ORIGINAL ARTICLE

MOLECULAR ECOLOGY WILEY

Amazonian birds in more dynamic habitats have less population genetic structure and higher gene flow

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Abstract

Understanding the factors that govern variation in genetic structure across species is key to the study of speciation and population genetics. Genetic structure has been linked to several aspects of life history, such as foraging strategy, habitat association, migration distance, and dispersal ability, all of which might influence dispersal and gene flow. Comparative studies of population genetic data from species with differing life histories provide opportunities to tease apart the role of dispersal in shaping gene flow and population genetic structure. Here, we examine population genetic data from sets of bird species specialized on a series of Amazonian habitat types hypothesized to filter for species with dramatically different dispersal abilities: stable upland forest, dynamic floodplain forest, and highly dynamic riverine islands. Using genome-wide markers, we show that habitat type has a significant effect on population genetic structure, with species in upland forest, floodplain forest, and riverine islands exhibiting progressively lower levels of structure. Although morphological traits used as proxies for individual-level dispersal ability did not explain this pattern, population genetic measures of gene flow are elevated in species from more dynamic riverine habitats. Our results suggest that the habitat in which a species occurs drives the degree of population genetic structuring via its impact on long-term fluctuations in levels of gene flow, with species in highly dynamic habitats having particularly elevated gene flow. These differences in genetic variation across taxa specialized in distinct habitats may lead to disparate responses to environmental change or habitat-specific diversification dynamics over evolutionary time scales.

KEYWORDS
Amazonia, birds, dispersal, genetic structure, habitat specialization

Resumo

A compreensão dos fatores que governam a variação da estrutura genética entre as espécies é fundamental para o estudo da especiação e da genética das populações. A estrutura genética tem sido ligada a vários aspectos da história da vida, tais como estratégia de forrageio, associação ao habitat, distância de migração e capacidade de dispersão, os quais poderiam influenciar a dispersão e o fluxo gênico. Estudos comparativos usando espécies que diferem nas suas histórias de vida oferecem uma

oportunidade para desvendar o papel da dispersão no estabelecimento do fluxo gênico e da estrutura genética da população. Aqui examinamos dados genéticos populacionais de diversas espécies de aves com diferentes capacidades de dispersão especializadas em três habitats amazônicos, incluindo florestas de terra-firme, florestas de várzea e ilhas fluviais, cujos ambientes ripários são altamente dinâmicos. Utilizando dados genômicos que incluem milhares de loci, mostramos que o tipo de habitat tem um efeito significativo na estruturação genética das populações; espécies de florestas de terra-firme, florestas de várzea e ilhas fluviais exibem níveis de estruturação progressivamente menores. Embora os traços morfológicos frequentemente usados como indicadores da capacidade de dispersão a nível individual não expliquem este padrão, as medidas genéticas populacionais de fluxo gênico são altas em espécies associadas a habitats ribeirinhos mais dinâmicos. Nossos resultados sugerem que o habitat no qual uma espécie é encontrada determina o grau de estruturação genética da população através de seu impacto nas flutuações de longo prazo do fluxo gênico, com espécies em habitats altamente dinâmicos tendo um fluxo gênico particularmente alto. As diferenças na variação genética dos táxons especializados em diferentes habitats podem resultar em respostas díspares às mesmas mudanças ambientais, ou dinâmicas de diversificação específicas a um determinado habitat ao longo de escalas de tempo evolutivas.

RESUMEN

Comprender los factores que rigen la variación de la estructura genética entre especies es clave para el estudio de la especiación y la genética de poblaciones. La estructura genética se ha relacionado con varios aspectos de la historia vital, como la estrategia de búsqueda de alimento, la asociación de hábitats, la distancia de migración y la capacidad de dispersión, factores todos ellos que podrían influir en la dispersión y el flujo genético. Los estudios comparativos de datos genéticos poblacionales de especies con historias vitales diferentes ofrecen la oportunidad de desentrañar el papel de la dispersión en la conformación del flujo genético y la estructura genética poblacional. En este trabajo examinamos los datos genéticos de poblaciones de especies de aves especializadas en una serie de hábitats amazónicos que, según la hipótesis, filtran especies con capacidades de dispersión radicalmente diferentes: bosques estables de tierras altas, bosques dinámicos de llanuras aluviales e islas fluviales altamente dinámicas. Utilizando marcadores genómicos, demostramos que el tipo de hábitat tiene un efecto significativo en la estructura genética de la población, y que las especies de los bosques de tierras altas, los bosques inundables y las islas fluviales presentan niveles de estructura progresivamente más bajos. Aunque los rasgos morfológicos utilizados como indicadores de la capacidad de dispersión individual no explican este patrón, las medidas genéticas poblacionales del flujo genético son más elevadas en las especies de hábitats fluviales más dinámicos. Nuestros resultados sugieren que el hábitat en el que se encuentra una especie determina el grado de estructuración genética de la población a través de su impacto en las fluctuaciones a largo plazo de los niveles de flujo genético, siendo las especies de hábitats muy dinámicos las que presentan un flujo genético particularmente elevado. Estas diferencias en la variación genética entre taxones especializados en hábitats distintos pueden dar lugar

a respuestas dispares al cambio ambiental o a dinámicas de diversificación específicas del hbitat a lo largo de escalas temporales evolutivas.

1 | INTRODUCTION

The genetic structuring of populations is key to the early stages of the speciation process, when, under an allopatric speciation model, populations subdivide into geographically isolated subpopulations (Hahn, 2018; Tobias et al., 2020). Population structure is typically quantified by examining genetic differences among spatially distributed subpopulations, and is governed by metapopulation dynamics, population connectivity, gene flow, mutation, and drift (Hahn, 2018; Wright, 1931), all of which have the potential to isolate or homogenize subpopulations across landscape barriers (Landis et al., 2022). Population structure is important for maintaining differences between incipient species (Lamichhaney et al., 2015; Poelstra et al., 2014; Toews et al., 2016). It also has consequential effects on speciation dynamics over evolutionary time; high levels of population structure are positively correlated with speciation rates, particularly in tropical birds (Harvey et al., 2017a).

Species show considerable variation in the degree of intraspecific population structure, and this variation has often been attributed to differences in movement patterns or dispersal (Hellberg, 2009; Miller et al., 2021; Salisbury et al., 2012; Seeholzer & Brumfield, 2018). Typically, an inverse relationship is found between dispersal ability and population genetic structure (Bohonak, 1999). This pattern suggests that dispersal ability is a reliable indicator of gene flow. If dispersal abilities, or proxies thereof, are high, then species will tend towards panmixia (Yamaguchi, 2022). In contrast, lower dispersal abilities will result in populations subdividing across geographic barriers in the landscape (Yamaguchi, 2022). This association is most obvious in species occupying heterogeneous landscapes with many barriers to dispersal. However, if dispersal occurs according to a stepping-stone model in which closer populations receive more immigrants than distant populations, then population structure can form within a homogeneous landscape, a process termed isolation-by-distance (IBD; Wright, 1943). Importantly, the dispersal that matters for gene flow is natal dispersal, or the movement of individuals away from their natal site to establish a breeding territory (Clobert et al., 2009). Natal dispersal is notoriously difficult to estimate (but see Paradis et al., 1998), so is typically measured using genetic estimators (Watts et al., 2007), or by means of proxies, which in birds include measures of wing shape, body mass, and diet (Claramunt, 2021; Paradis et al., 1998).

Amazonian birds are an ideal system in which to study the processes that govern population genetic structure and dispersal. The Amazon Basin has high avian species richness (Jenkins et al., 2013; Wallace, 1878), long-term evolutionary persistence (Bicudo et al., 2019; Harvey et al., 2017b), a wide diversity of habitat types (Tuomisto et al., 1995; Tuomisto et al., 2019), and species with a diversity of habitat preferences and specializations (e.g., Álvarez Alonso et al., 2013; Kratter, 1997; Rosenberg, 1990; Terborgh, 1985).

Additionally, experimental dispersal challenges over water are now available for some Neotropical birds (Moore et al., 2008; Naka et al., 2022). Claramunt et al. (2022) found that wing morphology was a significant predictor of overwater flight ability and Weeks et al. (2022) found a significant association between flight ability and natal dispersal distance in a phylogenetically diverse set of 114 bird species, supporting wing measurements from research specimens as a proxy for dispersal.

Previous comparative work on Neotropical birds found differences in genetic structure between canopy and understory bird species across landscape barriers, attributed to more dispersive canopy species tracking ephemeral food resources (Burney & Brumfield, 2009). Similarly, across many Central American bird species, diet—but not habitat—was a better predictor of dispersal ability (Miller et al., 2021), with species that track ephemeral food resources having greater dispersal ability and lower population structure. In contrast, habitat preference was associated with the amount of population genetic structure (Bates et al., 2003; van Els et al., 2021). In Amazonian birds, Harvey et al. (2017) found greater genetic structure in upland forest species than in floodplain forest species, and Barbosa et al. (2022) found differences in structure between species in distinct habitats within Amazonian floodplains. All these traits (canopy-living, noninsectivorous diet, and floodplain habitat specialization) are correlated with reduced species richness in Amazonian birds (Salisbury et al., 2012), illustrating the potential long-term evolutionary consequences of these aspects of life history.

