Climatic influences on the behavioral ecology and evolution of tropical lekking frugivores

by

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B.A., Wellesley College, 2013

AN ABSTRACT OF A DISSERTATION

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Division of Biology
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Abstract

A major question in evolutionary ecology is how organisms are affected by abiotic forces in their environment. The effects of climatic drivers on species' behavior and evolution are especially of concern because many species, particularly in the tropics, have begun to face increasingly extreme and unpredictable weather events. While many endotherms in the Neotropics experience strong selection through abiotic forces such as precipitation, sexually selected birds provide an opportunity to understand how these precipitation-related challenges interact with reproductive effort to influence avian behavioral ecology and evolution. Manakins are small birds from a family (Pipridae) of approximately 50 species distributed across Central and South America. The many species across the clade are known for exuberant display behaviors performed for females, who select mates without receiving direct benefits such as assistance with parental care, territory defense, or food acquisition. As a result, males are subject to strong reproductive skew, meaning that there is strong variation in siring success across males in these populations. In this dissertation, I investigate theoretical predictions of how abiotic drivers affect the behavior and evolution of two different species, white-ruffed manakins (*Corapipo altera*) and white-collared manakins (*Manacus candei*). Specifically, I address how individual life history trade-offs, population-level behavioral ecology, and genomic signatures of evolution are related to theoretical predictions.

I first test several predictions of life history theory among white-ruffed manakins. I focus on the survival benefits males may gain by migrating downslope during rainy non-breeding seasons and ask how males balance this priority against the reproductive benefits associated with remaining resident at breeding elevations. This study provided some support for the hypothesis that males that display at higher rates during the breeding season are more likely to migrate in

the following non-breeding season. However, I failed to provide support for the hypotheses that male quality or residual reproductive value are related to migratory tendency in this species.

On a broader level across a species' elevational range, I empirically evaluate expectations of hygric niche theory, which suggests that rainfall and elevation may interact to affect tropical endotherms by constraining reproductive activity. Specifically, I test the hypothesis that white-collared manakin males should display less frequently during rainfall at mid-elevation communal leks than at low-elevation communal leks, since thermoregulation (especially in the presence of rainfall) is expected to be more difficult at higher elevations where temperatures are lower. Across 287 observations, I found limited support for this hypothesis, as display behavior strongly decreased with increasing rainfall intensity at both elevational sites. Interestingly, an apparent interaction between rainfall and elevation was driven by a single outlier, suggesting the possibility of such an interaction but leading me to interpret this result cautiously.

Finally, I test whether climatic factors or population genetic structure drive the sex chromosome-to-autosomal diversity ratio in white-ruffed manakins across Costa Rica and Panamá. Because strong reproductive skew concentrates reproductive success among fewer males and disproportionately reduces Z-chromosome diversity relative to autosomal diversity, the Z/A diversity ratio is theorized to provide an indirect metric of reproductive skew. I expected that six *a priori* climatic metrics may be correlated with Z/A through their effects on differential male survival and genetic contribution after accounting for population genetic structure. I instead found that differences in Z/A among populations were strongly associated with population genetic structure when controlling for the six climatic factors. This study suggests a mechanism by which demographic history may contribute to variation in Z/A. Even as the potential effects of climate may be difficult to detect when obscured by the broader history of species

differentiation, this study emphasizes the ways in which population genetic structure can strongly affect evolutionary signatures on the Z-chromosome.

By studying theoretical predictions at multiple levels of biological organization, this dissertation demonstrates the challenges associated with testing theoretical predictions within complex ecological and evolutionary contexts. My findings emphasize both the importance of abiotic environmental challenges for Neotropical endotherms and the value of rigorously distinguishing climatic effects from alternative sources of ecological and evolutionary variation.

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Chapter 1 - Introduction

Across the globe and across taxa, abiotic environmental conditions play a critical role in shaping organisms' life history trade-offs, behavioral ecology, and evolution. With animals around the world facing increasingly sporadic and unpredictable weather events, understanding the effects of climatic factors on species is critical for informing our understanding of the behavioral and evolutionary consequences of climate change (Skagen & Adams, 2012; Van De Pol et al., 2017). Precipitation is an abiotic force whose influence is broadly under-studied but can have dramatic effects on animals' life history strategies, phenology, and range distributions (B. G. Freeman et al., 2024; Moreno-Palacios et al., 2025). Global patterns of precipitation have direct and indirect ecological effects; while precipitation during breeding can directly inhibit reproductive behavior, rainfall prior to breeding often enhances food availability, creating resource pulses that birds track when initiating reproduction (Silber & Boyle, 2026). Interestingly, the effects of rainfall are not as closely related to species' traits as they are to precipitation timing and biogeography, emphasizing the strong role of climatic factors in affecting reproductive behavior and evolution (Silber & Boyle, 2026). Hygric niche theory is a novel framework for understanding how and why endotherm populations respond to the direct and indirect consequences of temporal variation in rainfall (Boyle et al., 2020). Just as animals have optimal temperature ranges, they are expected to exhibit peak performance in the middle of the precipitation conditions they can tolerate (Boyle et al., 2020). While endotherms in arid environments are negatively influenced by drier seasons and respond positively to wetter years, theory predicts that endotherms in wet environments may experience negative effects when exposed to greater-than-normal rainfall. While the effects of water limitation for organisms inhabiting arid environments have been

comparatively well established, the consequences of excessive precipitation have until recently received considerably less attention (Boyle et al., 2020; Knapp et al., 2017).

Our understanding of organismal responses to climatic variation is disproportionately derived from temperate systems (Culumber et al., 2019). Tropical systems support much of the world's biodiversity and offer an opportunity to investigate organismal responses at the wet end of the hygric spectrum. At the lower latitudes, more consistent annual temperatures are offset by seasonal variation in rainfall amount and timing. So far in tropical species, we know relatively little about the direct and indirect effects of precipitation. In the Neotropics, ongoing changes in precipitation regimes and climatic variability appear to have important consequences for survival, reproduction, and distribution (Brawn et al., 2017; Shogren et al., 2019). In addition to the expectation that increased rainfall can affect endotherms, hygric niche theory predicts that rain should affect endotherms differently depending on elevation and therefore temperature, which tracks strongly with elevation in the tropics (M. T. Freeman et al., 2024). In humid air, temperature decreases with increasing elevation, about 0.6º C per 100 m (Clark et al., 2015). Because wetting can increase heat loss, the energetic costs of rainfall should be greater under the cooler conditions associated with higher elevations (Karr et al., 2025; Monteith et al., 2013).

Energetic and ecological constraints may be particularly consequential for animals performing energetically demanding behaviors under intense sexual selection. Sexually selected birds provide an opportunity to understand how abiotic forces interact with sexual selection to affect Neotropical endotherms. In this dissertation, I investigate theoretical predictions relating to how abiotic pressures influence behavioral ecology and evolutionary signatures within two different species of Neotropical passerines. Manakins are small birds from a family (Pipridae) of approximately 50 species distributed across Central and South America. Male manakins across

the clade perform exuberant displays for females, who select mates without receiving benefits such as assistance with parental care, territory defense, or food acquisition. Displays appear to require skill and athletic ability, and adult males (age four and older) can gain access to a display site. Males differ dramatically in their siring success across their leks (Durães et al., 2009; DuVal & Kempenaers, 2008; Jones et al., 2014); this differential reproductive success rate among males is termed reproductive skew.

To understand the life history mechanisms driving individual males' navigation of life history trade-offs and separately investigate how climatic factors influence genomic signatures of evolution, I studied white-ruffed manakins (*Corapipo altera*). These partial, facultative altitudinal migrants perform aerobatic displays in premontane primary forest and navigate seasonal decisions between survival and reproduction that are affected by substantial non-breeding season rainfall. In Chapter 2, I studied the drivers of life history trade-offs affecting migratory behavior, and in Chapter 4, I examined populations along the species' range to better understand the effect of climatic factors or population genetic structure on sex chromosome-to-autosomal ratios. For Chapter 3, I performed a behavioral observational study examining the influence of rainfall and elevation on display rates in white-collared manakins (*Manacus candei*). White-collared manakins are known for their loud, mechanical "snap-jump displays", which provide an easily quantifiable metric of display rate. Based on their small size and range across multiple elevations, these birds are an excellent study system for determining how rainfall interacts with thermogenic stress to affect reproductive effort.

Across these studies, I ask how abiotic environmental conditions interact with intense sexual selection to shape individual life history decisions, reproductive behavioral ecology, and genomic patterns of variation. By investigating theoretical expectations at multiple levels of

biological organization, this research strengthens our understanding of the behavioral ecology and evolution of animals exposed to challenging environments.

