<https://helda.helsinki.fi>

Postzygotic isolation drives genomic speciation between highly cryptic *Hypocnemis* antbirds from Amazonia

Cronemberger, Aurea A.

2020-11

Cronemberger , A A , Aleixo , A , Mikkelsen , E K & Weir , J T 2020 , ' Postzygotic isolation drives genomic speciation between highly cryptic *Hypocnemis* antbirds from Amazonia ' , *Evolution* , vol. 74 , no. 11 , pp. 2512-2525 . <https://doi.org/10.1111/evo.14103>

<http://hdl.handle.net/10138/335106>

<https://doi.org/10.1111/evo.14103>

acceptedVersion

Downloaded from Helda, University of Helsinki institutional repository.

This is an electronic reprint of the original article.

This reprint may differ from the original in pagination and typographic detail.

Please cite the original version.

Postzygotic isolation drives genomic speciation between highly cryptic *Hypocnemis* antbirds from Amazonia

Áurea A. Cronemberger,^{1,2,3} Alexandre Aleixo,^{3,4} Else K. Mikkelsen,^{1,2} and Jason T. Weir^{1,2,5,6}

¹Department of Ecology and Evolutionary Biology, University of Toronto, Toronto, Canada

²Department of Biological Sciences, University of Toronto Scarborough, Toronto, Canada

³Pós-graduação em Biodiversidade e Evolução, Museu Paraense Emílio Goeldi, Belém, Brazil

⁴Finnish Museum of Natural History, University of Helsinki, Helsinki, Finland

⁵Department of Natural History, Royal Ontario Museum, Toronto, Canada

⁶E-mail: jason.weir@utoronto.ca

Received February 19, 2020

Accepted August 28, 2020

How species evolve reproductive isolation in the species-rich Amazon basin is poorly understood in vertebrates. Here, we sequenced a reference genome and used a genome-wide sample of SNPs to analyze a hybrid zone between two highly cryptic species of *Hypocnemis* warbling-antbirds—the Rondonia warbling-antbird (*H. ochrogyna*) and Spix's warbling-antbird (*H. striata*)—in a headwater region of southern Amazonia. We found that both species commonly hybridize, producing F₁s and a variety of backcrosses with each species but we detected only one F₂-like hybrid. Patterns of heterozygosity, hybrid index, and interchromosomal linkage disequilibrium in hybrid populations closely match expectations under strong postzygotic isolation. Hybrid zone width (15.4 km) was much narrower than expected (211 km) indicating strong selection against hybrids. A remarkably high degree of concordance in cline centers and widths across loci, and a lack of reduced interspecific *F*_{st} between populations close to versus far from the contact zone, suggest that genetic incompatibilities have rendered most of the genome immune to introgression. These results support intrinsic postzygotic isolation as a driver of speciation in a moderately young cryptic species pair from the Amazon and suggest that species richness of the Amazon may be grossly underestimated.

KEY WORDS: Amazon, genetic incompatibility, hybridization, *Hypocnemis*, postzygotic reproductive isolation, speciation.

Species richness of birds and other groups is exceptionally high in Amazonia, and many phylogeographic assessments of widespread Amazonian taxa find that currently defined species comprise complexes of taxa which are deeply diverged genetically despite limited morphological and behavioral divergence (e.g. Silva et al. 2019). Some of these taxa are so similar morphologically that they have not previously been recognized taxonomically, even at the subspecies level (e.g. phylogroups within the Spot-backed Antbird; Fernandes et al. 2014). Whether such cryptic taxa are reproductively isolated and deserve species status, leading to even higher levels of Amazonian species richness, or simply represent geographically differentiated populations of

polytypic species is difficult to infer, but could have profound consequences on our understanding of species richness in what is already recognized as the most species-rich region of the planet.