In this study we compare bird species of three different Amazonian habitats that differ in their degree of geographic linearity, extent, temporal stability, and subdivision: (1) riverine islands; (2) seasonally flooded forests; and (3) upland forests. Riverine islands are common to many large river systems worldwide and are dynamic on human timescales, a drastic difference from the stability of upland terra firme forest in the Amazonian shields (Bicudo et al., 2019).

Amazonian riverine islands are continually reshaped by the dramatic forces of river erosion and sediment deposition (Peixoto et al., 2009), such that they form, grow, and disappear on timescales of tens to hundreds of years, or less (Figure 1; Kalliola et al., 1991, Peixoto et al., 2009). However, over longer timescales, these islands are cyclically destroyed and rebuilt through the effects of sea level change, precipitation, and erosion (Passos et al., 2020; Sawakuchi et al., 2022; Thom et al., 2020). In the Amazon basin these islands are formed primarily in "white water" river systems via sediment deposition in the river channel and subsequent plant colonization (Junk et al., 2011; Junk et al., 2012; Kalliola et al., 1991; Parolin et al., 2002; see also Appendix S1), where they host a specialized and globally unique avifauna (Remsen & Parker, 1983; Rosenberg, 1990) that is less diverse than that of upland forests (Diniz, 2021) but occurs at high densities (Rosenberg, 1990). The dynamism of riverine island habitat creates extreme pressure on riverine island species to move
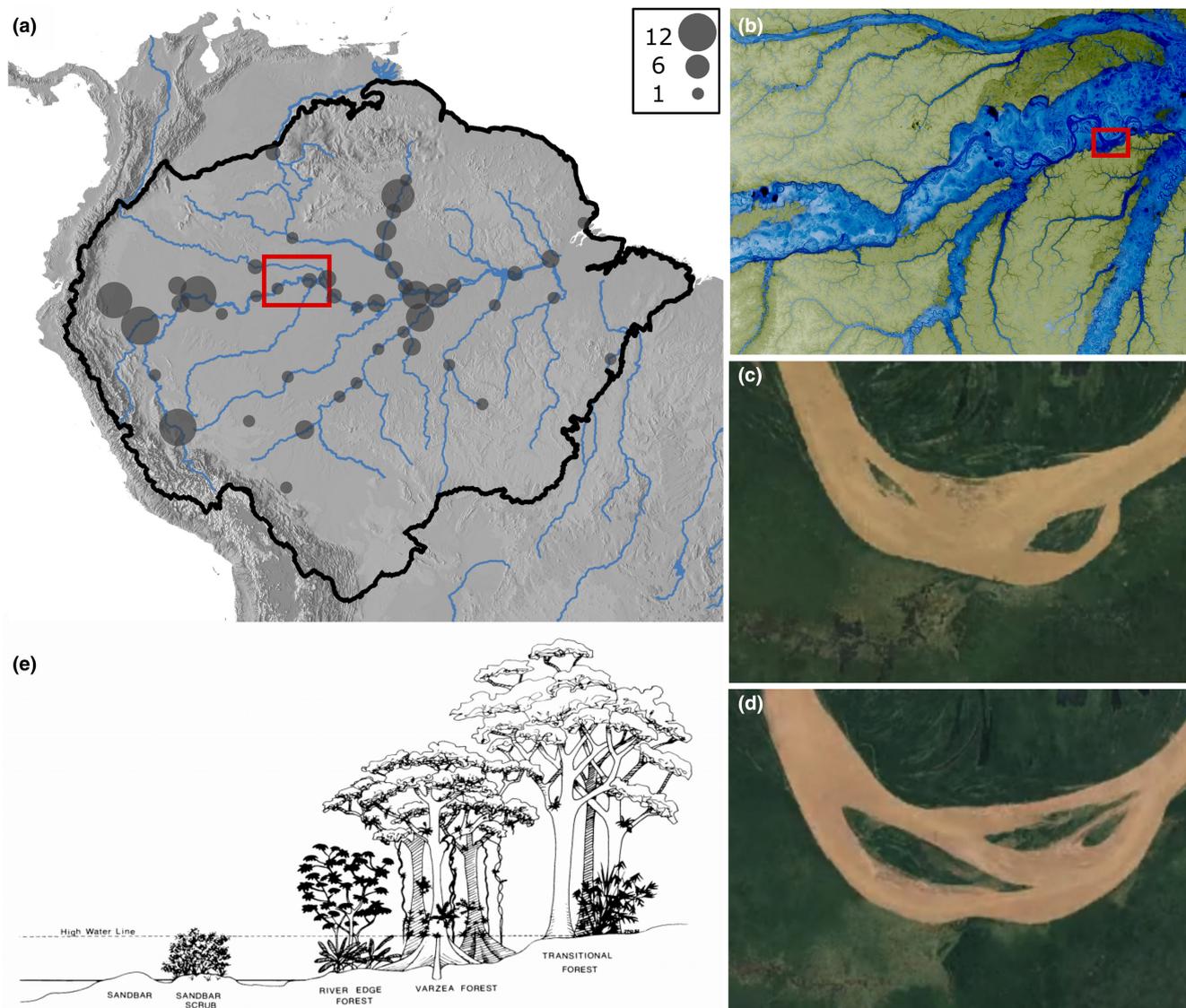
FIGURE 1 The extent and distribution of the three Amazonian habitats studied here. (a) Terrain map of northern South America, showing the extent of the Amazonian Biome (black border; https://github.com/gamamo/AmazonBasinLimits) and geographic sampling of riverine island birds (circles scaled to number of samples per locality; *n* = 145 samples). Blue lines denote major Amazonian rivers, which track the approximate distribution of floodplain forest and riverine islands. Locations of floodplain and upland forest bird samples are available in Harvey et al. (2017) and coordinates for each riverine island sample are available in Table S1. Red inset from (a) shown in (b): False colour imagery showing the distribution of upland forest (olive) and floodplain forest (blue). Data from Hess et al. (2015b) and the NASA/METI/ AIST/Japan Spacesystems and U.S./Japan ASTER Science Team (2018). Red inset from (b) shown in (c) and (d): The rapid movement of two riverine islands over a 30-year timelapse from 1987 (c) to 2017 (d). Data from Gorelik (2013). (e) The river-associated habitats of the Amazon Basin ordered left-to-right by distance from the river channel. Riverine islands are comprised of sandbar, sandbar scrub, and river edge forest. Floodplain forest is found on the river margins and is seasonally inundated (blue regions in (b)). Upland forest occurs above the high-water mark and would be off the figure to the right. Illustration by John P. O'Neill, from Remsen and Parker (1983), reproduced with permission from Biotropica.

to and from islands along river drainages at potentially interannual and longer timescales. However, the few genetic studies thus far provide mixed support for high gene flow and found some instances of high differentiation in riverine island birds (Choueri et al., 2017; Luna et al., 2021; Thom et al., 2020).

Characteristics that make riverine island habitat so dynamic—river erosion, flooding cycles, habitat linearity, and habitat succession—are found to a lesser degree in seasonally flooded forests on river margins (Figure 1, Junk et al., 2012). The flooded forest habitat is still strongly affected by riverine processes but is less dynamic and more extensive than riverine islands. Still, it is restricted to the river flood-plain and is subdivided by the river itself. Multiple studies found that some Amazonian floodplain forest species had relatively high levels of genetic structure across their distribution but not between oppo-site banks of the same river (Barbosa et al., 2022; Luna et al., 2022; Silva et al., 2021). Corroborating the influence of habitat association

of population history, Sawakuchi et al. (2022) found distinct dates of demographic expansion for birds associated with riverine islands and floodplain forests in river margins, and that dates of demographic expansion for river island specialists agree with dates of sediment accumulation on riverine islands throughout Amazonia.

Even more stable, less linear, and more geographically subdivided by rivers, are upland forests found on higher ground away from the floodplains (Bicudo et al., 2019; Pupim et al., 2019). Large Amazonian rivers have long been recognized as strong dispersal barriers for vertebrates of upland forests (Capparella, 1991; Moraes et al., 2016; Wallace, 1852), and are an especially strong barrier for bird species specialized in understory habitats (Maximiano et al., 2020; Musher et al., 2022; Naka & Brumfield, 2018; Silva et al., 2019). These three habitat types, therefore, form a gradient in geographic linearity, temporal stability, and habitat subdivision (Figure 1).

Here, we use a comparative framework to investigate the effect of habitat association on intraspecific genetic diversity, population structure, gene flow, and dispersal ability of Amazonian birds by examining genetic and morphological data from 636 samples across 66 species specialized on three distinct Amazonian habitat types. We hypothesize that more dynamic habitats select for species capable of more frequent and longer-distance dispersal, thereby increasing range-wide gene flow and inhibiting the formation of population genetic structure. Specifically, we test the hypotheses that (1) birds specialized on dynamic riverine islands exhibit less genetic structure than floodplain and especially upland forest birds, and (2) gene flow and proxies for dispersal increase in more dynamic habitats, being highest in riverine island species and lowest in upland forest species.