Chapter 2 — Tests of three hypotheses explaining migratory variation in a facultative altitudinal migrant

Life history theory centers on animals' needs to balance current and future reproduction in the context of natural selection (Stearns, 1989, 1992; Williams et al., 2010). These trade-offs can play out through decisions that prioritize reproduction in the short term or promote survival and therefore preserve longer-term reproductive potential. White-ruffed manakins (*Corapipo altera*) are partial, facultative altitudinal migrants who offer a unique opportunity to test theoretical expectations about how animals balance reproductive effort and survival. I hypothesized that residual reproductive value, intrinsic quality, or seasonal carryover effects might affect males' annual migratory decisions. I tested these hypotheses via population- and individual-level predictions regarding how age and/or courtship display activity are associated with altitudinal migratory behavior. Contrary to the residual reproductive value hypothesis, migratory behavior across the adult population did not decline with age but instead remained consistent. Migratoriness was also unrelated to display rates at the population level, contrary to the expectations of both the intrinsic quality and seasonal carryover hypotheses. However, individual birds tended to be slightly more migratory following years in which they displayed more frequently, as predicted by the seasonal carryover hypothesis. While my results did not identify a single hypothesis that strongly explained variation in migratory behavior, these findings help narrow the range of likely explanations for facultative altitudinal migration in this study system.

Chapter 3 — Rainfall reduces reproductive effort at the wet end of a species' hygric niche

Hygric niche theory proposes several direct and indirect mechanisms by which rain may affect tropical endotherms. I empirically tested two direct mechanisms in an observational study of how white-collared manakins (*Manacus candei*) respond behaviorally to rainfall and to its interaction with low- and mid-elevation. Using audio recording devices and rain gauges set up at white-collared manakin display courts in Costa Rica, I analyzed occurrence and rates of rainfall at each of 13 display courts. I analyzed the relationship of these two rainfall metrics to court display rates using a zero-inflated Bayesian mixture model. Manakins reduced their display behavior by 31% when rain was falling, demonstrating that rainfall strongly inhibits reproductive effort in white-collared manakins. Contrary to the expectation that display rate would be negatively influenced by the interaction of rainfall and thermogenic stress at cooler higher-elevation sites, I found that display rate declined similarly at both low and mid elevations as rainfall increased. Notably, one outlier among 291 events reversed the interaction effect, emphasizing the complexity of disentangling the role of ecological factors in a system driven by interacting incentives and behavioral drivers.

Chapter 4 — Population genetic structure is a key factor when interpreting sex chromosome-to-autosomal diversity ratios

Selection and demography can leave informative signatures of evolution across the genome. One complex signature of evolution is the ratio between sex chromosome and autosomal diversity, which reflects differences between sex chromosomes and autosomes in their relative exposure to selection. Such diversity metrics can show evidence of processes such as selection, population bottlenecks, and speciation (Balakrishnan et al., 2026; Payseur et al., 2018; Wright et al., 2015). Birds have a ZW sex chromosome system in which males are homogametic (ZZ) and females are heterogametic (ZW); low Z/A diversity ratios are associated with strong reproductive skew, in

which reproductive success is concentrated among relatively few males. To disentangle how abiotic influences and population genetic structure affect Z/A diversity ratios, I performed whole-genome sequencing on 150 white-ruffed manakins and studied the patterns of the Z/A diversity metric across 15 sampling sites throughout Costa Rica and Panamá. If climatic factors such as precipitation and elevation (as a proxy for temperature) affected male patterns of tenure and reproductive opportunity, I expected that sampling site-level Z/A ratios may have directional relationships with six separate *a priori* climatic metrics after accounting for broad genetic cluster membership. I evaluated this hypothesis against the competing prediction that population genetic structure and history, measured through population structure analyses, genetic differentiation and absolute sequence divergence, play a strong role in driving Z/A ratios and evolution on the Z chromosome. Overall, I found little support for the hypothesis that climate regimes influence Z/A ratios. Instead, genetic cluster contributed a large proportion of the variance in models that reciprocally tested the relative contributions of six climatic metrics while addressing all sampling sites. A particular region on the Pacific slope of Costa Rica that appeared to be associated with a demographic event, such as a population bottleneck or founder effect, also showed a sharp decrease in Z/A diversity. This finding is consistent with theory: the Z chromosome is present in fewer copies in the population relative to the autosomes, meaning that demographic events and genetic drift can disproportionately affect Z-linked diversity and therefore cause Z/A ratios to retain striking signatures of population history (Irwin, 2018). While my current analyses cannot confidently distinguish between related phenomena such as population bottlenecks, founder effects, and genetic drift, I have found further empirical evidence suggesting that the Z/A metric may be used in conjunction with other metrics of evolutionary history to inspire hypotheses about historical demography.

Chapter 5 — Conclusions and Broader Implications

In my final chapter, I discuss the overall conclusions of my dissertation and the broader implications for evolutionary ecology. I consider how these findings inform our understanding of abiotic drivers of behavior and evolution, as well as future directions for rigorously testing theoretical predictions and disentangling climatic mechanisms in complex natural systems.

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Chapter 2 - Tests of Three Hypotheses Explaining Migratory Variation in a Facultative Altitudinal Migrant

Abstract

1. A critical challenge in life history theory involves understanding the drivers that influence individuals' life-history decisions. As facultative, partial altitudinal migrants, white-ruffed manakins (*Corapipo altera*) offer a unique opportunity to test which ecological and behavioral factors influence the prioritization of reproductive effort versus survival. In this Neotropical species, females prefer males that remain resident year-round at higher breeding elevations. However, uphill residency during the rainy, non-breeding season increases survival risks. It is unknown why males make different altitudinal migratory decisions each year.
2. We hypothesized that residual reproductive value, intrinsic quality, or seasonal carryover effects might affect males' annual migratory decisions. We tested these hypotheses via population- and individual-level predictions regarding relationships between age, courtship display activity, and altitudinal migratory behavior.
3. Over six breeding seasons in Costa Rica, we marked individual males and determined their known or estimated minimum age, collected behavioral data on display rates, and related these factors to isotope data derived from individuals' claw tissues. Isotopic analysis provided an index of migratoriness enabling comparisons within and among years.
4. Contrary to the residual reproductive value hypothesis, migratory behavior did not decline across adulthood, but instead remained consistent. Also at the population level,

migratoriness was unrelated to display rates as expected under the intrinsic quality and seasonal carryover hypotheses. At the individual level, we detected no decrease in migratoriness with increasing age in adult individuals. However, birds tended to be slightly more migratory following years in which they displayed more frequently, as predicted by the seasonal carryover hypothesis.

5. Our results did not identify a single hypothesis that consistently explained variation in migratory behavior, although we found some evidence that increased display rate may influence individual migratory decisions across years. These findings help constrain plausible explanations for facultative altitudinal migration that were not strongly supported in this study system. The contrast between our population- and individual-level findings also underscores the importance of testing competing hypotheses across biological scales.

Keywords: age; carryover effects; Neotropical birds; life history trade-offs; migratory decision-making; quality; residual reproductive value; stable isotopes

Introduction

Life history theory postulates that animals have limited energy, and that allocation to one component of fitness comes at a cost to others (Kleiber, 1975; J. B. Williams et al., 2010). At its core, this framework centers on animals' needs to balance current and future reproduction in the context of selective pressures. Other life-history trade-offs can be viewed as arising from this primary constraint, including species' allocation of resources between reproduction and growth, survival, and number and quality of offspring (Stearns, 1992; G. C. Williams, 1966). Identifying the factors that mediate such trade-offs is an ongoing challenge, in part because multiple intrinsic

and extrinsic factors interact to influence individual decisions in complex natural systems. However, populations with context-dependent behaviors provide a powerful framework for distinguishing among alternative hypotheses, as variation among individuals allows for direct evaluation of the factors that drive life-history decisions.

One hypothesis rooted in classic life history theory suggests that individuals' decisions change as they age due to declining chances of future reproductive opportunities. *Residual reproductive value* is the expected reproductive output remaining in an individual's life (Williams, 1966). Younger individuals have high residual reproductive value and are predicted to allocate to behaviors that increase survival and future reproductive opportunities at the expense of current reproduction. In contrast, older individuals are expected to allocate more heavily to current reproduction, even if it is risky, because they have fewer remaining chances to reproduce (Candolin, 1998; Fischer et al., 2008; Hall et al., 2015).

A second major factor influencing life-history decisions is an animal's intrinsic "quality". Quality can be defined as among-individual heterogeneity that is positively correlated with fitness (Wilson & Nussey, 2010). The *quality* hypothesis posits that some individuals appear to be good at everything. Greater resource acquisition allows these "high-quality" individuals to simultaneously invest in multiple costly traits or activities, thereby masking trade-offs that become apparent among lower-quality individuals (van Noordwijk & de Jong, 1986). For example, guppies in favorable environments can simultaneously exhibit higher growth, reproduction, and performance (Reznick & Endler, 1982), while in mute swans, longer-lived females lay earlier and larger clutches (McCleery et al., 2008).