Recent attempts to address speciation in morphologically cryptic taxa of Amazonian birds have largely focused on behavior, with detailed analyses of vocalizations often showing minor, but diagnosable differences which have often been used to reassess species boundaries under the assumption that consistent variations in song or calls between allopatric populations have rendered them reproductively isolated (e.g. *Hypocnemis*, Isler et al. 2007a; *Willisornis*, Isler and Whitney 2011; *Percnostola*, Isler et al. 2007b). In some cases, additional clear evidence

for speciation for specific taxa has been uncovered (e.g. broad sympatry rather than allopatry was demonstrated for *Hypocnemis subflava* and *H. peruviana* and these appear to mate assortatively in sympatry; Isler et al. 2007a), but for many taxa no additional evidence is available. However, recent comparative analyses involving playback experiments on male birds found that Amazonian species continue to respond to playback of the songs of closely related taxa for much longer on average than at high latitudes, suggesting that the slight variations in song that differentiate many cryptic, allopatric pairs of Amazonian taxa may not be serving as strong discriminatory cues, at least for male birds (Weir and Price 2019). Whether weak species discrimination will be true of females also is much harder to test in the field. A slow rate of song divergence near the equator (Weir and Wheatcroft 2011; Weir et al. 2012) coupled with a slow rate of male song discrimination (Weir and Price 2019) might argue against song-based premating isolation as a key driver of speciation in Amazonian birds (see also Pulido-Santacruz et al. 2018).

Parapatric contact zones provide a useful tool for assessing levels of reproductive isolation between cryptic Amazonian taxa. A lack of frequent hybridization along contact zones would provide evidence that cryptic taxa have acquired strong premating isolation and are behaving as distinct species. Genome-wide datasets were recently used to document local syntopy between seven pairs of species and subspecies which are largely allopatric, but which form narrow contact zones in the headwater region between the Teles Pires and Xingu rivers of southern Amazonia (Weir et al. 2015). Four of the seven pairs of taxa were highly cryptic and required multilocus analyses to detect local syntopy. Hybrids were discovered in all seven species pairs, while those pairs sampled in detail in regions of syntopy revealed that hybridization was frequent, even between taxa as old as four million years (Pulido-Santacruz et al. 2018).

The presence of old hybrid zones in the Amazon demonstrates that premating isolating barriers are often poorly developed, even after several million years of divergence. However, the dynamics of hybridization along parapatric contact zones can also provide important insight into levels of intrinsic postzygotic isolation. A recent genome-wide assessment of hybrid zones in two pairs of cryptic Amazonian bird species revealed evidence that speciation was essentially complete and was driven primarily by the accumulation of intrinsic postzygotic isolation. First, while plenty of F_1 hybrids and backcrosses of these into parental populations were documented, entire classes of early generation hybrids representing the most heavily recombined genotypes between the parental species were rare or missing from hybrid zones. Simulations with high levels of assortative mating (based on genomic similarity) were incapable of producing an absence of these hybrid classes (due to a lack of selection against F_2 and other early generation backcrosses), but simulations with postzy-

gotic isolation (i.e. in the form of genetic incompatibilities) produced nearly identical results (F_2 hybrids and other early generation hybrid classes with heavily recombined genomes were selected against). Second, these hybrid zones also revealed remarkably little variation in geographic and genomic cline centers or widths across individual loci. Substantial variability in hybrid zone dynamics is expected under neutral dynamics and is often observed in natural hybrid zones (e.g. Payseur 2010). Its absence suggests that most of the genome is behaving in a concerted fashion. The accumulation of many genetic incompatibilities across the genome causes neutral regions that should otherwise be free to introgress to be closely linked to loci that are selected against in the wrong genetic background. These results support the role of intrinsic postzygotic isolation in driving speciation in cryptic Amazonian taxa, but the taxon-pairs studied were old (2.5 to 4 million years), and postzygotic isolation, which is expected to accumulate as a function of genome differentiation (Orr 1995; Orr and Turelli 2001), might be expected to have accumulated to high levels in such old taxa pairs. It remains to be determined if postzygotic isolation is also capable of driving speciation on shorter time scales of 1 to 2 million years—a time frame over which a great many cryptic pairs of Amazonian taxa (e.g. subspecies and closely related congeneric species) have been diverging (Silva et al. 2019).