2 | MATERIALS AND METHODS

2.1 | Sampling design

In Amazonia, we sampled 20 upland forest species and 20 floodplain species or species complexes (Table 1) by using genetic data collected by Harvey et al. (2017). To these data we added new genetic data from 18 Amazonian riverine island species (Table 1; D. Lane personal communication; Remsen & Parker, 1983; Rosenberg, 1990; B. Whitney personal communication). Three of the riverine island species (*Conirostrum bicolor*, *Stigmatura napensis*, and *Thamnophilus nigrocinereus*) are found in nonriverine habitats outside the Amazon basin but are restricted to riverine islands within the Amazon (del Hoyo et al., 2014; Remsen & Parker, 1983); our samples are restricted to Amazonian localities. In selecting sample localities, we maximized the number of river systems sampled and the geographic distance between samples, and we only included species for which tissue samples from at least four localities were available. Previous phylogenetic data have shown that *Thamnophilus nigrocinereus* and *T. cryptoleucus* form a species complex (Brumfield & Edwards, 2007), with gene flow at a broad hybrid zone (Choueri et al., 2017; Thom et al., 2020), so we here consider these a species complex for analyses. The data from Harvey et al. (2017) likewise include multiple

species complexes, and for simplicity we refer to all species complexes as "species" or "lineages" in the analyses (Table 1). When available, we sampled an outgroup taxon for tree visualization purposes.

2.2 | Ultraconserved element data collection

We downloaded ultraconserved element (UCE) read data for all upland forest and floodplain species (*n* = 458), and for 45 riverine island samples, from the GenBank Sequence Read Archive (BioProject Accessions PRJNA389814, PRJNA655842, and PRJNA595086; Harvey et al., 2017, Harvey et al., 2020; Thom et al., 2020). We extracted total DNA for 125 new riverine island samples using c. 25 mg of pectoral muscle and we quantified DNA concentration using a Qubit 2.0 Fluorometer (Life Technologies). Samples were standardized to 10 ng/μL. For 101 of the 125 samples, we prepared and sequenced genomic libraries. We sheared DNA into approximately 600 bp fragments using an Episonic 1100 bioprocessor. We built genomic libraries using a KAPA Biosystems Hyper Prep kit and enriched them for UCEs (Faircloth et al., 2012; Smith, Harvey, et al., 2014) using a set of 5742 probes that target 5060 loci in vertebrates, following the protocol of Faircloth et al. (2012). We pooled enriched samples at equimolar ratios and sequenced them on three lanes of HiSeq 2500 or 3000 sequencers at the Oklahoma Medical Research Foundation Clinical Genomics Center (OMRF; Oklahoma City, Oklahoma, USA). For the remaining 24 samples we shipped DNA extracts to Rapid Genomics for UCE library preparation and sequencing using a MiSeq sequencer. This latter sample set was enriched using a custom probe set consisting of 2321 vertebrate UCEs and 96 exons. Rapid Genomics and OMRF demultiplexed samples using custom scripts. All sequencing lanes contained DNA libraries used in other projects. We processed all samples, regardless of data source, identically in the remainder of analyses.

We trimmed reads of adapter contamination and low-quality bases using illumiprocessor (Faircloth, 2013) and trimmomatic (Bolger et al., 2014). Because we obtained reads from a variety of sources with sequencing done with different lane sizes, we subsampled cleaned reads to 2.0 million reads per individual to normalize read depth of assemblies across samples to mitigate effects of sequencing source and sample quality. We assembled contigs in SPAdes (Nurk et al., 2013). Because samples were sequenced with two different probe sets, we matched contigs to the "Tetrapods-UCE-2.5Kv1" (uce-2.5 k-probes.fasta) probe set, which consists of 2560 baits targeting 2386 UCE loci that are a subset of the other probe sets.

To confirm identification of samples we used the Phyluce 1.6.7 (Faircloth, 2015) tool match_contigs_to_barcodes to match contigs from each sample to a mitochondrial COI barcode sequence of each species obtained from GenBank (Table S2) and to map those contigs against the Barcode of Life Database (BOLD; Ratnasingham & Hebert, 2007). We used the Phyluce tool get_trinity_coverage to calculate per-contig coverage and extracted those contigs matching UCE probes and mitochondrial loci. We removed potentially

TABLE 1 Species and genetic sample sizes used in this study. Species are grouped by habitat then by taxonomy.

Name	Habitat	Sample size	Theta/bp	Heterozygosity
Olive-spotted Hummingbird (*Talaphorus* [*Leucippus*] *chlorocercus*)	Riverine island	4	0.0011	0.35
Zimmer's Woodcreeper (*Dendroplex kienerii*)	Riverine island*	7	0.0014	0.27
Lesser Hornero (*Furnarius minor*)	Riverine island	7	0.0008	0.28
Parker's Spinetail (*Cranioleuca vulpecula*)	Riverine island	7	0.0006	0.27
White-bellied Spinetail (*Mazaria propinqua*)	Riverine island	8	0.0038	0.21
Leaden Antwren (*Myrmotherula assimilis*)	Riverine island*	7	0.0017	0.23
Black-and-white Antbird (*Myrmochanes hemileucus*)	Riverine island	7	0.0033	0.25
Klages's Antwren (*Myrmotherula klagesi*)	Riverine island*	7	0.0019	0.25
Castelnau's/Blackish-grey antshrikes (*Thamnophilus cryptoleucus/ nigrocinereus*)	Riverine island*	18	0.0027	0.09
Ash-breasted Antbird (*Myrmoborus lugubris*)	Riverine island*	8	0.0016	0.19
Riverside Tyrant (*Knipolegus orenocensis*)	Riverine island	12	0.0021	0.16
Drab Water Tyrant (*Ochthornis littoralis*)	Riverine island	12	0.0007	0.20
Lesser Wagtail-Tyrant (*Stigmatura napensis*)	Riverine island	10	0.0011	0.25
Brownish Elaenia (*Elaenia pelzelni*)†	Riverine island*	2	0.0023	0.55
River Tyrannulet (*Serpophaga hypoleuca*)	Riverine island	10	0.0009	0.22
Bicolored Conebill (*Conirostrum bicolor*)	Riverine island*	15	0.0015	0.20
Pearly-breasted Conebill (*Conirostrum margaritae*)	Riverine island*	4	0.0015	0.39
Undulated Tinamou (*Crypturellus undulatus*)	Floodplain forest	11	0.0021	0.15
Squirrel Cuckoo (*Piaya cayana*)	Floodplain forest	11	0.0038	0.13
White-beared Hermit (*Phaethornis hispidus*)	Floodplain forest	11	0.0023	0.16
Tropical Screech-Owl (*Megascops choliba*)	Floodplain forest	11	0.0010	0.14
Ferruginous Pygmy-Owl (*Glaucidium brasilianum*)	Floodplain forest	11	0.0014	0.17
Black-fronted Nunbird (*Monasa nigrifrons*)	Floodplain forest	11	0.0020	0.16
Cream-coloured Woodpecker (*Celeus flavus*)	Floodplain forest	11	0.0013	0.22
Crimson-crested Woodpecker (*Campephilus melanoleucos*)	Floodplain forest	11	0.0020	0.14
Collared Trogon (*Trogon collaris*)	Floodplain forest	11	0.0027	0.11
White-browed Antbird (*Myrmoborus leucophrys*)	Floodplain forest	11	0.0029	0.12
Plumbeous Antbird (*Myrmelastes hyperythrus*)	Floodplain forest	11	0.0014	0.17
Spot-backed Antbird (*Hylophylax punctulatus*)	Floodplain forest	11	0.0026	0.16
Black-faced Antthrush (*Formicarius analis*)	Floodplain forest	11	0.0032	0.10
Striped Woodcreeper (*Xiphorhynchus obsoletus*)	Floodplain forest	11	0.0017	0.17
Plain-crowned Spinetail (*Synallaxis gujanensis*)	Floodplain forest	11	0.0025	0.12
White-tailed/Band-tailed/Crimson-hooded manakins (*Pipra filicauda/fasciicauda/ aureola*)	Floodplain forest	11	0.0033	0.13

TABLE 1 (Continued)