Finally, animals' life-history decisions are often influenced by *carryover effects*, in which experiences during one stage of an animal's life influence performance in later life stages (Imlay

et al., 2021; Inger et al., 2010; O'Connor et al., 2014). Delayed costs of physiological or environmental stressors may result from a variety of factors, such as breeding-season effort or micronutrient exposure (Louhi et al., 2023; Lundsgaard et al., 2023; Saboret & Ingram, 2019). Seasonal carryover effects are those in which processes in one season influence individuals' success in the following season, such as when non-breeding season food availability influences subsequent breeding season behavior, or when body condition at the end of the dry season influences migratory tendency in the following season (Ceriani et al., 2015; Gaidet & Lecomte, 2013). Carryover effects may be especially influential when they limit the range of future decisions an individual can make while still surviving (Daan et al., 1996).

Birds are common models in the study of life histories as they exhibit a wide variety of life history strategies, are often conspicuous and diurnal, and have external development (Martin, 2004). As small-bodied endotherms, they are subject to considerable energetic and survival challenges of inclement weather that affect condition (Frazar, 1881; Tejeda-Cruz & Sutherland, 2005). In particular, migrant species are frequently subject to seasonal carryover effects that can influence allocation; for example, individual birds' late breeding efforts can lead to a decrease in stopovers during migration, putatively influencing condition upon arrival to their wintering grounds (Imlay et al., 2021). Migratory decisions can be influenced by the survival risk of not migrating, along with the reproductive risk of temporarily leaving one's breeding area (Hulthén et al., 2022; Ketterson & Nolan, 1983). Partial, facultative migrants are especially amenable to testing life history hypotheses; in partial migrants, some individuals migrate while others remain resident, and in facultative migrants, individuals adjust their migratory behavior across years based on ecological or physiological context (Chapman et al., 2011). Such traits allow

researchers to examine drivers of migratory variation, since individuals can make different decisions from year to year.

White-ruffed manakins (*Corapipo altera*) are small (~11 g), partial, facultative altitudinal migrants living in wet forests of Central America. Like others in the manakin family (Pipridae), male white-ruffed manakins are subject to strong sexual selection during the breeding season. Males engage in complex aerobatic displays in leks comprised of loose aggregations of multiple display logs within earshot of one another. Females select a mate and subsequently undertake all other reproductive duties (Jones et al., 2014; Rosselli et al., 2002). Following three distinct pre-adult plumage stages, four-year-old males molt into definitive adult plumage, gaining the opportunity to attract females if they can command a display log within a lek (Jones et al., 2014; Ryder & Durães, 2005). Males' aerial courtship displays include upright "butterfly" flights and high-speed dives from the canopy synchronous with acoustic signals. Displays appear to require skill and athletic ability, and males differ dramatically in their siring success and in the rates at which they display (Jones et al., 2014; Rosselli et al., 2002).

White-ruffed manakins' annual migration decisions reflect a trade-off between survival and reproduction (Boyle et al., 2011). During the rainy non-breeding season on the Atlantic slope of Costa Rica, higher breeding elevations receive twice the rainfall as adjacent lowlands. Migratory individuals arrive downhill to lowland sites between July and November and return uphill in February for the drier breeding season (Boyle et al., 2010b). White-ruffed manakins in poorer condition (i.e. lighter, molting later, higher ectoparasite loads) are more likely to migrate than those in better condition. Brightly-colored males are more likely to migrate than cryptic green females (Boyle, 2008). Additionally, males in their third year (on the cusp of gaining adult

definitive plumage and a display log), are the least likely plumage class of males to migrate (Boyle, 2008).

White-ruffed manakin migration is initiated synchronously with the onset of heavy storms at higher elevations. Resident manakins captured following rainstorms had greater baseline corticosterone, had more stored fat, and were mobilizing those fat stores more than migrant birds captured in the lowlands (Boyle et al., 2010b). These results suggest that rainfall presents a survival risk for these frugivorous birds, whose high metabolic rates and need to forage frequently may increase their vulnerability during prolonged or heavy storms (Boyle, 2008). Although altitudinal migration likely confers survival benefits, migrant males (as opposed to resident males) can suffer reproductive costs by losing social dominance at display sites and being less likely to attract females to display sites (Boyle et al., 2011). Classic work on partial migration has emphasized that such decisions can be shaped by a variety of mechanisms including density-dependence (Lundberg, 1987)**.** In frugivorous species, density dependence is commonly expected to operate through competition for food resources. However, white-ruffed manakins feed almost exclusively on small berries, which are more abundant at the breeding locations than at the elevations they migrate to (Boyle, 2010a). As such, density-dependent foraging success does not explain these downhill migrations. Furthermore, because the migrations occur during the non-breeding season, competition for mates or nest sites likewise cannot explain their movements. Thus, the specific mechanisms that influence the substantial variation in individual adult male migratory behavior remain unknown. Systems with facultative, partial migration provide a unique opportunity to infer the mechanisms underlying individual, context-dependent migration decisions, providing a window into the factors that lead to migration more broadly.

We aimed to understand variation in male migratory behavior by testing predictions of the (1) *residual reproductive value*, (2) intrinsic *quality*, and (3) seasonal *carryover* hypotheses. If *residual reproductive value* explains variation in migratory behavior among reproductively viable males (ages four and older), we expected that migratory propensity would decline with age as younger birds would be expected to prioritize survival over opportunities for reproduction. The intrinsic *quality* hypothesis predicts that high-quality individuals able to invest heavily in display behaviors would also be those able to allocate energy to maintaining residency, withstanding the rains of the non-breeding season. The seasonal *carryover* hypothesis predicts the opposite pattern; birds investing the most in breeding-season display should suffer condition-related costs of that effort, making them more likely to migrate downhill in the non-breeding season to avoid the stressors of high-elevation rainfall.

We used a six-year dataset of male display behavior and claw isotope data collected from the Caribbean slope of Costa Rica. We first determined whether overall interannual variation in migratoriness in white-ruffed manakins was correlated with non-breeding season rainfall at the higher elevation breeding location, as predicted previously (Boyle et al., 2010b). We then tested core predictions of our three hypotheses at the population level, using all sampled males to evaluate the roles of age and display rate in predicting migratoriness (predictions *a* and *b*). We tested individual-level predictions of our three hypotheses by relating individuals' migratoriness across consecutive years to adult age and to display rates (predictions *c* and *d*). Finally, we evaluated the generality of plumage-related differences in migration found previously (Boyle, 2008) using this larger dataset of individuals sampled in multiple years. Figure 2.1 illustrates the temporal and spatial structure of the study system and sampling efforts, depicting the alignment

between predictions and study design. Table 2.1 provides an overview of the alternative hypotheses, their corresponding predictions, and the outcomes of each analysis.

Table 2.1. Summary of ecological relationships tested to evaluate factors driving variation in male migratory behavior. Using both population- and individual-level predictions, we evaluated three alternative hypotheses explaining variation in annual migratory decisions of male white-ruffed manakins. Results are summarized in the right-hand column.

	Hypotheses			
Ecological Relationship	*(1) residual reproductive value*	(2) *intrinsic quality*	(3) seasonal *carryover*	Results
(a) Adult age and migratoriness during the following non-breeding season	Negative relationship between adult age (age 4 and older) and migratoriness	No explectation	No explectation	***No*** relationship between adult age and migratoriness
(b) Display rates and migratoriness during following non-breeding season	No explectation	Negative relationship	Positive relationship	***No*** relationship between display rates and migratoriness
(c) Age-related changes in individual birds' migratoriness across consecutive years	Decline in migratoriness across consecutive years among adult males	No consistent change	No consistent change	***No*** consistent change
(d) Correlates of individual change in migratoriness across consecutive years	No explectation	No explectation	Increase in migratoriness across consecutive years, correlated with higher display rates during intervening breeding season	Slight increase in individual migratoriness following years of higher display rates