The *Hypocnemis cantator* complex possesses cryptic taxa that span a range of divergence dates and this provides an excellent opportunity to study the process of Amazonian speciation that involves minimal morphological or behavioural divergence. Six currently recognized species in this complex (*H. cantator*, *H. flavescens*, *H. peruviana*, *H. rondoni*, *H. ochrogyna*, and *H. striata*) are fully allopatric or parapatric (we exclude the sister group to this complex, *H. subflava*, which, though generally included, differs substantially in plumage and is now known to be sympatric with *H. peruviana* over a major portion of its lowland geographic distribution), and are highly similar in plumage (with some essentially undiagnosable even in the hand; see Whitney et al. 2013). Though they differ consistently in songs and/or calls—the key reason they were recently split into distinct species (Isler et al. 2007a; del Hoyo and Collar 2016)—vocal differences do not seem strongly pronounced (e.g. Fig. 1), and recent playback experiments between several taxon pairs in this complex (songs of various taxa were played to territorial males or male/female pairs of *H. peruviana* and response to heterotypic versus conspecific song was recorded) generally suggested weak song discrimination (Weir and Price 2019). Two members of this complex, *H. ochrogyna* and *H. striata striata* (Fig. 1), form a parapatric contact zone in the headwaters between the Teles Pires and Xingu Rivers (Figs. 2, 3) where both species and a single hybrid between them were recently discovered in syntopy (Weir et al. 2015). This species pair is closely related

A *Hypocnemis striata striata*

B *Hypocnemis ochrogyna*

Figure 5. Analyses of geographic clines for SNPs with (A) ΔP greater than 0.5 and (B) ΔP greater than 0.8 (see text for details). Loci with widths greater (green) or narrower (pink) than expected or with cline centers significantly shifted toward *H. ochrogyna* (red) or *H. striata* (blue) are shown.

6.3 km. For 2362 highly differentiated loci (Fig. 5) with P 0.8 (and with $P_{ochrogyna}$ greater than 0.9 and $P_{striata}$ less than 0.1), none had narrower and 22 had broader cline widths than the genome-wide cline and 3 loci had cline centers shifted significantly toward *H. ochrogyna* and 3 toward *H. striata striata*. The central 95% of cline widths occurred between values of 13.4 and 37.8 km and cline centers between 4.2 and 2.7 km.

Assessment of gene flow into parental populations: Interspecific Hudson's F_{st} calculated from across the genome was nearly identical and overlapped in 95% confidence intervals for close ($F_{st} = 0.552$, 95% CI 0.548-0.556) versus far ($F_{st} = 0.553$, 95% CI 0.549-0.557) parental populations. Likewise, Hudson's F_{st} calculated for each genome scaffold were very similar between close and far populations and paired t -tests were nonsignificant when either all scaffolds with 10 or more SNPs were used ($n = 1074$, mean of difference between far versus close = 0.00084, $t = 0.384$, $p = 0.70$) or when only highly differentiated scaffolds with an interspecific Hudson's F_{st} exceeding 0.5 were used ($n = 633$, mean of difference between far versus close = 0.00089, $t = 0.345$, $p = 0.73$).

Discussion

Here, we investigate levels of reproductive isolation along a contact zone between two highly cryptic lineages of *Hypocnemis* antbird. We found multiple lines of evidence that despite being highly cryptic and despite extensive hybridization at the contact zone as revealed by many individuals exhibiting a range of admixture values, speciation is essentially complete with high levels of postzygotic isolation resulting in only a very small amount of the genome introgressing across species barriers.