Name	Habitat	Sample size	Theta/bp	Heterozygosity
Varzea Schiffornis (*Schiffornis major*)	Floodplain forest	11	0.0054	0.13
Buff-breasted Wren (*Cantorchilus leucotis*)	Floodplain forest	11	0.0032	0.14
White-shouldered Tanager (*Loriotus [Tachyphonus] luctuosus*)	Floodplain forest	11	0.0032	0.15
Blue-grey Saltator (*Saltator coerulescens*)	Floodplain forest	11	0.0040	0.12
Variegated Tinamou (*Crypturellus variegatus*)	Upland forest	11	0.0036	0.14
Black-bellied Cuckoo (*Piaya melanogaster*)	Upland forest	11	0.0060	0.11
Straight-billed/Needle-billed hermits (*Phaethornis bourcieri/philippi*)	Upland forest	11	0.0029	0.10
Tawny-bellied Screech-Owl (*Megascops watsonii*)	Upland forest	11	0.0021	0.14
Amazonian Pygmy-Owl (*Glaucidium hardyi*)	Upland forest	11	0.0009	0.15
White-fronted/Black nunbirds (*Monasa morphoeus/atra*)	Upland forest	11	0.0020	0.12
Scaly-breasted/Waved woodpeckers (*Celeus grammicus/undatus*)	Upland forest	11	0.0025	0.17
Red-necked Woodpecker (*Campephilus rubricollis*)	Upland forest	11	0.0027	0.14
Black-throated Trogon (*Trogon rufus*)	Upland forest	11	0.0036	0.09
Black-faced Antbird (*Myrmoborus myotherinus*)	Upland forest	11	0.0032	0.10
Sooty Antbird (*Hafferia fortis*)	Upland forest	11	0.0020	0.12
Dot-backed Antbird (*Hylophylax naevius*)	Upland forest	11	0.0041	0.09
Rufous-capped Antthrush (*Formicarius colma*)	Upland forest	11	0.0023	0.10
Elegant/Spix's woodcreepers (*Xiphorhynchus elegans/spixii*)	Upland forest	11	0.0026	0.12
Ruddy Spinetail (*Synallaxis rutilans*)	Upland forest	11	0.0021	0.11
Golden-headed/Red-headed/Round-tailed manakins aaaaaa(*Ceratopipra erythrocephala/rubrocapilla/chloromeros*)	Upland forest	11	0.0031	0.11
Brown-winged Schiffornis (*Schiffornis turdina*)	Upland forest	11	0.0037	0.10
Coraya/Whiskered wrens (*Pheugopedius coraya/genibarbis*)	Upland forest	11	0.0050	0.10
Flame-crested Tanager (*Loriotus [Tachyphonus] cristatus*)	Upland forest	11	0.0030	0.18
Slate-coloured Grosbeak (*Saltator grossus*)	Upland forest	11	0.0027	0.13
Total		585		

Note: Asterisk (*) indicates riverine island species primarily occurring in the later stages of ecological succession within islands. Cross (†) indicates species excluded from analyses due to low sample size. Taxonomy follows Remsen et al. (2022). All genetic parameters are shown in Table S4.

misidentified or contaminated samples from the data set where mitochondrial contigs matched the incorrect species and where mitochondrial contigs matched multiple species with high coverage (greater than two standard deviations above the mean mitochondrial coverage: Figure S35). Some mitochondrial contigs that matched the incorrect species were sequenced at a low coverage (less than two standard deviations below the mean mitochondrial coverage), indicating either inaccurate matches to mitochondrial barcodes due

to poor assembly or low levels of contamination. Within each sequencing lane, we minimized the possibility of contamination of UCE contigs by using the maximum coverage value of these low coverage mitochondrial contigs as a filter and removed UCE contigs with mean coverage below that threshold.

To phase UCE loci, we selected as a reference the individual for each species that contained the greatest number of UCE loci after filtering, and we reassembled contigs for these individuals using

itero (https://github.com/faircloth-lab/itero) to further increase the number of loci recovered. We removed low-coverage UCE contigs from these reference individuals using the same threshold as with the SPAdes assemblies (5.5x). After aligning and edge-trimming the itero assemblies across species, we phased UCE loci within each species using the seqcap_pop pipeline (https://github.com/mgharvey/seqcap_pop; Faircloth, 2015; Harvey et al., 2016; Li et al., 2009; Li & Durbin, 2009; McKenna et al., 2010) to obtain a SNP data set. We then filtered this data set in VCFtools (Danecek et al., 2011) to remove SNPs with quality scores less than 30 and read depth less than 9.5, as well as those with >50% missing data. We restricted the SNPs to those with biallelic loci, and we removed indels. Because this data set contains multiple SNPs per locus, we refer to this as the "linked SNP data set." We then sampled at random one SNP per UCE locus to obtain the final SNP data set for each species, which we refer to as the "unlinked SNP data set."

To obtain phased alignments we used Phyluce 1.6.7 (Faircloth, 2015) to phase UCE loci following Andermann et al. (2019), phasing data within each species by mapping reads against the reference individual using the Phyluce tools snp_bwa_align and snp_phase_uces (Li et al., 2009; Li & Durbin, 2009). To produce a final 75% complete data matrix, we used MAFFT 7.130b (Katoh & Standley, 2013) in Phyluce align_seqcap_align to align and edge-trim the contigs, treating the two alleles as separate individuals and allowing ambiguous sites in alignments.

2.3 | Mitochondrial data collection

We used off-target reads from the UCE sequencing to assemble draft mitochondrial genomes in MITObim 1.9 (Hahn et al., 2013), which is a Perl wrapper for MIRA 4.0.2 (Chevreux et al., 1999), using as a reference the complete mitochondrial genome of the most closely related species available on GenBank (Table S2) and the --quick option. We annotated the assembled mitochondrial genomes using the MITOchondrial genome annotation Server (MITOS) 2 (Bernt et al., 2013) and aligned the 13 protein-coding genes in MAFFT 1.3.7 (Katoh et al., 2002) as implemented in Geneious 10.2.3 (https://www.geneious.com) to create a partitioned mitochondrial alignment for each species.

2.4 | Population genetics

We used Dendropy 4.2.0 (Sukumaran & Holder, 2010) to estimate nucleotide diversity, mutation-scaled effective population size (Watterson's Theta; θ), the number of segregating sites, the average number of pairwise differences between individuals (π), and Tajima's D for each species. We calculated the degree of sequence divergence, D_{xy}, with the R package PopGenome (Pfeifer et al., 2014) and the number of SNPs per base pair with VCFtools (Danecek et al., 2011). We calculated observed per-individual heterozygosity both as the species average and as the average for each genetic

cluster within a species (as defined by DAPC; see below) in VCFtools (Danecek et al., 2011) and adegenet (Jombart & Ahmed, 2011), from the linked SNP data set.

We calculated F_{ST} with the R package PopGenome (Pfeifer et al., 2014) using the phased partitioned alignments, treating each individual (each consisting of the two phased alleles) as a population, to obtain an overall measure for each species. We estimated both the nucleotide-based F_{ST} and Nei's estimator for multiple alleles (Nei's G_{ST}; Nei, 1973). The relationship between genetic distance and geographic distance (IBD) provides a more explicit measure of gene flow across a population by accounting for spatial sampling patterns, with lower slope values of a regression analysis indicating a greater degree of gene flow. We estimated IBD with the $\hat{e}$ estimator of genetic dispersal rate (Watts et al., 2007) using Euclidean distances between sampling locations. This estimator is less biased than the $\hat{a}$ estimator of Rousset (2000), especially under scenarios of high dispersal, as would be expected in riverine island birds. We conducted this analysis on the linked SNP data set in genepop (Rousset, 2008).

To quantify patterns of population structure within species and to assign individuals to populations, we used three methods that each relies on a different analytical clustering framework: STRUCTURE (Pritchard et al., 2000), DAPC (Jombart et al., 2010), and BAPS (Corander et al., 2003). For the latter two methods we analysed the unlinked SNP data set to minimize biases resulting from linkage of SNPs. For STRUCTURE analyses we analysed the linked SNP data set and implemented the linked model, providing the distance in base pairs between SNPs within each locus. We selected the best K value based on the method of Evanno et al. (2005). If an individual was assigned to multiple populations (i.e., admixed), we assigned that individual to the population with the greatest percentage assignment, for downstream analyses. We conducted a DAPC analysis in the R package adegenet (Jombart & Ahmed, 2011). Following the recommendations of Jombart et al. (2010) we selected the optimal number of clusters based on the lowest Bayesian Information Criterion (BIC) score. We conducted a PCA for visualization purposes, with samples coded by DAPC group assignments (Figures S5–S36). BAPS uses a Bayesian model to estimate the genetic population structure in multiallelic data sets. It has the advantage of speed even for many thousands of molecular markers. We conducted a genetic mixture analysis on the unlinked SNP data set, conducting 80 runs on all values of K from 1 through 10, and selected the optimal K value based on the log of the marginal likelihood of the resulting partitions. We report the average number of population clusters per species across each of the three clustering methods to minimize biases from each of the clustering methods. For downstream analyses that require samples assigned to population genetic clusters, we use the sample assignments from DAPC.

2.5 | Phylogenetics

For analyses that control for phylogenetic covariation (see below), we estimated a phylogenetic tree of all study species by selecting

a single individual per species that had the greatest number of assembled loci after filtering and aligning. This tree was rooted on the branch leading to two tinamou species. We visualized the tree in FigTree 1.4.4 (http://tree.bio.ed.ac.uk/software/figtree; Figure S38).