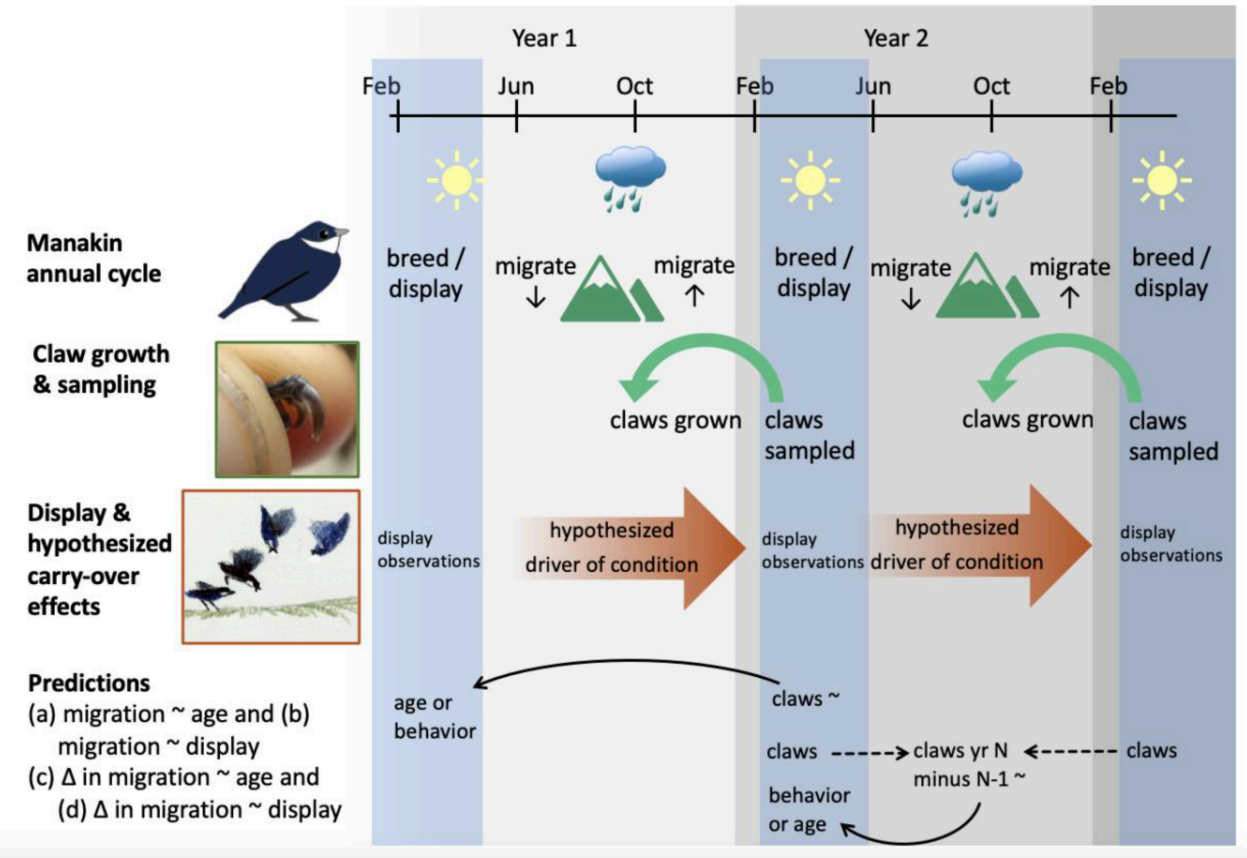


Figure 2.1. Diagram depicting the major events in the white-ruffed manakin annual cycle, including temporal patterns of claw growth and sampling, male breeding displays, and hypothesized mechanisms affecting migratoriness. Predictions indicate the temporal logic of expected outcomes for population-level (*a* and *b*) and individual-level (*c* and *d*) analyses.

Methods

Study Sites and Ethical Approval

We studied white-ruffed manakins from 2008–2013 at Rara Avis Rainforest Lodge and Reserve and the adjacent SelvaTica reserve (10°1703″N, 84°02047″W), located on the Caribbean slope of north-central Costa Rica. We collected behavioral data during the 2008–2012 breeding seasons and collected claw samples in 2009–2013 to learn about the birds' locations in the prior non-breeding seasons. The reserves span an elevational gradient of ~500 to 900 meters above sea

level and are contiguous with approximately 50,000 hectares of primary forest in Parque Nacional Braulio Carrillo. Mean annual rainfall at the higher-elevation breeding site is substantial at 8267 mm annually (Boyle et al., 2010b). Most of this annual rainfall occurs in the second half of the year, peaking in June–July and in December, corresponding with facultative downhill migration during the manakins' non-breeding season. Migratory individuals arrive to non-breeding lowland elevations (i.e. 0 to ~200 m.a.s.l.) between July and November and return uphill in February (Boyle et al., 2010b). Meanwhile, the driest months of February through April correspond with the manakin breeding season at higher elevations (Jones et al., 2014; Rosselli, 1994).

The Costa Rican authorities granted the following permits: 034-2008-SINAC, 176-2008-SINAC, 041-2010-SINAC, 061-2010-SINAC, 027-2011-SINAC, 049-2012-SINAC, and 005-2913-ACCVC-PI. Ethical approval was granted from the Animal Use Committee of the University of Western Ontario (2008-013-Guglielmo).

Manakin Capture and Aging

From February–June we captured manakins at breeding elevations (650–850 m a.s.l.) using mist nets placed near display logs and at fixed capture locations. Upon capture and claw sampling, we marked each bird with a unique combination of one numbered aluminum band and 1–3 colored plastic leg bands. We sexed birds based on plumage (Skutch, 1967), molt limit (Ryder & Durães, 2005), and interpubic bone distance (Mendenhall et al., 2010). We classified males as hatch-year (HY), second-year (SY), third-year (TY), or after-third-year (ATY, adult) based on plumage (Mendenhall et al., 2010). White-ruffed manakins exhibit delayed plumage maturation, with four distinct plumage classes that permit reliable age assignment through the first three years of life and classification of definitively plumaged males as at least four years old. Age classes followed

the calendar year, with individuals advancing to the next age class on 1 January. For all analyses, age assignments corresponded to the year of behavioral observation and subsequent migration rather than the year of claw sampling. Accordingly, isotopic values used to assess migratory tendency were derived from claws collected one year later and paired with the corresponding age and plumage assignments for the preceding behavioral year.

To ensure that uncertainty associated with estimating minimum ages did not obscure patterns in the data, we analyzed population-level prediction *a* regarding adult age using two datasets. We first used the full dataset including adult males with known and minimum estimated ages, and then a reduced dataset including only the known-aged adult males. We did not fit models to the known-aged dataset for the individual-level age-related prediction (*c*) because too few adults of known ages had been sampled in consecutive years. Finally, to address migratoriness based on plumage class, we used the full dataset of birds for which behavioral-year plumage classes could be assigned.

Behavioral Data

To quantify individual investment in courtship, we observed display behavior from camouflaged blinds placed 8-10 meters from display logs. We performed multiple 2- or 3-hour long observations at each display log each season between 0700 and 1500. Logs were observed at a variety of times on observation days. While there was a slight tendency for females to visit in the early afternoon, we did not note consistent daily patterns of activity amongst the males. Observers determined identities of attending individuals via their unique color band combinations and counted the number of times that each individual performed aerial display elements: butterfly flights, aerial flaps, and flap-chee-waahs (Jones et al., 2014). These maneuvers involve high-speed and specialized aerobatic skills compared to typical avian flight

behaviors, and we assume they are energetically expensive as demonstrated by physiological studies on related manakin species (Barske et al., 2014). We calculated individual-specific display rates for each breeding season by dividing the total number of individuals' aerial display elements by the number of hours of behavioral observation of that bird in that year.

Claw Sampling and Stable Isotope Analysis

We analyzed ratios of the stable hydrogen isotope deuterium relative to hydrogen in claw tissue. These deuterium ratios are expressed as δD, in parts per thousand [‰], relative to the Vienna Standard Mean Ocean Water (VSMOW) reference standard, in which less negative values indicate greater enrichment in deuterium. As moist air masses ascend mountain slopes, the heavier deuterium isotope disproportionately condenses and precipitates at lower elevations, resulting in deuterium-depleted precipitation at higher elevations (i.e., more negative δD values further from the reference standard; (Hobson & Wassenaar, 2008). Less negative δD values (closer to the reference standard) indicate that claw tissue was formed while birds spent relatively more time at lower elevations, where precipitation is enriched in deuterium relative to higher elevations. The δD difference over the relatively small elevational gradient is augmented by precipitation patterns; rainout leads to decreasing δD with increasing precipitation (Bowen, 2010). In our system, this depletion amplifies the elevational signal because higher breeding elevations receive over 8 meters of rain annually compared to approximately 4 meters annually at the base of the mountain (Boyle et al., 2010b). Previous empirical studies in white-ruffed manakins sampled at both higher breeding (~750 m) and lowland non-breeding (~50 m) sites demonstrated the following: (a) rainwater ~ elevation relationships (derived at four sampling locations) were highly linear and yielded an estimated 11.7‰ difference between the lowlands and breeding elevations, (b) claws of non-migratory frugivores of other species differed by 5-

10‰, and (c) there was a ~16‰ difference in δD between claw samples collected from white-ruffed manakins at the breeding as compared to low-elevation non-breeding sites. Those data, in combination with direct (recapture) evidence of birds making these migrations, support the inferences derived from δD in claws as an indicator of elevational movements (Boyle et al., 2011). This degree of isotopic differentiation observed between white-ruffed manakin breeding and non-breeding elevations is also broadly consistent with elevational differences reported in other tropical systems. For example, feather δD values in Andean hummingbirds decline significantly with elevation, with differences on the order of ~20–30‰ across ~3,000 m, illustrating that stable hydrogen isotopes can reliably track elevational variation (Hobson et al., 2003).