Our first line of evidence for reproductive isolation comes from comparing levels of interchromosomal linkage disequilibrium (D') within our hybrid zone populations to linkage disequilibrium values from simulations with or without reproductive isolation and under low to high levels of gene flow from parental populations. Because we compared D' across SNPs which are fixed between parental populations outside of contact zones, a lack of F_2 and later generation hybrids (including backcrosses with parental populations), would result in a D' value of 1.0 for each SNP pair within the hybrid zone. Our empirical values (Fig. 4A) are less than 1.0 for all but a small proportion of SNP pairs indicating that admixture has led to some breakdown of linkage disequilibrium. However, in the absence of any reproductive isolation, our simulations indicate that geneflow from parental populations can only maintain low to moderate levels of D' through time (Fig. 4C, D). Increasing migration rate resulted in higher D' . Our high immigration scenario results in approximately 30% of individuals in each year representing immigrants from outside parental populations. While we have not measured immigration rates directly, low dispersal capabilities typically associated with Amazonian understory-dwelling forest birds, such as *Hypocnemis* (Develey and Stouffer 2001; Woltmann et al. 2012), render it highly unlikely that 30% or more of contact zone individuals are replaced by parental immigrants each year. Rather, our empirical distribution of D' had a larger mode than even the high dispersal simulations suggesting that

immigration alone cannot account for the empirical pattern (compare Fig. 4A and D). In contrast, adding premating isolation had a modest effect and postmating isolation a major effect on maintaining an elevated distribution of D' .

Triangle plots of observed heterozygosity and hybrid index in hybrid populations provide strong evidence for selection against hybrids. Our empirical results closely match those simulated with Batesian—Dobzhansky—Muller incompatibilities under low to moderate levels of immigration (Fig. 4E). The empirical triangle plot revealed six F_1 -like hybrids as well as a series of individuals representing successive generations of backcrossing into each parental species (Fig. 4B). Surprisingly, we only encountered one individual consistent with an F_2 -like hybrid and found no additional individuals that occur in the off-diagonal positions of the triangle plot where a large proportion of individuals in a hybrid zone should occur if there is no selection against hybrids (Fig. 4D,F,G). Importantly, hybrids in off-diagonal positions possess highly recombined genomes and their near absence in the hybrid zone is a strong indicator that genetic incompatibilities, often most deleterious in a heavily recombined state (e.g. the “pathway” model of epistatic incompatibilities sensu Lindtke and Buerkle 2015; or recessive Batesian—Dobzhansky—Muller incompatibilities), generally render individuals with highly recombined genotypes inviable. Our empirical results closely match those simulated with Batesian—Dobzhansky—Muller incompatibilities under low to moderate levels of immigration.

Previous analyses under moderate levels of immigration have demonstrated that even very high levels of assortative mating (up to 99%) are incapable of preventing the formation of numerous off-diagonal hybrids (Pulido-Santacruz et al. 2018), thus, their near absence is an indicator for selection against heavily recombined genotypes rather than strong premating barriers. Our triangle plot simulations here confirm this result under a broader range of immigration rates. While high immigration and strong assortative mating (Fig. 3G) are capable of generating a bivariate distribution of hybrid indices, they continue to exhibit an excess of off-diagonal hybrids (as evidenced by the excess of simulated individuals with H_o near 0.5) that fail to match our empirical data. However, high levels of immigration would also lead to emigration of hybrids into adjacent parental populations, likely accelerating hybrid zone expansion rather than narrowing it, an effect we did not consider in our model but which has been modeled and confirmed elsewhere (Irwin 2020). These results, together with the fact that we have a hybrid zone where hybridization is common, argues against strong premating isolation for our species pair but does not eliminate a secondary contribution of assortative mating to total reproductive isolation.