For each species we estimated an unrooted gene tree for each UCE locus in RAxML 8.2.10 (Stamatakis, 2014) using a GTR+γ finite-sites model of sequence evolution. We calculated the average root-to-tip distance for each resulting gene tree in R (R Core Team, 2013), using the nodeHeights function in phytools (Revell, 2012). We calculated the average gene tree depth across all UCE gene trees. For the mitochondrial data, we estimated the optimal partitioning scheme for the mitochondrial genome alignment using PartitionFinder 2.1.1 (Lanfear et al., 2016), providing an initial scheme of all genes partitioned separately by codon position, and analysing only the models available in RAxML using the greedy algorithm. We used the resulting partitions and the estimated overall best model of rate variation to estimate a phylogenetic tree in RAxML 8.2.10 (Stamatakis, 2014) using the autoMRE bootstrapping criterion and 20 runs on distinct starting trees. For the mitochondrial gene tree, we calculated tree depth in R (R Core Team, 2013), using the nodeHeights function in phytools (Revell, 2012).

Two studies (Aleixo, 2006; Brumfield & Edwards, 2007) reported that riverine island bird species tend to be found on long terminal branches that are sister to species-rich clades and they hypothesized that the pattern could be a long-term evolutionary effect of low population structure. Visual assessment of the phylogenetic position of riverine island species in a recent species-level phylogeny of suboscine birds (Harvey et al., 2020) seems to support this. To test the hypothesis that riverine island species occur disproportionately on long terminal branches, we extracted the stem age of each species as the divergence time from the sister clade or species using the R package phytools (Revell, 2012). We restricted this analysis to the 36 suboscine species in our data set and utilized the phylogeny of Harvey et al. (2020). To address the expected correlate, that long branches would be sister to species-rich clades, we tallied the number of species in the sister clade from species-level phylogenetic studies, which were available for all species except the two *Monasa* and the two *Crypturellus* (Barker et al., 2015; Chaves et al., 2013; DaCosta & Klicka, 2008; Harvey et al., 2020; Mann et al., 2006; McGuire et al., 2014; Shakya et al., 2017; Sorenson & Payne, 2005; Wink et al., 2004).

2.6 | Morphology

We used the hand-wing index (HWI; Kipp, 1959) as a proxy for dispersal ability, as it correlated with natal dispersal distance (Claramunt, 2021; Weeks et al., 2022). We calculated the HWI as:

$$HWI = (K / W) * 100$$

where K is the Kipp's distance, which is the distance from the tip of the outermost secondary feather (S1) to the wingtip on the closed wing,

and W is the wing chord. The index approximates the aspect ratio of the wing and can be obtained from museum specimens. Lower values indicate shorter, rounder wings less effective for flight, whereas larger values indicate the longer, more pointed wing that is more effective for flight. We obtained measures of the HWI from all study species from the AVONET database (Tobias et al., 2022), with an average of 17.3 (SE: 2.9) individuals per lineage, and we used the average across all individuals from each lineage. Because diet may be correlated with dispersal ability (Dawideit et al., 2009; Miller et al., 2021), we also obtained from the AVONET database (Tobias et al., 2022) four measures that describe or may be associated with diet: trophic niche (a broad categorization of diet), mass, bill depth, and bill length. Trophic niche was coded by increasing degree of carnivory as a pseudo-ordinal variable in the following order: frugivore, nectarivore, omnivore, and invertivore. Lastly, we obtained from the AVONET database (Tobias et al., 2022) a measure of range size, which may be correlated with population size. We note that these measures of range size are based on coarse range maps from BirdLife International and probably overestimate range size, especially for linearly distributed species.

2.7 | Comparative analyses

We first assigned variables to broad categories: data attributes (e.g., contig length), genetic structure, gene flow, genetic diversity/population size, species traits, and speciation dynamics. For each of these categories, we used variance inflation factors (VIF) on linear models to calculate multicollinearity of variables. We also tested a linear model including all genetic variables (i.e., excluding species traits and data attributes). We removed variables if VIF values were greater than 10.

We used the R package phytools (Revell, 2012) to test for mean differences in each trait and genetic parameter across habitat categories with one-way ANOVAs, accounting for phylogenetic covariation. We rescaled all variables to Z-scores with the R function *scale* and provided as an input these variables and the phylogenetic tree estimated from one individual of each species. We tested each parameter separately and compared pairwise differences between the three habitats with Holm's post hoc *t*-tests (Holm, 1979).

We tested for correlations between increasing habitat stability and increasing genetic structure with phylogenetic generalized least squares (PGLS) regressions in the R package caper (Orme et al., 2013). We categorized habitats according to our hypothesized pattern of increasing spatial structuring and stability by using dummy variables in the model, with riverine islands = 0, floodplain forest = 1, and upland forest = 2. As with the phylogenetic ANOVAs, we rescaled all variables to Z-scores and ran the analysis for all traits and genetic parameters. To assess the relative importance of each variable, we used AIC weights with the akaike. weights function in the R package qpcR (Spiess, 2018). We calculated the AIC weights for the genetic metrics for which we had full species-level sampling and excluded the three descriptive statistics of total base pairs, number of loci, and average contig length.

We calculated AIC weights separately for each of three categories of measurements: genetics, species traits, and speciation dynamics. In addition to PGLS and ANOVA tests for the sister clade richness parameter, we conducted a sign test within each habitat to ask whether the number of species in the sister clade was greater than expected by chance. This is a rough way of measuring whether the sister clade has diversified more than the focal clade, which is comprised of a single species. The test does not account for potentially confounding factors such as differing divergence times and speciation rates that certainly vary among lineages.

A box plot of HWI (Figure 2b) showed outliers with high HWI from all habitats, representing species such as hummingbirds and trogons that have wing shapes distinctly different from most birds in this study (Passeriformes). We therefore reran the HWI ANOVAs separately for species with HWI >30 and <30 (removing outliers), and again separately for passerine and nonpasserine species.

Due to interspecific variation in the degree of specialization on certain habitats (Remsen & Parker, 1983), we reran ANOVA and PGLS analyses using different habitat assignments for some species that occur in multiple habitats to varying degrees. First, we reassigned *Dendroplex kienerii* and *Ochthornis littoralis* to the flooded forest habitat category, as both species can be found in that habitat (Remsen & Parker, 1983; A. Aleixo, O. Johnson personal observation). We also ran analyses with these two species removed. Next, we divided the riverine island species into early- and late-successional species

(Table 1) based on their habitat preferences within islands (Remsen & Parker, 1983; Rosenberg, 1990; C. Ribas, L. N. Naka personal observation). We considered the early-successional riverine island habitat to be the most dynamic, as it is most strongly affected by river erosion and sediment deposition.

3 | RESULTS

3.1 | Sequencing results and data attributes

The final data set contained 587 samples of 66 species (Table 1, Table S1, Figure 1a), plus an additional 41 samples of closely related species used as outgroups (total = 627). This data set, including outgroup samples, contained 757.5 million reads before subsampling and trimming. We removed 44 samples due to failed sequencing, misidentification, potential contamination, or an excess of single copy alleles (Table S3), leaving 546 ingroup (Table 1) and 38 outgroup samples used in the analyses. The phased UCE alignments contained an average of 2074 loci per species (range 1852–2204), with an average locus length of 594 base pairs (bp; range 111–1205 bp), and a total of 1.37 billion bp of DNA. We obtained complete or nearly-complete mitochondrial genomes for 550 samples (97% of samples), and an average mitochondrial genome length of 17,174 bp (range 16,109–19,520 bp). The alignments of 13 mitochondrial protein-coding genes had an average
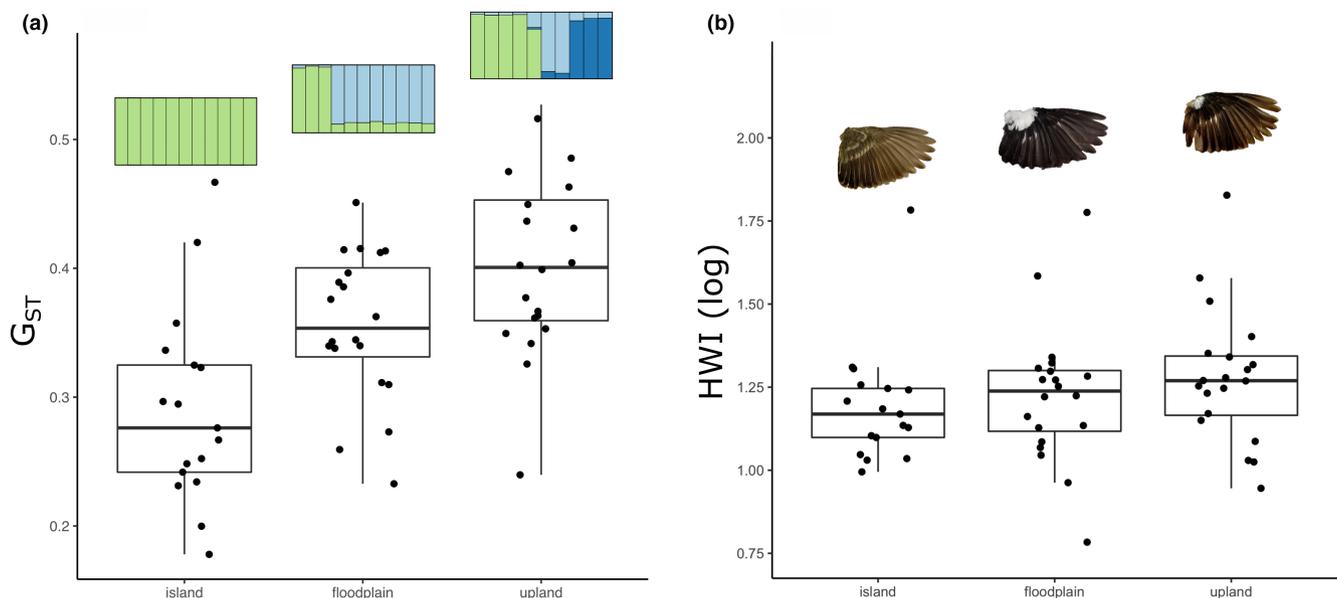