Migrant manakins join resident manakins at breeding elevations in late February to early March, corresponding to the start of the breeding season (Fig. 2.1). Animals incorporate the isotopic signatures of local precipitation during tissue growth, providing an integrated record of the elevation at which the tissue was formed (Hobson & Wassenaar, 2008). We captured individuals at breeding elevations as early as possible each season and clipped the distal 1.5 mm from the central claw on each foot. Because claws take approximately four months to grow, deuterium ratios in claw tips give an integrative, continuous index of the elevations at which they were grown during the 3-4 months prior to sampling (Fig. 2.1; Boyle et al., 2011; Hobson, 2005; West et al., 2006). Consequently, individuals exhibiting less negative δD values are inferred to have grown more of their claw tissue at lower elevations due to downhill altitudinal migration during the non-breeding season during previous months (Hobson & Wassenaar, 2008).

We surface-sterilized claws using a 2:1 methanol solution, air-dried them under a fume hood, and weighed out 350 mg of tissue into silver capsules. We used the comparative

equilibration method to determine the δD of non-exchangeable H in claws (Hobson & Wassenaar, 2008), which uses keratinous lab standards previously calibrated for δD of non-exchangeable H. Samples and standards were measured using high temperature (1350°C) flash pyrolysis of claw tissue and continuous flow-isotope ratio mass spectrometry. This was performed at Environment and Climate Change Canada's Stable Isotope Hydrology and Ecology Research Laboratory and at the Northern Arizona University Stable Isotope Lab. Values are reported in δ notation as $\delta D = [(R_{sample}/R_{standard}) - 1] \times 1000$ ‰, where R is the $^{2}H/^{1}H$ ratio of the sample relative to the international standard VSMOW laboratory standards (Hobson & Wassenaar, 2008).

Statistical Analyses

We performed all statistical analyses in R (version 4.4.2, R Core Team, 2021), and visualized results using the packages ggplot2, RColorBrewer, and visreg (Breheny & Burchett, 2017; Neuwirth, 2022; Wickham, 2016). To test our core predictions, we separately analyzed two continuous response variables: δD (population-level predictions a and b), and year-to-year changes in δD (individual-level predictions c and d). We used linear models to examine their relationships with adult age and/or display rate, with model structure determined by whether predictors were continuous, categorical, or a combination of both. We treated year of claw sample, age, and plumage class as factors throughout the analyses.

Prior to analysis, we removed observations that appeared to represent biologically implausible measurements. Two claw δD values (−20‰ and −80‰) from separate birds were inconsistent with previously reported mean claw δD values for white-ruffed manakins sampled across this elevational gradient (approximately −55‰ and −71‰ for birds sampled at low and breeding elevations, respectively; Boyle et al., 2011). Upon later investigation, we confirmed

that these observations also fell at least three standard deviations outside the distribution of the remaining observations and were biologically unlikely given the expected isotopic variation across the elevational gradient in this system. While we cannot conclusively determine their origin, such extreme values may reflect analytical artifacts or sample-specific issues associated with keratin-based isotope measurements. Following exclusion of these outliers, we conducted model diagnostics to check assumptions of linearity, homogeneity of residual variance, and normality of residuals. We also checked all models for multicollinearity and for leverage points.

We expected that there may be interannual differences in overall propensity to migrate and therefore annual δD values (Hobson & Wassenaar, 2008). Thus, we conducted a one-way analysis of variance (ANOVA) with δD as the response variable and year of claw sample as the categorical predictor. To determine whether non-breeding season rainfall at higher (i.e. breeding) elevations was associated with interannual variation in downhill migratoriness, we assessed the relationship between cumulative July through December rainfall each year (2008–2012) at Rara Avis (collected daily via a manual rain gauge) and individuals' claw δD values collected from 2009–2013. This non-breeding season period from July through December is associated with notably increased rainfall at the Rara Avis breeding location and is hypothesized to cause the stressors leading to altitudinal migration in this system (Boyle et al., 2010b).

Because claws continue to grow following uphill migration, isotopic signatures of migration are expected to decay over time. To evaluate how the relationship between δD values and ordinal date varied across years, we fit separate linear models for each year of claw sampling with δD as the response variable and ordinal date as the predictor.

To test prediction *a*, we fit a one-way ANOVA with δD as the response variable and categorical adult age as the predictor. We first used the full dataset of birds who were at least

four years old in the year that we quantified display behavior, then fit a similar model including only adults with known ages. To test prediction *b*, we regressed the δD data from individuals' claw samples with display rate data from the previous year. Additionally, we analyzed both age and display rate in a single model to determine whether our conclusions differed when we accounted for both factors simultaneously.

Approximately 60% of individuals were sampled in more than one year, resulting in a mixed data structure. To evaluate potential nonlinear (e.g., curvilinear) relationships between age and deuterium values (prediction *a*) while accounting for repeated sampling of individuals across years, we additionally fit generalized additive mixed models (GAMMs) using the *mgcv* package in R. We modeled age as a penalized smooth term to test for nonlinear age effects and included individual bird identity as a random effect. For the display rate analysis (prediction *b*), we also modeled display rate as a linear predictor and again included individual identity as a random effect to account for repeated measures across years. We fit models using restricted maximum likelihood. Because both modeling approaches led to the same conclusions regarding our main predictions and fixed-effect estimates, we excluded random effects from population-level analyses *a* and *b* (Zuur, 2009). We therefore treated repeated observations from the same individuals in different years as independent samples.

To test the age- and display-related predictions regarding individual-level change in migratoriness (*c* and *d*), we first extracted data for individuals for whom we had claw samples in more than one year. We then regressed each bird's δD values from a given year against ordinal date for that year to obtain a residual δD value representing a year-specific standardized index of migratoriness. We calculated the difference in the standardized δD between pairs of subsequent years for each individual, with negative values indicating a shift toward residency, and positive

values indicating a shift toward migratoriness. We tested prediction *c* by regressing the standardized δD differences from year to year against individuals' adult ages, and tested prediction *d* by regressing these standardized δD differences against individuals' display rates during the intervening breeding season. Finally, to verify previous findings of plumage class differences in male migratory tendency (Boyle, 2008), we fit a one-way ANOVA comparing δD values among four categorical plumage classes: hatch-year, second-year, third-year, and adult (after-third-year or ATY) males.

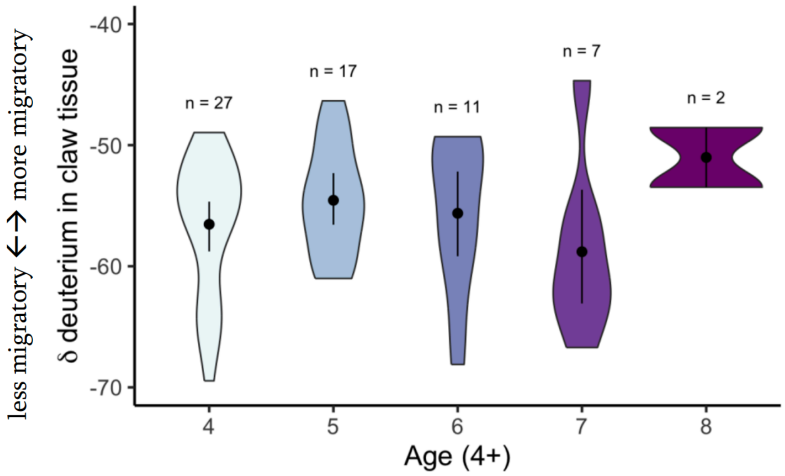
Based on the distinct slopes for δD and ordinal date amongst different years, we tested additional models for predictions *a* and *b* which included year, ordinal date, and an interaction between ordinal date and year. In one case, the sample size for the subset of known-aged individuals made the model unreliable, so we did not proceed with the analysis including the interaction term. We did the same with our model regarding birds of distinct plumage classes. Since these temporal aspects did not affect our results, we present those from the simpler models here but provide those additional results in the Supplementary Information.

We interpreted statistical results primarily based on estimated effect sizes and associated uncertainty, rather than relying solely on dichotomous significance thresholds. We avoided strong biological inference when model support was limited, interpreting estimates cautiously when they involved substantial uncertainty. When biologically relevant patterns were suggested in small datasets, we presented these results as tentative evidence and as hypotheses warranting further investigation rather than as strong evidence of an effect. Sample sizes for tests of different predictions varied because we did not observe display behavior in the year prior to capture for every individual for which we had obtained claw isotopic data.