The presence of substantial numbers of backcrossed individuals along the diagonals of the triangle plot might indicate

that introgression into a heterospecific background is still possible despite selection against heavily recombined genotypes. How much of the genome is capable of introgressing through successive generations of backcrossing should largely depend on the proportion of the genome that is not closely linked to genetic incompatibilities. During early stages of genomic divergence, few incompatibilities allow most of the genome to introgress. During late stages of the speciation process, large numbers of incompatibilities render most of the genome immune to introgression. We calculated geographic cline width of individual loci to determine the number of loci for which bidirectional introgression is eroding away species differences. Expected cline widths under neutral dynamics are governed by postnatal dispersal distance (larger distances result in broader clines) and the number of generations since secondary contact. Postnatal dispersal distances have been estimated for only one avian Neotropical species, *Myrmeciza exsul*, which disperses on average only 1.1 km (Woltmann et al. 2012). This species belongs to the same family as *Hypocnemis*. Assuming *Hypocnemis* disperses similar distances, has a generation time of two years, and that secondary contact for *Hypocnemis* and the other six known hybrid zones in the headwaters between the Teles Pires and Xingu rivers formed approximately 11,500 years ago following postglacial expansion of forest along the southern edge of Amazonia (see Weir et al. 2015), hybrid zone widths should be about 211 km on average. Only 1 of 5387 loci had a cline width exceeding this expectation (Fig. 5), while 95% of loci had cline widths less than 45 km and the median cline width was only 21 km. These results suggest selection acting against introgression is strong and that very few loci are able to introgress as deeply as a reasonable neutral expectation.

Cline centers for individual loci are also expected to show considerable variability due to neutral processes when they are not closely linked to genetic incompatibilities, while selection for a locus to introgress into a heterospecific background may cause its cline center to be greatly displaced relative to other loci. However, we found very little variability in cline center (Fig. 5). This tight coupling of cline centers and very narrow cline widths are further reflected in nearly identical interspecific Hudson's F_{st} values between parental populations located far (greater than 100 km away) versus near the contact zone center (50 to 100 km away) and suggest that very few loci are capable of introgressing even short geographic distances into a heterospecific background. Together these results suggest that these two species have left the intermediate stage of the speciation process where neutral parts of the genome remain semipermeable to gene flow in regions not closely linked to genetic incompatibilities and instead argue strongly for a late stage in the speciation process, where, despite what appears to be a lack of strong levels of premating isolation, sufficient numbers of genetic incompatibilities

spread across the genome have rendered most of the genome immune to introgression.

Hypocnemis ochrogyna and *H. striata* together with five other currently recognized species that make up the *Hypocnemis cantator* complex were long considered conspecific given their high degree of similarity in plumage and territorial song (see Fig. 1). Their current treatment as full species rests largely on the presence of diagnostic differences in song and call (Isler et al. 2007a) which, though detectable on sonograms (e.g. Fig. 1), are nevertheless remarkably similar. However, playback experiments of songs between several allopatric taxa pairs in this complex continue to illicit strong aggressive responses suggesting that song differences may often not be recognized by the birds themselves (Weir and Price 2019), though premating isolation generated by call differences between allopatric taxa has not been tested. The presence of a hybrid zone between *H. ochrogyna* and *H. striata*—whose pace differences (evident in Fig. 1) in male song render them one of the most vocally divergent pairs of taxa within the complex (Isler et al. 2007a)—demonstrates this level of vocal differentiation is insufficient to drive strong levels of premating isolation, though some premating isolation likely results. Rather, our evidence for strong levels of intrinsic postzygotic isolation support full species status for this pair and probably more generally for taxon pairs of similar age within the *H. cantator* complex. We are not discrediting a contribution of vocal difference to reproductive isolation, simply that it is unlikely that vocal differences are sufficient on their own to prevent species collapse upon secondary contact without postmating isolation (see also Irwin 2020).

Our results have important implications for our understanding of species richness in Amazonian birds because they suggest that taxon pairs which are morphologically cryptic, weakly differentiated behaviorally, and which appear to have evolved incomplete species discrimination are nevertheless speciating primarily through the evolution of intrinsic postzygotic isolation, with premating isolation playing a more minor role due to its slower accumulation. While a past study on Amazonian hybrid zones in a cryptic pair of antbirds and woodcreepers suggested the same (Pulido-Santacruz et al. 2018), the two pairs in that earlier study were older (i.e. 2.5 to 4.1 mya), and the buildup of high levels of intrinsic postzygotic isolation might be expected for such old taxon pairs. Here, our results for *H. ochrogyna* and *H. striata*—estimated to have diverged between one and two million years ago (Weir et al. 2015; Silva et al. 2019)—suggest that speciation, driven primarily by strong levels of intrinsic postzygotic isolation, is possible in younger pairs of cryptic Amazonian taxa. Whether this will generally be true depends on whether the buildup of high levels of intrinsic postzygotic isolation in *H. ochrogyna* and *H. striata* was at a pace typical for Amazonia. Genetic incompatibilities are expected to accumulate through time