FIGURE 2 (a) Significant effect of habitat preference on Nei's G_{ST} based on PGLS regression ($p < .001$, $t = 5.51$, $e = 0.52$). The image above each boxplot is a representative STRUCTURE plot from a lineage in that habitat, highlighting the increasing amount of genetic structure. STRUCTURE plots are for (L to R) *Ochthornis littoralis*, *Synallaxis gujanensis*, and *Phaethornis bourcieri/philippii*. (b) PGLS regression illustrates that habitat type had no significant effect on the hand-wing index (HWI; $p < .07$, $t = 1.83$, $e = 0.6$), and in fact the trend was opposite of our expectation. HWI was not significantly different across habitats in a phylogenetic one-way ANOVA, and no pairwise habitat-level comparisons were significantly different in Holm's post hoc *t*-tests (Table 2). Spread wing photographs above (b) are representative spread-wings from species in each habitat, showing similar wing shapes in different habitats. Spread wing photographs are (L to R) *Elaenia pelzelni* (LSUMZ 228944), *Loriotus luctuosus* (LSUMZ 195789), and *Loriotus cristatus* (LSUMZ 195782). Spread wing photographs courtesy of J. V. Remsen Jr. and D. Vander Pluym.

length of 11,414 bp (range 11,124–11,663 bp). The linked SNP data set contained an average of 6827 SNPs per species (range 1430–14,067) and the unlinked SNP data set contained an average of 1714 SNPs per species (range 859–2074). The number of loci recovered did not differ among habitats, but the contig length and total number of base pairs recovered was higher in riverine island samples (Table 2).

3.2 | Multicollinearity

VIFs greater than 10 indicated that six genetic variables (pairwise differences, SNPs per bp, SNPs per locus, total SNPs, nucleotide diversity, and segregating sites) contributed significantly to multicollinearity among variables. These six were removed from analyses. In a linear model of all genetic variables, only D_{xy} and F_{ST} had VIFs greater than 5 (8.1 and 7.7, respectively) indicating moderate collinearity. Because these two variables are of particular interest in this study we retained them in analyses.

3.3 | Genetic diversity

Because longer contigs are probably a result of higher quality read data for the newly sequenced riverine island samples, which may improve accuracy of population genetics estimates, we analysed the per-individual heterozygosity from VCFtools (Danecek et al., 2011) and found that heterozygosity estimates were significantly higher for the newly sequenced riverine island samples than those from other data sources (Welch two sample t-test: $t = 10.12$, df = 114.4, $p \ll .001$), suggesting that genetic diversity in these samples may be inflated by recovery of more alleles due to greater sequencing depth. However, when comparing only samples from older sequencing platforms, riverine island samples still showed higher heterozygosity estimates than did samples from floodplain and upland forests (Welch two sample t-test: $t = 7.04$, df = 54.57, $p \ll .001$) suggesting that the pattern of elevated heterozygosity in island birds is real and is independent of sequencing platform. Both species-level and per-population heterozygosity were higher in riverine island species, and the inbreeding coefficient was lower (Table 2, Figures S1 and S39).

Although the geographic extent of the three habitats scales approximately with habitat stability (Figure 1, Hess et al., 2015a), we found significant differences in range size between habitats, but this was not correlated with habitat stability, perhaps due to inflated range size estimates for floodplain forest and riverine island birds which are based on coarse range maps (Table 2, Figure S1). Effective population size (Watterson's θ) corrected for number of base pairs was positively correlated with habitat stability (higher in upland forest birds), while heterozygosity was negatively correlated (Table 2). Tajima's D was negative for all species, and more so for floodplain and upland species (Figure S1), although we found mixed statistical support for this relationship (Table 2).

3.4 | Population genetic structure

Species in the more dynamic riverine island habitat had lower population structure (Figure 2a). Across all population clustering analyses, riverine island birds consistently showed less range-wide population structure than species from either upland or floodplain forests (Figure 2a, Figure S1, Table 2). Phylogenetic ANOVAs revealed significant differences in population structure and genetic variation between habitat types for three parameters that describe the degree of population genetic structure (Table 2), all of which also showed significant positive correlations in PGLS analyses when habitats were ordered by increasing spatial structure and stability (riverine islands -> flooded forest -> upland forest). These variables are listed in Table 2, and in order of decreasing AICc weights are: mitochondrial tree length, UCE gene tree length, and D_{xy}. Two variables (pairwise differences and average genetic groups) were not significantly correlated with habitat (Table 2). A variable that describes genetic structure (D_{xy}) was moderately correlated with F_{ST} based on VIF values. The flanking regions of UCE loci have more variable sites (Faircloth et al., 2012), so longer contigs may produce greater variation in these samples. This would make our estimates of lower population genetic structure in riverine island birds a conservative estimate in comparison to the floodplain and upland forest estimates.

3.5 | Gene flow

Two genetic variables that measure rates of gene flow — F_{ST} and IBD — showed strong differences across habitats (ANOVA) and were associated with habitat stability in PGLS analyses (Figure 2a, Table 2, Table S5). For both variables, more dynamic habitats showed higher gene flow estimates. Tree length of the rapidly evolving mitochondrial genome, although it measures the amount of genetic structure, is also strongly affected by gene flow, and was likewise significantly associated with habitat in all analyses (Table 2).

3.6 | Species traits correlated with dispersal

No traits hypothesized to predict dispersal ability were significantly correlated with habitat in any analysis (Table 2; Figure S2), including the HWI (Figure 2b). Separately filtering to species with a HWI < 30 and to passerine species did not change the HWI results (Figure S2). PGLS analysis of the HWI showed a weak correlation with increasing habitat stability ($p = .07$, slope = 0.6), with more rounded wings in riverine island species.

3.7 | Sensitivity analyses

Habitat specialization varies within Amazonian floodplains, and some species use more than one kind of habitat (Remsen & Parker, 1983). Reassigning two riverine island species (*Ochthornis littoralis* and

TABLE 2 Phylogenetic one-way ANOVAs and PGLS for each population genetic, dispersal, and summary statistic showing differences across habitat categories.

Parameter	PGLS		ANOVA		Island vs. floodplain		Island vs. upland		Floodplain vs. upland	
	t	p	F	p	|t|	p	|t|	p	|t|	p
Data attributes										
Contig length	−0.52	<.001	96.80	.001	12.31	.003	12.11	.003	0.21	.55
Total base pairs	−0.59	<.001	93.34	.001	11.87	.003	12.11	.003	0.24	.45
Loci	−0.25	.088	3.26	.093	1.71	.224	2.52	.11	0.85	.081
Genetic structure										
mtDNA tree length	0.44	<.001	10.74	<.001	1.74	.22	4.52	.003	3.00	.003
UCE gene tree length	0.44	<.001	6.48	.007	1.91	.16	3.60	.006	1.76	.003
Dxy	0.38	.0015	5.27	.018	2.02	.13	3.23	.012	1.27	.006
Average genetic groups	0.15	.15	2.53	.15	1.25	.38	2.25	.17	1.04	.036
Gene flow										
F_{ST}	0.55	<.001	12.48	<.001	2.81	.021	5.00	.003	2.28	.003
Isolation-by-distance	0.32	.0011	4.59	.025	1.20	.40	2.98	.014	1.89	.003
Genetic diversity and population size										
Range size	−0.042	.72	41.61	<.001	8.80	.003	6.90	.003	2.01	.003
Observed heterozygosity	−0.46	<.001	26.84	.001	5.65	.003	6.98	.003	1.39	.003
Tajima's D	−0.11	.32	16.93	.001	5.28	.003	4.91	.003	0.38	.27
Theta	0.41	.002	7.26	.002	2.59	.023	3.75	.003	1.21	.004
Species traits										
Beak length at nares	0.045	.86	2.98	.08	2.17	.23	2.11	.23	0.06	.84
Mass	−0.42	.17	2.83	.117	2.31	.25	1.72	.25	0.62	.25
Trophic niche (diet)	−0.49	.63	1.40	.29	1.61	.66	1.24	.66	0.39	.66
Hand Wing Index	0.60	.073	0.52	.56	0.42	.93	1.01	.93	0.62	.29
Speciation dynamics										
Sister clade richness	−0.21	.25	0.75	.42	0.81	.837	1.20	.84	0.40	.84
Stem branch length	−0.37	.093	0.61	.40	0.24	.81	1.09	.53	0.75	.46
Subtending branch length	−0.091	.73	0.01	.98	0.11	.00	0.03	1.00	0.12	1.00

Note: Habitat-specific contrasts are Holm's post hoc *t*-tests with *p*-values adjusted for multiple comparisons (Holm, 1979). Variables with variance inflation factors (VIF) greater than 5 are removed for clarity, except for F_{ST} and D_{xy} which had a VIF of 7.3 in a linear model with other genetic variables but are of particular interest in this study. AICc weight data for PGLS analyses are shown in Table S5.