Results

Between 2009–2013, we collected 1055 claw samples and analyzed isotope data from a subset of 333 of individuals of multiple ages and both sexes. For this study, we were able to analyze data from 52 individual male white-ruffed manakins (107 claw samples) for which we had also located the corresponding display log and quantified behavioral observations from the preceding breeding season. Thirty-one of the 52 individuals were sampled in multiple years; 86 of our 107 claw samples were from individuals sampled more than once. Of the 107 claw measurements, 61 came from (30) males of known ages and 46 samples came from (22) males for which we calculated minimum ages. In the full dataset including both known age and minimum-estimated-age males, two birds were at least eight years old during the behavioral year (the year in which display behavior and subsequent migration occurred)**.** Known-aged birds ranged from 1–7 years old during the behavioral year**,** corresponding to 2–8 years old in the year of claw sampling. We related 66 claw δD measurements to display rates from the prior year. Males differed greatly in their display rates, with estimates ranging from 0–16.8 display elements per hour. We obtained an average of 16.2 hours of observation data from each individual displaying in a given breeding season (mean ± SE = 16.2 ± 1.6 h). Claw δD values ranged from -69.46 to -42.25.

A.



B.

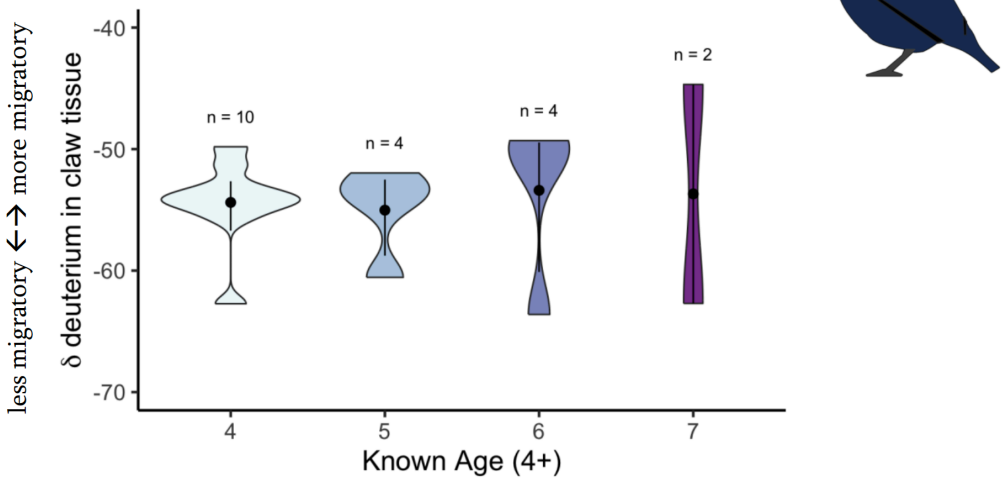




Figure 2.2. Relationship between adult age during the breeding season and δ deuterium values during the subsequent migratory period that same year. By the time white-ruffed manakins reach adulthood (after-third year; ATY), males have glossy blue-black plumage with a white ruff and may command a display log where they court females. **A**: Relationship between adult age and migratoriness for the full dataset of adults with known or minimum estimated behavioral-year ages (n = 64 claw samples). Ages were assigned based on the behavioral year rather than the year of claw sampling. **B**: Relationship between adult age and migratoriness for the subset of known-age adults only (n = 20 claw samples). Higher (less negative) δ deuterium values are associated with greater migratoriness to lower elevations during the rainy non-breeding season.

Mean values of δD differed somewhat among years (Supp. Fig. S2.1). We found inconsistent relationships between claw sampling dates and δD values for different years; in 2010 and 2012, δD values were significantly related to ordinal date, but in 2009, 2011, and 2013, they were not (Supp. Table S2.1; Supp. Fig. S2.2). In analysis of all individuals, cumulative non-breeding season rainfall per year collected at the breeding site (Rara Avis) explained very little of the inter-annual variation in δD ($F_{1, 105} = 0.8$, $P = 0.383$, $r^2 = 0.01$).

Under the residual reproductive value hypothesis, we predicted a decline in migratory behavior with adult age (prediction *a*). We found no evidence that migratory behavior varied with adult age in either the full dataset of known and minimum estimated aged individuals, (prediction *a*; $F_{4,59} = 1.2$, $P = 0.326$, $r^2 = 0.08$; Fig. 2.2A) nor in analyses of known-aged adults only ($F_{3,16} = 0.1$, $P = 0.974$, $r^2 = 0.01$; Fig. 2.2B). Contrary to predictions of both the seasonal carryover and quality hypotheses, δD values were unrelated to display rate at the population level (prediction *b*; $F_{1, 64} = 0.1$, $P = 0.798$, $r^2 = 0.001$; Fig. 2.3). Adding age to this model did not influence this relationship ($F_{5,51} = 0.9$, $p = 0.469$, adjusted $R^2 = -0.01$).

Among individuals, migratoriness across consecutive years did not decrease with adult age (prediction *c*; $F_{4, 29} = 0.8$, $P = 0.549$, $r^2 = 0.10$). However, individuals that exhibited higher display rates during a given breeding season tended to be more migratory the subsequent year (prediction *d*; $F_{1, 35} = 3.6$, $P = 0.067$, $r^2 = 0.09$; Fig. 2.4). Finally, males of different plumage classes (i.e., HY, SY, TY, ATY) did not differ significantly in their mean δD values ($F_{3, 103} = 1.8$, $P = 0.143$, $r^2 = 0.05$; Fig. 2.5A).

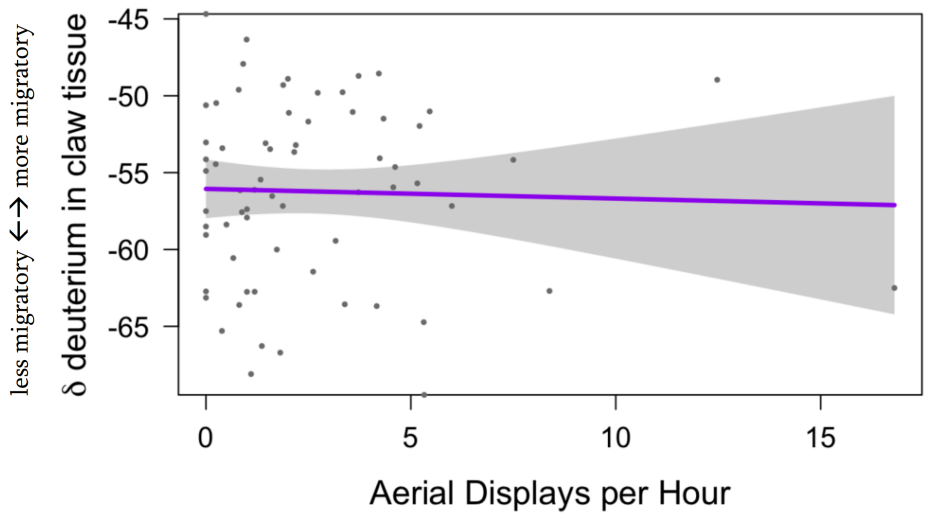


Figure 2.3. Relationship between males' aerial displays per hour performed during the breeding seasons and their claws' δ deuterium values representing migratoriness during the following non-breeding seasons (n = 66 comparisons between display rates and δ deuterium values). Higher (less negative) δ deuterium values are associated with migratoriness to lower elevations during the rainy non-breeding season.

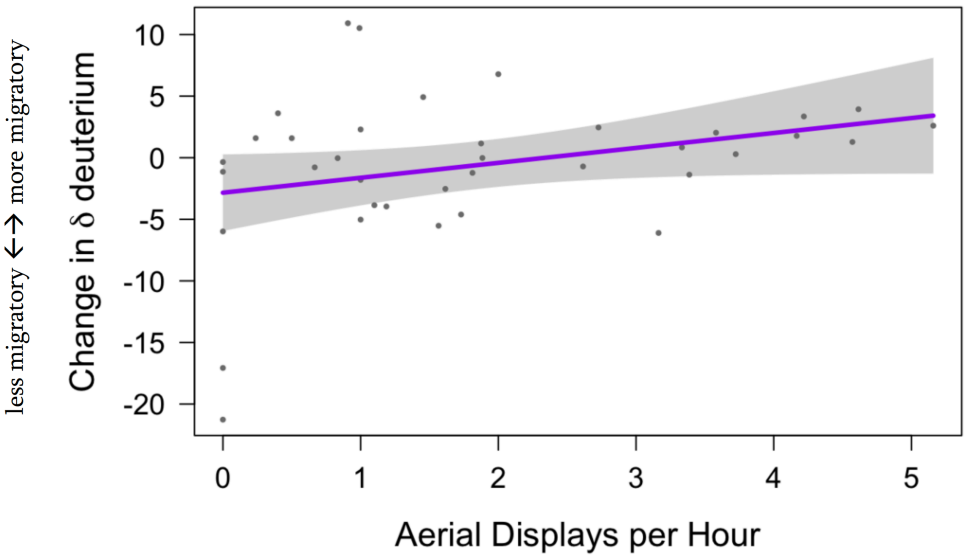


Figure 2.4. Individual year-to-year changes in δ deuterium values as compared to aerial displays per hour during the intervening breeding season (n = 37 individual males' claws sampled in consecutive years). Positive values (above 0) indicate a shift toward downhill migratoriness from year to year, while negative values indicate a shift to breeding-elevation residency.