as a byproduct of neutral genome differentiation between all pairs of allopatric taxa. While divergent selection can speed up this process, we have no reason to expect that *H. ochrogyna* and *H. striata* experienced higher levels of divergent selection than other Amazonian taxa. Divergent selection driven by climatic niche, for example, is estimated to be low in birds near the equator in general (Lawson and Weir 2014). If our results for *H. ochrogyna* and *H. striata* hold true for other hybridizing taxa pairs of similar age, then it is likely that the plethora of cryptic Amazonian taxa that continue to be detected by detailed phylogeographic studies will also possess intrinsic reproductive isolation and their recognition as full species would substantially increase species richness in what is already recognized as the most species-rich region on the planet. Our results thus suggest the strong possibility that species richness could be dramatically underestimated in Amazonia, especially for antbirds and other understory specialists.

AUTHOR CONTRIBUTIONS

J.T.W. designed the study. A.A.C., A.A., and J.T.W. collected field samples. A.A.C. and E.M. generated GBS libraries, A.A.C. perform analysis of population structure and admixture, and J.T.W. performed hybrid zone simulations and cline analysis. J.T.W. and A.A.C. wrote and all authors edited the manuscript.

ACKNOWLEDGMENTS

We gratefully acknowledge the many landowners in Brazil who allowed sampling on their property, in particular the owners and/or managers of Fazenda Juruna and Fazenda Jarina where our greatest field effort was concentrated. The Brazilian National Research Council (CNPq) granted field research permits (4253-1, 40173-1, and 6581-1). Research was funded by the following grants: PDSE-CAPES #88881.189565/2018-01 (A.A.C.); CNPq (‘INCT em Biodiversidade Uso da Terra da Amazonia’ #574008/2008-0; #471342/2011-4; #310880/2012-2; and #306843/2016-1) and the Amazonia Paraense Foundation—FAPESPA (grant no. ICAAF 023/2011) (A.A.); Natural Sciences and Engineering Research Council of Canada (NSERC) CGS-D fellowship (411293437) (E.M.); NSERC Discovery Grants (402013-2011, RGPIN-2016-0653) and an NSERC Accelerator Grant (no. 492890) (J.T.W.). We thank Maya Faccio, Paola Pulido-Santacruz, Alfredo Barrera-Guzmán and Sidnei Dantas for assistance with field sample collection; Alfredo Barrera-Guzmán and Carlynne Simões for assistance with DNA isolation and quantification; and Jordan Bemmels for discussions about F_{st} .

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA ARCHIVING

Illumina short read data have been deposited in the National Center for Biotechnology Information Sequence Read Archive under BioProject PRJNA665047.