Dendroplex kienerii) that also use floodplain forests to the flooded forest habitat had no effect on the significance level of any trait in the PGLS or ANOVA analyses. Removing these two species likewise had no effect on any variable. Riverine island species may use distinct micro-habitats within islands, with species more closely associated with early- and late-successional stages of ecological succession within islands (Rosenberg, 1990). When dividing riverine island species into those found in early and late stages of ecological succession, most of the same genetic measures had a significant overall effect across habitats in ANOVA analysis and no variables differed between early- and late-stage island species (Table S6).

All analyses of stem branch length, subtending branch length, and sister clade richness were not significant for any habitat comparison (Table 2, Table S5, Figure S3). Sign tests of sister clade richness were not significant (island *p* = .63, floodplain *p* = 1, upland *p* = .81). Significance levels for ANOVAs and post hoc tests for each parameter are shown in Table 2, results from PGLS for each genetic parameter are in Table 2 and Table S5, and boxplots for all variables across habitat are in Figures S1–S4.

4 | DISCUSSION

We found that the habitat preferences of Amazonian bird species have a significant and predictable effect on the amount of intraspecific genetic structure. On average, birds of upland forest had

greater population structure than birds of flooded forests, and birds of flooded forests had greater population structure than birds of riverine islands. We tested the hypothesis that this general pattern reflects dispersal ability of the species. Although often-used morphological proxies for dispersal ability, such as the HWI, did not vary across habitat types, we found that population genetic measures of gene flow were greater in species inhabiting the more dynamic riverine islands, suggesting higher natal dispersal in these species. Our data build on previous comparative population genetic work in Amazonian birds (Bates et al., 2003; Burney & Brumfield, 2009; Harvey et al., 2017) by adding sampling from birds of riverine islands, one of the most dynamic habitats in the Amazon basin.

4.1 | Species in more dynamic habitats have less population genetic structure

We observed lower genetic structure in floodplain and especially riverine island birds compared to those of upland forests. In fact, some riverine island species, like *Mazaria propinqua*, exhibited almost no genomic differentiation and had identical mitochondrial haplotypes across their entire Amazonian distributions. These results agree with prior studies on birds of riverine islands that have found similarly low levels of population genetic structure (Aleixo, 2006; Barbosa et al., 2022; Cadena et al., 2011; Choueri et al., 2017, but see Luna et al., 2021). These prior studies included a mix of highly specialized riverine island species like *M. propinqua* (Barbosa et al., 2022) and more generalist species like *Hypocnemoides melanopogon* (Choueri et al., 2017) and *Chrysomus icterocephalus* (Cadena et al., 2011) that also occur in other riverine habitats. A few of our study species, as well as some species examined in prior studies, did have higher levels of population genetic structure (Luna et al., 2022; Thom et al., 2020). We explore this population differentiation and its causes below. Regardless, it is noteworthy that our examination of a broader suite of riverine island specialists demonstrated lower structure on average in this dynamic habitat.

The observed trend in population genetic structure across habitats could be influenced by the selection of species from each habitat that we examined. Due to variation in habitat specificity on multiple axes and the unique distributions of each species, there are no objective criteria for identifying sets of habitat specialists. We relied on prior classifications (Remsen & Parker, 1983; Rosenberg, 1990) combined with expert opinion to identify our riverine island species set, similar to the strategy used to select the floodplain and upland species (Harvey et al., 2017). We expect that sampling more species in each habitat would improve the reliability of our results, but do not anticipate a difference in the pattern of average differentiation across habitats. Although the diversity of Amazonian bird communities makes them an optimal system for comparative studies, we are also fundamentally limited by the finite set of species that exist in each habitat.

The lower population genetic structure in habitats thought to be more dynamic suggests that something about habitat dynamism inhibits the formation of genetic structure. The exact mechanism responsible, and whether the process involved is ecological or evolutionary in temporal scale, is unclear. Below, we utilize our other population genetic and morphological results to examine possible mechanisms for this pattern, including individual-level ecological traits such as dispersal ability and species-level evolutionary processes such as gene flow or colonization rate.

4.2 | Dispersal ability and gene flow

Dispersal has been considered central to avian speciation and diversification (Brumfield, 2012; Smith, McCormack, et al., 2014, but see Crouch et al., 2019), but the concept of dispersal used is often vague. Dispersal is used to refer both to the movement of individuals and the movement of alleles (e.g., Barton & Shpak, 2000; Hellberg, 2009). The latter is of primary interest for studies like ours examining evolutionary differences. Natal dispersal (Dawideit et al., 2009), or the dispersal of individuals from where they were born to where they breed, is the type of individual movement most relevant for the movement of alleles. However, data on natal dispersal are hard to find. In birds, measuring natal dispersal requires tracking individuals from their nest site to their ultimate breeding territories. Although this has been accomplished in some systems (Paradis et al., 1998), such data are lacking for most Neotropical species. Moreover, the dispersal events critical to genetics and evolution may be rare, long-distance, natal dispersal events rather than the short-distance events that are the norm (Hellberg, 2009; Paradis et al., 1998; Watts et al., 2007) and require a large sample size to accurately capture.

Genetic and evolutionary studies commonly use proxies of dispersal ability. These proxies are measured as a population- or species-wide metric that is thought to reflect the movement of individuals on an ecological scale. This metric is typically based either on morphology, such as wing shape (Claramunt, 2021; Kipp, 1959), or on ecological traits thought to predispose species to dispersive lifestyles, such as foraging ecology and diet (Burney & Brumfield, 2009; Miller et al., 2021). Recently, researchers have also conducted dispersal challenge experiments to measure an individual's relative flying ability (Moore et al., 2008; Naka et al., 2022). Naka et al. (2022) found no correlation in these experiments between flight distance and riverine island specialization, suggesting that wing shape, rather than ecological preference, better explains the capacity of birds to cross an open gap of water. Although this experimental design – a captured bird attempting to escape a perceived human predator in open space – may reflect escape strategy rather than dispersal integrated over longer timescales ability (J. V. Remsen, Jr. personal communication), some studies have found correlations between wing shape and both natal dispersal distance (Belliure et al., 2000) and evolutionary processes like speciation rate (Claramunt et al., 2012).

We suggest that dispersal proxies like wing shape may not accurately capture dispersal ability of evolutionary relevance for all species. Species with wing shapes not conducive to long-distance

flight may also be capable of dispersing long distances through contiguous habitat. Our genetic data indicate that riverine island birds can disperse along Amazonian rivers and perhaps along vegetated riverbanks, maintaining range-wide genetic connectivity despite similar wing shapes to species in other habitats. Two anecdotal observations illustrate this well. Naka et al. (2022) observed an individual of *Mazaria propinqua*, a species with a very low HWI value (10.8), swimming a few meters to reach a river island during a 100-m dispersal experiment. More dramatically, an individual of this species was found on the Oyapok River in French Guiana (Ingels et al., 2012), 265 km from the nearest known population (Aguiar et al., 2010; Barbosa et al., 2022). Targeted searches along this river and in the intervening regions did not find populations of this species, supporting the hypothesis that this was a wandering individual (Ingels et al., 2012). Proxy measures like the HWI probably remain powerful predictors of dispersal potential generally, as evidenced in recent comparative (Arango et al., 2022; Claramunt, 2021; Dawideit et al., 2009; Sheard et al., 2020; Weeks et al., 2022) and experimental approaches (Naka et al., 2022) but may not be useful in all habitats or for all species, or when other historical or ecological variables (see for example Capurucho et al., 2020) overcome their effect. Many of these potential ecological variables, such as generation time and reproductive behaviour, are not available for many Amazonian species, highlighting the need for additional natural history data. Together, these shortcomings may explain why we did not find elevated dispersal ability based on any morphological metric in riverine island birds.

A recent study from the Rio Branco in northern Amazonia provides an interesting perspective on this issue. Luna et al. (2022) showed that three passerine bird species specialized on white-water floodplains hold populations on the Rio Branco, and are isolated from populations on other white-water rivers by the black waters of the Rio Negro. Despite different dispersal abilities, all three species were able to colonize the Rio Branco during the late Pleistocene, but the two species that live on allegedly more dynamic habitats such as sand-bar scrub (*Mazaria propinqua* and *Stigmatura napensis*) showed lower population structure than the species that occurs in relatively less dynamic *Cecropia*-dominated flooded forests (*Conirostrum bicolor*). These data suggests that over historical times, species may be able to cope with dispersal restrictions if appropriate habitat is available, even if for a restricted period of time.

