mol. Ecol. Resour.* **17**: 142–152.

Manthey, J.D., Campillo, L.C., Burns, K.J. & Moyle, R.G. 2016. Comparison of target-capture and restriction-site associated DNA sequencing for phylogenomics: a test in cardinalid tanagers (Aves, Genus: *Piranga*). *Syst. Biol.* **65**: 640–650.

Manuel, C.G. 1939. Studies of Philippine kingfishers, I: Genus *Ceyx*. *Philippine J. Sci.* **69**: 377–387.

van Marle, J.G. & Voous, K.H. 1988. *The birds of Sumatra: an annotated check-list. No. 10*. Pembroke: British Ornithologists' Union.

Miller, M.R., Dunham, J.P., Amores, A., Cresko, W.A. & Johnson, E.A. 2007. Rapid and cost-effective polymorphism identification and genotyping using restriction site associated DNA (RAD) markers. *Genome Res.* **17**: 240–248.

Miller, M.A., Pfeiffer, W. & Schwarz, T. 2010. Creating the CIPRES science gateway for inference of large phylogenetic trees. In *Proceedings of the Gateway Computing Environments Workshop (GCE), 14 Nov. 2010*: 1–8. New Orleans, LA.

Moyle, R.G., Fuchs, J., Pasquet, E. & Marks, B.D. 2007. Feeding behavior, toe count, and the phylogenetic relationships among alcedinine kingfishers (Alcedininae). *J. Avian Biol.* **38**: 317–326.

Nguyen, L.-T.T., Schmidt, H.A., von Haeseler, A. & Minh, B.Q. 2014. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Mol. Biol. Evol.* **32**: 268–274.

Paris, J.R., Stevens, J.R. & Catchen, J.M. 2017. Lost in parameter space: a road map for stacks. *Methods Ecol. Evol.* **8**: 1360–1373.

Poelstra, J.W., Vijay, N., Bossu, C.M., Lantz, H., Ryll, B., Müller, I., Baglione, V., Unneberg, P., Wikelski, M., Grabherr, M.G. & Wolf, J.B.W. 2014. The genomic landscape underlying phenotypic integrity in the face of gene flow in crows. *Science* **344**: 1410–1414.

Portik, D.M., Leaché, A.D., Rivera, D., Barej, M.F., Burger, M., Hirschfeld, M., Rödel, M.O., Blackburn, D.C. & Fujita, M.K. 2017. Evaluating mechanisms of diversification in a Guineo-Congolian tropical forest frog using demographic model selection. *Mol. Ecol.* **26**: 5245–5263.

Pritchard, J.K., Stephens, M. & Donnelly, P. 2000. Inference of population structure using multilocus genotype data. *Genetics* **155**: 945–959.

Pryke, S.R. & Griffith, S.C. 2006. Red dominates black: Agonistic signalling among head morphs in the colour polymorphic Gouldian finch. *Proc. R. Soc. B Biol. Sci.* **273**: 949–957.

Rasmussen, P.C. & Anderton, J.C. 2005. *Birds of South Asia: The Ripley Guide*. Washington, D.C. & Barcelona: Smithsonian Institution & Lynx Edicions.

Rheindt, F.E., Gwee, C.Y., Baveja, P., Ferasyi, T.R., Nurza, A., Rosa, T.S. & Haminuddin 2020. A taxonomic and conservation re-appraisal of all the birds on the Island of Nias. *Raffles Bull. Zool.* **68**: 496–528.

Ripley, S.D. 1942. A revision of the kingfishers *Ceyx erithacus* and *rufidorsus*. *Fortschr. Zool.* **27**: 55–59.

Ripley, S.D. 1944. The bird fauna of the West Sumatra Islands. *Bull. Mus. Comp. Zool.* **97**: 307–430.

Ripley, S.D. & Beehler, B.M. 1987. Species status of the Malaysian three-toed Kingfishers (*Ceyx*) – a re-assessment. *Bull. Br. Ornithol. Club* **107**: 145–151.

Rochette, N.C., Rivera-Colón, A.G. & Catchen, J.M. 2019. STACKS 2: analytical methods for paired-end sequencing improve RADseq-based population genomics. *Mol. Ecol.* **28**: 4737–4754.

Sanchez-Donoso, I., Ravagni, S., Rodríguez-Teijeiro, J.D., Christmas, M.J., Huang, Y., Maldonado-Linares, A., Puigcerver, M., Jiménez-Blasco, I., Andrade, P., Gonçalves, D., Friis, G., Roig, I., Webster, M.T., Leonard, J.A. & Vilà, C. 2022. Massive genome inversion drives coexistence of divergent morphs in common quails. *Curr. Biol.* **32**: 462–469.

Sheldon, F.H., Lohman, D.J., Lim, H.C., Zou, F., Goodman, S.M., Prawiradilaga, D.M., Winker, K., Braile, T.M. & Moyle, R.G. 2009. Phylogeography of the magpie-robin species complex (Aves: Turdidae: *Copsychus*) reveals a Philippine species, an interesting isolating barrier and unusual dispersal patterns in the Indian Ocean and Southeast Asia. *J. Biogeogr.* **36**: 1070–1083.

Sheldon, F.H., Lim, H.C. & Moyle, R.G. 2015. Return to the Malay Archipelago: The biogeography of Sundaic rainforest birds. *J. Ornithol.* **156**: 91–113.

Sims, R.W. 1959. The *Ceyx erithacus* and *rufidorsus* species problem. *J. Linn. Soc. Lond.* **44**: 212–221.

Smythies, B.E. 1999. *The Birds of Borneo, Revised by Geoffrey WH Davison*. Sabah: Natural History Publications (Borneo).

Stoltz, M., Baeumer, B., Bouckaert, R., Fox, C., Hiscott, G. & Bryant, D. 2021. Bayesian inference of species trees using diffusion models. *Syst. Biol.* **70**: 145–161.

Theron, E., Hawkins, K., Bermingham, E., Ricklefs, R.E. & Mundy, N.I. 2001. The molecular basis of an avian plumage polymorphism in the wild: a melanocortin-1-receptor point mutation is perfectly associated with the melanic plumage morph of the bananaquit, *Coereba flaveola*. *Curr. Biol.* **11**: 550–557.

Toews, D.P.L., Taylor, S.A., Vallender, R., Brelsford, A., Butcher, B.G., Messer, P.W. & Lovette, I.J. 2016. Plumage genes and little Else distinguish the genomes of hybridizing warblers. *Curr. Biol.* **26**: 2313–2318.

Tuttle, E.M., Bergland, A.O., Korody, M.L., Brewer, M.S., Newhouse, D.J., Minx, P., Stager, M., Betuel, A., Cheviron, Z.A., Warren, W.C., Gonser, R.A. & Balakrishnan, C.N. 2016. Divergence and functional degradation of a sex chromosome-like supergene. *Curr. Biol.* **26**: 344–350.

Uy, J.A.C., Moyle, R.G., Filardi, C.E. & Cheviron, Z.A. 2009. Difference in plumage color used in species recognition between incipient species is linked to a single amino acid substitution in the melanocortin-1 receptor. *Am. Nat.* **174**: 244–254.

Voous, K.H. 1951. Distributional and evolutionary history of the kingfisher genus *Ceyx* in Malaysia. *Ardea* **39**: 182–196.

Wells, D.R. 1999. *The Birds of the Thai-Malay Peninsula, Volume 1, Non-passerines*. London: Academic Press.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1 Location details of individuals used in this study, along with colour of forehead, neck patch, mantle and coverts of each bird.