LITERATURE CITED

- Alcaide, M., E.S.C. Scordato, T.D. Price, and D.E. Irwin. 2014. Genomic divergence in a ring species complex. *Nature* 511:83–85.
- Alexander, D.H., J. Novembre, and K. Lange. 2009. Fast model-based estimation of ancestry in unrelated individuals. *Genome Res.* 19:1655–1664.
- Bolger, A.M., M. Lohse, and B. Usadel. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 30:2114–2120.
- Catchen, J., P.A. Hohenlohe, S. Bassham, A. Amores, and W.A. Cresko. 2013. Stacks: an analysis tool set for population genomics. *Mol. Ecol.* 22:3124–3140.
- Catchen, J.M., A. Amores, P. Hohenlohe, W. Cresko, and J.H. Postlethwait. 2011. Stacks: building and genotyping loci de novo from short-read sequences. *Genes Genomes Genetics* 1:171–182.
- Danecek, P., A. Auton, G. Abecasis, C.A. Albers, E. Banks, M.A. DePristo, R.E. Handsaker, G. Lunter, G.T. Marth, S.T. Sherry, et al. 2011. The variant call format and VCFtools. *Bioinformatics* 27:2156–2158.
- Develey, P.E., and P.C. Stouffer. 2001. Effects of roads on movements by understory birds in mixed-species flocks in central Amazonian Brazil. *Conserv. Biol.* 15:1416–1422.
- delHoyo, J., and N.J. Collar. 2016. HBW and Bird Life international illustrated checklist of the birds of the world volume 2: Passerines. Barcelona. *J. Field Ornithol.* 88:421–424.
- Derryberry, E.P., G.E. Derryberry, J.M. Maley, and R.T. Brumfield. 2014. Hzar: hybrid zone analysis using an R software package. *Mol. Ecol. Resour.* 14:652–663.
- Ellegren, H. 2010. Evolutionary stasis: the stable chromosomes of birds. *Trends Ecol. Evol.* 25:283–291.
- Ellegren, H., L. Smeds, R. Burri, P.I. Olason, N. Backström, T. Kawakami, A. Künstner, H. Mäkinen, K. Nadachowska-Brzyska, A. Qvarnström, et al. 2012. The genomic landscape of species divergence in *Ficedula* flycatchers. *Nature* 491:756–760.
- Elshire, R.J., J.C. Glaubitz, Q. Sun, J.A. Poland, K. Kawamoto, E.S. Buckler, and S.E. Mitchell. 2011. A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. *PLoS One* 6:1–10.
- Fernandes, A.M., M. Wink, C.H. Sardelli, and A. Aleixo. 2014. Multiple speciation across the Andes and throughout Amazonia: the case of the spot-backed antbird species complex (*Hylophylax naevius*/*Hylophylax naevioides*). *J. Biogeogr.* 41:1094–1104.
- Gompert, Z., and A.C. Buerkle. 2010. introgress: a software package for mapping components of isolation in hybrids. *Mol. Ecol. Resour.* 10:378–384.
- Hammer, Ø., D.A.T. Harper, and P.D. Ryan. 2001. Past: paleontological statistics software package for education and data analysis. *Palaeontol. Electron.* 4:1–9.
- Hudson, R.R., M. Slatkin, and W.P. Maddison. 1992. Estimation of levels of gene flow from DNA sequence data. *Genetics* 132:583–589.
- Irwin, D.E. 2020. Assortative mating in hybrid zones is remarkably ineffective in promoting speciation. *Am Natural.* 195:E150–E167.
- Isler, M.L., P.R. Isler, and B.M. Whitney. 2007a. Species limits in antbirds (Thamnophilidae): the warbling antbird (*Hypocnemis cantator*) complex. *Auk* 124:11–28.
- Isler, M.L., P.R. Isler, B.M. Whitney, and K.J. Zimmer. 2007b. Species limits in the “*Schistocichla*” complex of *Pernostola* Antbirds (Passeriformes: Thamnophilidae). *Wilson J. Ornithol.* 119:53–70.
- Isler, M.L., and B.M. Whitney. 2011. Species limits in antbirds (Thamnophilidae): The scale-backed antbird (*Willisornis poecilinotus*) complex. *Wilson J. Ornithol.* 123:1–14.
- Li, H. 2018. Minimap2: pairwise alignment for nucleotide sequences. *Bioinformatics* 34:3094–3100.