We did find significantly higher values of population genetic measures thought to capture gene flow in floodplain and especially in riverine island birds. F_{ST} and IBD both showed this pattern. Prior work shows that while both metrics capture gene flow in simple models (Wright, 1931, 1943), the $\hat{e}$ estimator of IBD that we used here is a more explicit estimator of gene flow (Watts et al., 2007; Whitlock & McCauley, 1999). Moreover, genetic metrics may capture the effects of rare dispersal events over longer timescales (Slatkin, 1985), and they may be more relevant to evolutionary questions. Measures of dispersal at this evolutionary scale begins to blend with colonization of previously unoccupied areas (range expansion or re-colonization), and it may not always be clear in the data whether one or the other

process is at work (Crouch et al., 2019; Slatkin, 1985). Future studies should focus on more spatially explicit models of gene flow, such as effective migration surfaces (Petkova et al., 2016), that may better account for gene flow in linear systems, while accounting for the unique geography of particular species distributions. Regardless, genetic metrics of dispersal capture movements of alleles across geographic space that are relevant to evolutionary questions. The pattern observed in our results suggests that genetic dispersal, be it through gene flow or colonization, is greater in more dynamic habitats, even if the individual-level dispersal abilities of different species are not.

4.3 | Other mechanisms: Habitat shape and history, population size, and genetic diversity

Riverine habitats seem to have fewer geographic barriers to dispersal, and this probably contributes to lower genetic structure in these species. The distribution of riverine island species spans the width of the Amazon Basin, 2500 km from east to west, but is linear in shape and largely contiguous along rivers (Hess et al., 2015a). There are stretches of Amazonian rivers that lack islands, such as at the mouth of the Rio Tapajós (Irion et al., 2009), and these regions have been hypothesized to cause genetic breaks that are observed in some riverine species (Thom et al., 2020). Similarly, Luna et al. (2022) demonstrated that river colour can also represent a meaningful barrier for white- or black-water river specialists, as suggested by Laranjeiras et al. (2019).

This points to landscape barriers as a primary determinant of genetic structure. The geographic distribution of floodplain forests is similar to that of riverine islands, albeit with greater extent, so similar processes may explain the lower genetic structure in that habitat. Case studies from other organisms found in similar linear habitats have also demonstrated low levels of structure (Albernaz et al., 2012; Beheregaray et al., 2015; Vargas-Ramírez et al., 2020), but comparative studies are lacking. Upland forests, conversely, occur in extensive but noncontiguous patches delimited by rivers, termed interfluves. Genetic breaks in these species typically occur across rivers rather than within the interfluves, and these rivers are thought to pose strong barriers to dispersal for birds (Capparella, 1991; Maximiano et al., 2020; Musher et al., 2022; Naka & Brumfield, 2018; Silva et al., 2019), which may drive the higher genetic structure observed in that habitat.

The past as well as current distribution of riverine habitats may drive low genetic structure. The extent of riverine island and floodplain forests has fluctuated over geological time with cycles of isolation and expansion on scales of tens to hundreds of thousands of years (Sawakuchi et al., 2022). Recent studies of historical demography in Amazonian floodplain birds indicate that species associated with riverine island habitats have gone through recent and steep demographic expansion in the Holocene when compared with floodplain forest specialists (Barbosa et al., 2022; Sawakuchi et al., 2022). This points to habitat-level differences in historical

demographics, driven by differences in sedimentation dynamics and habitat history (Sawakuchi et al., 2022). Cycles of range size reduction and expansion could suppress the formation of population structure. Our estimates of low Tajima's D in species in these habitats support this possibility. We note, however, that we did not detect evidence of past bottlenecks or reductions in genetic diversity in riverine island species, although broader genomic sampling may be necessary to detect bottlenecks. Observed heterozygosity, which measures the genetic diversity within rather than between individuals, was higher in riverine island species (Table 2). Other metrics of population size such as Watterson's Theta did not show this pattern, but are probably driven by diversity partitioned among populations rather than within. The high diversity within riverine island species may stem partly from the high population densities of these species, an order of magnitude greater than those of upland forest species (Rosenberg, 1990). It is possible that measures of genetic diversity may be higher within genetic clusters of upland birds, but we lack sufficient sampling to accurately estimate within-population diversity. Future studies should focus on greater sampling within the genetic clusters that we have identified here, to test whether genetic parameters such as effective population size differ between comparable genetic clusters across species found in different habitats. While historical population or range fluctuations may contribute to the lack of structure in floodplain habitats, they have not been so severe as to wipe out genetic diversity in most of these species.

4.4 | Genetic differentiation in dynamic riverine habitats

Although we found significantly lower population genetic structure in species from the most dynamic habitat, riverine islands, some outlier species in this habitat did show higher genetic structure. This was especially true for *Thamnophilus nigrocinereus/ cryptoleucus*, *Myrmoborus lugubris*, and *Knipolegus orenocensis* (see Appendix S1). Some of these divergences have been reported previously (Choueri et al., 2017; Thom et al., 2020), and demonstrate that gene flow may be reduced in some regions, facilitating population differentiation, despite estimates of high species-wide gene flow. Similarly, Luna et al. (2022), examining populations in the geographically isolated Rio Branco drainage, found that populations of the riverine island species *Mazaria propinqua*, *Stigmatura napensis*, and *Conirostrum bicolor* are genetically distinct from those of the rest of the Amazon Basin. We sampled those same populations in this study and found they clustered separately in DAPC analyses (see Appendix S1), but the differentiation was less pronounced than in many floodplain or upland forest species. Prior authors (Luna et al., 2022; Thom et al., 2020) have hypothesized that population differentiation in riverine island birds can be driven by water type, with distinct populations in sediment-rich "white-water" versus sediment-poor, tannin-heavy "black-water" systems. Our results are consistent with this idea, with breaks

evident at transitions in water type between the Rio Negro and Rio Amazonas and between the Rio Branco and Rio Negro (e.g., Figures S24, S30 and S35). Both habitat structure and floristics are quite distinct in the two habitats (Junk et al., 2015; Laranjeiras et al., 2019), which may drive divergent selective pressures between the areas. Other breaks appear to be associated instead with geographic barriers, in isolated rivers or stretches of river lacking islands. For example, we observed breaks in *Knipolegus orenocensis* between the geographically isolated Rio Xingu and other populations along the main Amazonian tributaries (Figures S14 and S16). The genetic breaks we identified in riverine island birds warrant further investigation to identify the mechanisms responsible and to resolve their taxonomic implications.

4.5 | Implications and significance

Here, we show that population histories of birds on riverine islands of the Amazon Basin are distinct from those of species in floodplain and in upland habitats and are characterized by higher gene flow and lower genetic structure. This suggests that they may require distinct conservation strategies to maintain natural patterns of population connectivity. It is encouraging that some riverine island species can expand into second-growth habitats after human disturbance (Armacost & Capparella, 2012; Melo et al., 2021), indicating some resilience to habitat changes. However, the ongoing construction of hydroelectric dams in the Amazon Basin is likely to be detrimental to the persistence of riverine island and floodplain forest birds (Melo et al., 2021) and will certainly disrupt population connectivity and decrease population sizes of riverine bird species due to changes in river flow and silt load (Naka et al., 2019). Our data show that the effective population sizes of riverine island species are already lower than those of species in other habitats, further highlighting the need to preserve population connectivity. The population fluctuations of riverine island birds could also have long-term evolutionary implications. Low genetic structure coupled with the high rates of gene flow could inhibit population differentiation and speciation leading to evolutionary stasis over longer timescales (Aleixo, 2006; Brumfield & Edwards, 2007), although we did not find evidence of longer stem branches in these species (Table 2). Still, these processes may lead to different modes of speciation, such as speciation with gene flow, and unique sets of traits in the species that evolve in riverine islands, among the most dynamic of Amazonian habitats.

CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to declare.

OPEN RESEARCH BADGES

This article has earned an Open Data Badge for making publicly available the digitally-shareable data necessary to reproduce the reported results. Data files associated with this project, including sequence alignments, vcf files, and phylogenetic trees are available at Dryad (https://doi.org/10.5061/dryad.rxwdbrvc1) and scripts used for data processing and analysis are available at GitHub (https://github.com/henicorhina/Riverine_islands_code), archived at Zenodo: https://doi.org/10.5281/zenodo.7626736.

DATA AVAILABILITY STATEMENT

Raw sequence reads and metadata for samples newly sequenced have been deposited in the GenBank Sequence Read Archive (BioProject Accession PRJNA849703). Sequence read data downloaded from the GenBank Sequence Read Archive are available from BioProject Accessions PRJNA389814, PRJNA655842, and PRJNA595086. Data files associated with this project, including sequence alignments, vcf files, and phylogenetic trees have been made available at Dryad (Johnson et al., 2023; https://doi.org/10.5061/dryad.rxwdbrvc1) and scripts used for data processing and analysis are available at GitHub (https://github.com/henicorhina/Riverine_islands_code), archived at Zenodo: https://doi.org/10.5281/zenodo.7626736.

BENEFIT-SHARING STATEMENT

A research collaboration was developed with the scientists who provided genetic samples, with all collaborators included as coauthors of this manuscript. The results of this research have been shared with broader scientific community through public databases, as described above. Translated versions of this manuscript have been shared with the agencies that facilitated research.

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