Results were substantively unchanged when we modeled age using GAMMs that allowed for nonlinear age effects and included individual identity as a random effect. We found no relationship between migratoriness and either age among all adults (F = 1.08, $P = 0.507$) or known age among the subset of known-age individuals (F = 0.09, $P = 0.767$). Likewise, display rates were not associated with migratoriness after accounting for repeated observations of individuals (β = -0.04 ± 0.23 SE, P = 0.864).

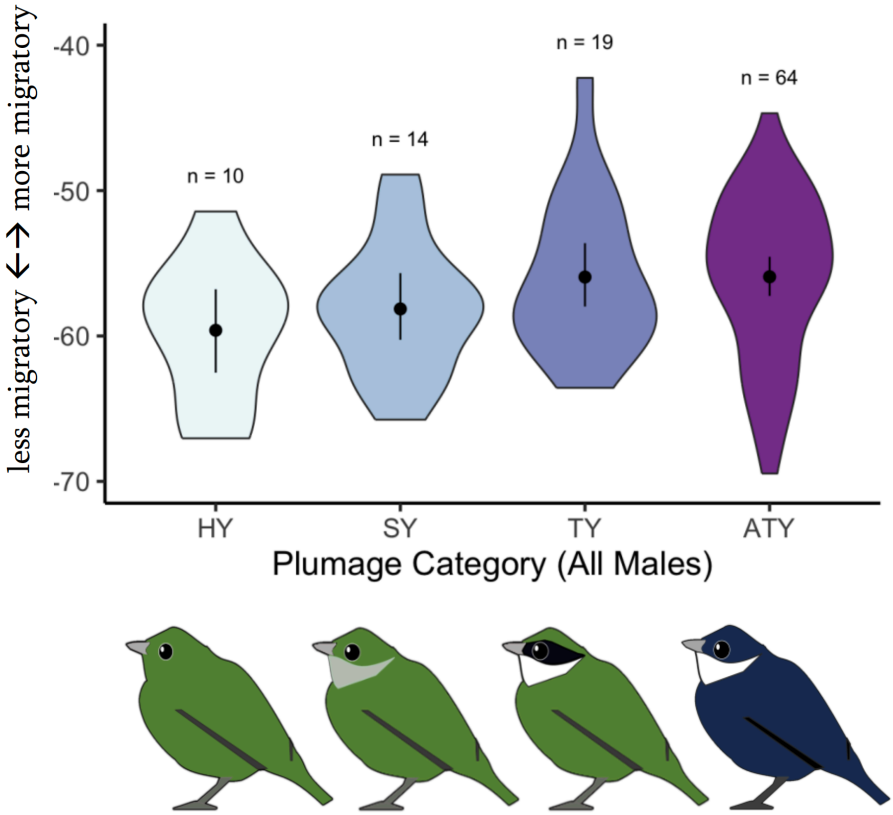


Figure 2.5. Relationship between plumage class during the breeding season and δ deuterium values representing the subsequent migratory period. By the time white-ruffed manakins are adults (after-third year; ATY), males have glossy blue-black plumage with a white ruff and may command a display log where they court females. Plumages were assigned based on age during behavioral year rather than the year of claw sampling. Higher (less negative) δ deuterium values are associated with greater migratoriness to lower elevations during the rainy non-breeding season.

Discussion

The results of this study did not conclusively support any one of our three hypotheses explaining variation among male white-ruffed manakins in migratory behavior (Table 2.1). Migratoriness did not decrease throughout adulthood, as we had predicted based on the residual reproductive value hypothesis. Male breeding season display rates were neither positively nor negatively related to their downhill migratory propensity in the following non-breeding season, as we had predicted under the intrinsic quality and seasonal carryover hypotheses, respectively. Analyses at the individual level also failed to support the residual reproductive value hypothesis, since individual adult males sampled in consecutive years did not become less migratory based on increasing age. Only the seasonal carryover hypothesis received limited support at the individual level.

What factors explain individual variation, then? Our results are not consistent with our initial predictions based on residual reproductive value (i.e., a steady decline in migratory propensity with age). It is possible that studies capable of tracking a larger sample of old individuals would detect age-related patterns consistent with the residual reproductive value hypothesis. However, we also found no evidence that males with higher display rates were less migratory, as predicted under the quality hypothesis. We used display rate as a proxy for quality, but this metric may not capture the most critical traits relevant to male quality and associated fitness. Female preference in some manakin species has been linked to traits such as display speed or accuracy (Barske et al., 2011), while siring success in blue-crowned manakins (*Lepidothrix coronata*) is associated with vocalization rates within specific leks (Durães et al., 2009). Variation in quality may still contribute to migratory decisions in this system, but our results suggest that these relationships may rely on a broader set of variables than we evaluated

here. In addition, differences in resource acquisition may obscure expected quality-related tradeoffs among individuals (van Noordwijk & de Jong, 1986), making the quality hypothesis difficult to evaluate using a single proxy. Reproductive success in manakins likely depends on multiple interacting factors, including age, experience, display performance, and the acquisition or retention of display sites. Because these components are likely to change over time, a single measure of display rate may not fully capture the aspects of individual quality that influence migratory decisions.

We detected a signal of seasonal carryover effects associated with display activity within individual white-ruffed manakins. Birds with higher display rates tended to show greater year-to-year shifts toward downhill migratoriness, with display rate accounting for 9.3% of the variance in isotopic value differences across consecutive years. We interpret this pattern cautiously, however, as the estimated effect was modest relative to the unexplained variability in the model. Despite the modest effect size, the relationship is noteworthy because its direction is consistent with predictions from life-history theory in a species shaped by both natural selection associated with migration and strong sexual selection on reproductive behavior.

Although we did not find evidence for differences in migratoriness among adults of different ages, our larger study allowed us to re-evaluate previous findings related to plumage class and migratory tendency. Earlier findings using migratory data from a single non-breeding season indicated that among male white-ruffed manakins, adults (after-third-years) are most likely to migrate, followed by birds in green plumage (hatch and second years), with third-year males being the least likely to migrate (Boyle, 2008). These previous results were interpreted as reflecting a tendency for third-year males to maintain residency in anticipation of molting into definitive adult plumage that year and becoming more competitive in obtaining a display site and

attracting females the following breeding season. Although we did not detect statistically supported differences among plumage classes, mean δD values in our larger six-year dataset tended to increase from hatch-year through third-year males before leveling off between third-year age and adulthood, a qualitative pattern that differed from Boyle (2008). Male manakins' migratory decisions may change across the years in ways consistent with the changing predation risk associated with a shift from highly cryptic green coloration to bright, contrasting blue-black and white plumage (Kirwan & Green, 2012). Females and other green-plumaged birds can carry high fat loads during adverse conditions (Boyle, 2008) which may help them survive rain storms, but also would slow take-off speeds and maneuverability which would increase predation risk (Gosler et al., 1995; Rogers, 2015).

By focusing on a partially migratory species subject to conflicting selection pressures, we were able to test alternative hypotheses to better understand context-dependent life history decisions. Recent reviews on differential migration emphasize the importance of focusing on competing, non-overlapping hypotheses to evaluate migratory decision-making (Paprocki & Conway, 2025). In partially migratory populations of fishes and other animals, it is widely accepted that multiple competing factors are at play as individuals evaluate the costs and benefits of different strategies that maximize lifetime reproductive effort (Brönmark et al., 2014; Chapman et al., 2011). In American kestrels (*Falco sparverius*), partial migration varies with factors including sex, body size, and winter weather, with individuals switching strategies from year to year (Ranck et al., 2023). In partially migratory red kites (*Milvus milvus*), multiple factors affecting migratory phenotype include both body size and arrival time on the breeding grounds (Witczak et al., 2024). In other words, partial migration is often condition-dependent, affected by

interacting effects of intrinsic states (e.g. sex, age, body size/condition) and extrinsic contexts (e.g. weather, resource availability, predation risk), rather than being mediated by a single driver.