- Li, H., B. Handsaker, A. Wysoker, T. Fennell, J. Ruan, N. Homer, G. Marth, G. Abecasis, and R. Durbin. 2009. The sequence alignment/map format and SAM tools. *Bioinformatics* 25:2078–2079.
- Lawson, A.M., and J.T. Weir. 2014. Latitudinal gradients in climatic-niche evolution accelerate trait evolution at high latitudes. *Ecol. Lett.* 17:1–10.
- Lindtke, D., and C.A. Buerkle. 2015. The genetic architecture of hybrid incompatibilities and their effect on barriers to introgression in secondary contact. *Evolution (N. Y.)* 69:1987–2004.
- Orr, H.A. 1995. The population genetics of speciation: the evolution of hybrid incompatibilities. *Genetics* 139:1805–1813.
- Orr, H.A., and M. Turelli. 2001. The evolution of postzygotic isolation: accumulating Dobzhansky–Muller incompatibilities. *Evolution (N. Y.)* 55:1085–1094.
- Payseur, B.A. 2010. Using differential introgression in hybrid zones to identify genomic regions involved in speciation. *Mol. Ecol. Resour.* 10:806–820.
- Pulido-Santacruz, P., A. Aleixo, and J.T. Weir. 2018. Morphologically cryptic Amazonian bird species pairs exhibit strong postzygotic reproductive isolation. *Proc. R. Soc. B Biol. Sci.* 285: 20172081.
- Shin, J.-H., S. Blay, J. Graham, and B. McNeney. 2006. LDheatmap: an R function for graphical display of pairwise linkage disequilibria between single nucleotide Polymorphisms. *J. Stat. Softw.* 16. <https://doi.org/10.18637/jss.v016.c03>
- Silva, S.M., A.T. Peterson, L. Carneiro, T.C.T. Burlamaqui, C.C. Ribas, T. Sousa-Neves, L.S. Miranda, A.M. Fernandes, F.M. D’Horta, L.E. Araújo-Silva, et al. 2019. A dynamic continental moisture gradient drove Amazonian bird diversification. *Sci. Adv.* 5:1–10.
- Whitney, B.M., M.L. Isler, G.A. Bravo, N. Aristizaba, F. Schunck, L.F. Silveira, V.D.Q. Piacentini, M. Cohn-haft, and M.A. Rego. 2013. A new species of antbird in the *Hypocnemis cantator* complex from the Aripuana-Machado interfluvium in central Amazonian Brazil. Pp 282–285. In: J. del Hoyo, A. Elliott, and D. Christie, eds. *Handbook of Birds of the World*, Vol. 8. Lynx Edicions, Barcelona.
- Weir, J.T., M.S. Faccio, P. Pulido-Santacruz, A.O. Barrera-Guzmán, and A. Aleixo. 2015. Hybridization in headwater regions, and the role of rivers as drivers of speciation in Amazonian birds. *Evolution (N. Y.)* 69:1823–1834.
- Weir, J.T., and T.D. Price. 2019. Song playbacks demonstrate slower evolution of song discrimination in birds from Amazonia than from temperate North America. *PLoS Biol.* 17:1–19.
- Weir, J.T., and D. Wheatcroft. 2011. A latitudinal gradient in rates of evolution of avian syllable diversity and song length. *Proc. R. Soc. B Biol. Sci.* 278:1713–1720.
- Weir, J.T., D.J. Wheatcroft, and T.D. Price. 2012. The role of ecological constraint in driving the evolution of avian song frequency across a latitudinal gradient. *Evolution (N. Y.)* 66:2773–2783.
- Weisenfeld, N.I., V. Kumar, P. Shah, D.M. Church, and D.B. Jaffe. 2017. Direct determination of diploid genome sequences. *Genome Res.* 27:757–767.
- Woltmann, S., T.W. Sherry, and B.R. Kreiser. 2012. A genetic approach to estimating natal dispersal distances and self-recruitment in resident rainforest birds. *J. Avian Biol.* 43:33–42.

Associate Editor: E. P. Derryberry
Handling Editor: T. Chapman

Supporting Information

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

Table S1. List of *Hypocnemis* specimens used in the present study, corresponding to the distribution of the samples represented in Figure 1(c). The acronym MPEG represents the Museu Paraense Emílio Goeldi institution where the samples are deposited.