Interestingly, we found little evidence supporting the residual reproductive value or intrinsic quality hypotheses, and only limited support for the seasonal carryover hypothesis at the individual level. Although no single hypothesis consistently explained migratory variation, evaluating these competing alternatives narrows the range of likely mechanisms operating in this system while identifying a plausible role for seasonal carryover effects that merits further investigation. To the extent that this pattern reflects an underlying biological process, variation in migratory behavior may be influenced by male investment in display, which presumably is or has been under strong directional selection via female choice (Barske et al., 2011; DuVal & Kempenaers, 2008). Thus, investment in display may come at a cost that plays out during the subsequent rainy season and influence the complex decisions that individuals make regarding altitudinal migration. However, carryover effects appear to be subtle, context dependent, or obscured by substantial background variability in natural systems. Our results emphasize the importance of evaluating partial migration hypotheses at both the individual and the population level, while also demonstrating how evaluating multiple hypotheses can be particularly informative in complex ecological contexts.

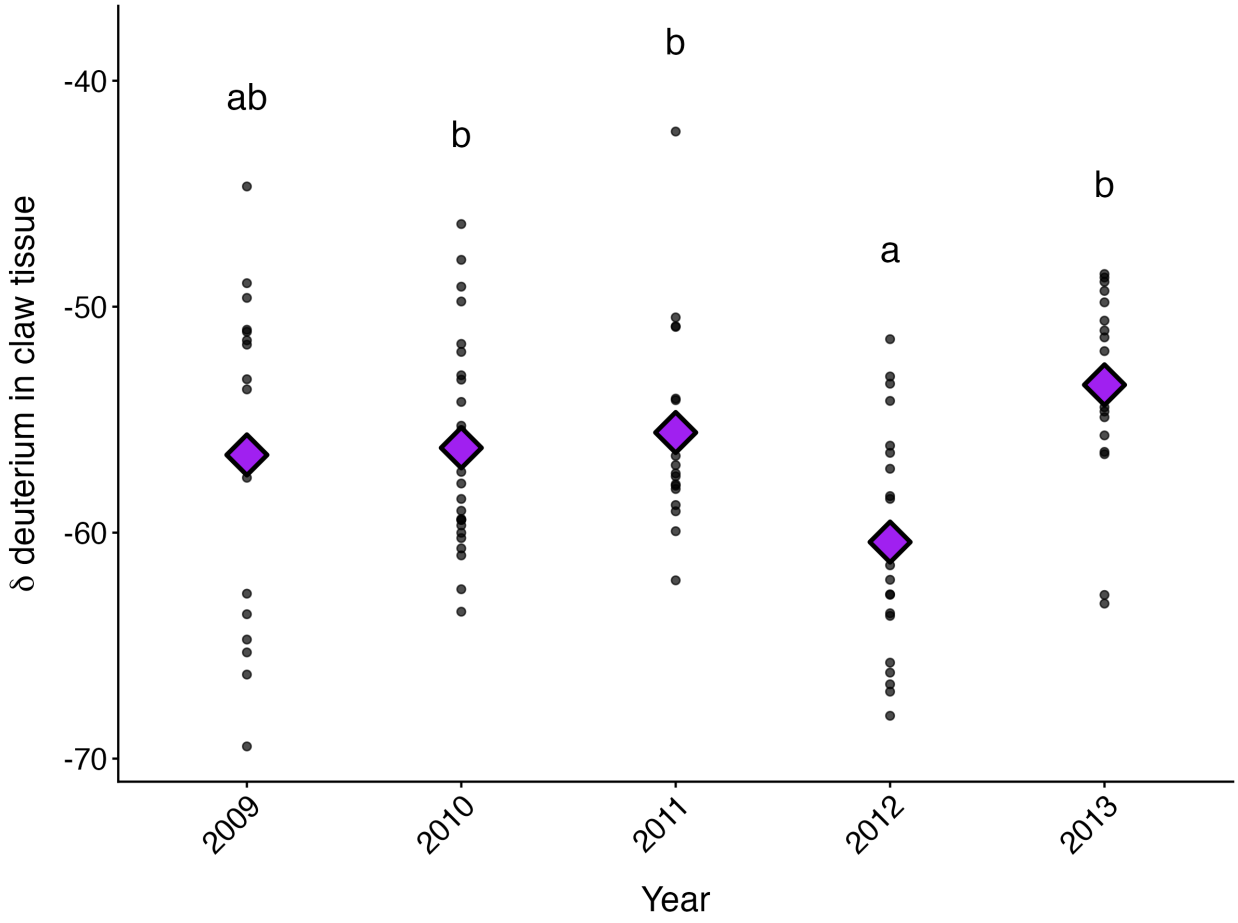


Figure S2. 1. Variation in δ deuterium proportion in claw samples by year. White-ruffed manakins were captured at a mid-elevation site on the Caribbean slope of Costa Rica (n = 107 claw samples from 52 individual male birds). Mean δ deuterium values are shown in purple diamonds for each year; means with the same letter do not differ from one another ($P < 0.05$). Higher (less negative) δ deuterium values indicate that birds were more migratory in this system (moving downhill to less rainy sites).

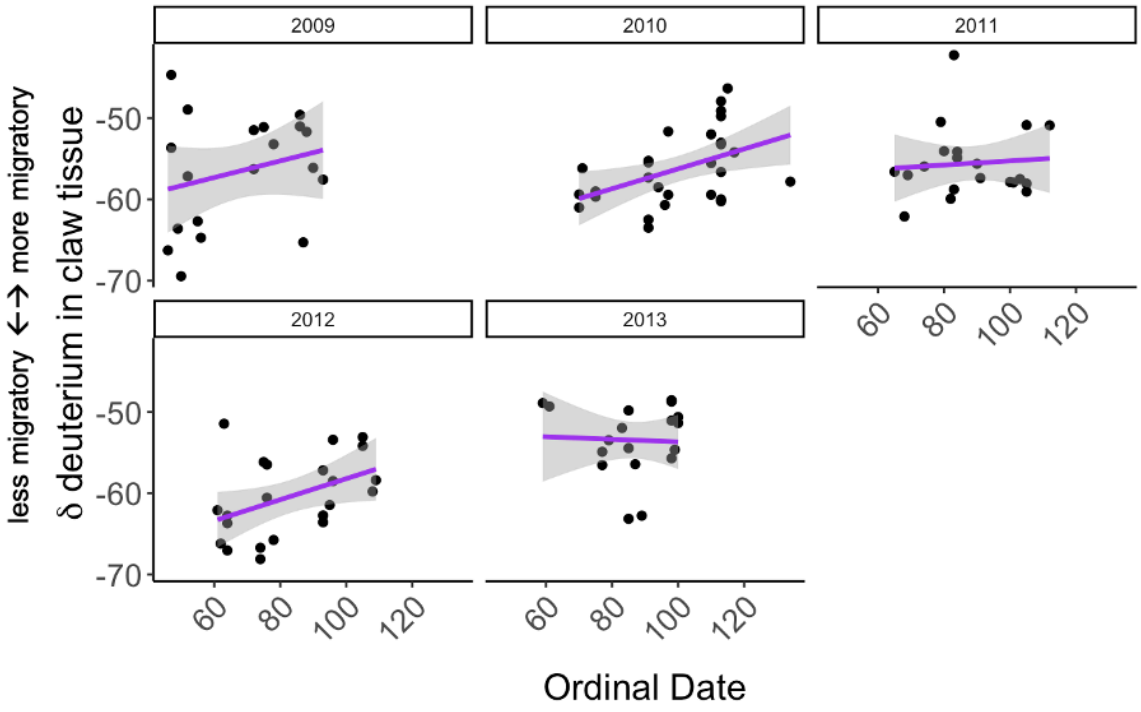


Figure S2. 2. Relationship between δ deuterium in claw samples and ordinal date of claw sampling by year. White-ruffed manakins were captured at a mid-elevation site on the Caribbean slope of Costa Rica (n = 107 claw samples from 52 individual male birds). Higher (less negative) δ deuterium values indicate that birds were more migratory in this system (moving downhill to less rainy sites).

Table S2. 1. Relationship between ordinal date of claw sampling and δ deuterium (migratoriness) values for each year of claw sampling (δD ~ Ordinal Date). White-ruffed manakins were captured at a mid-elevation site on the Caribbean slope of Costa Rica (n = 107 claw samples from 52 individual male birds).

Year	Estimate	SE	t	P	r^2	DF
2009	0.102	0.093	1.1	0.285	0.07	17
2010	0.122	0.047	2.6	0.014	0.21	26
2011	0.025	0.073	0.3	0.734	0.01	18
2012	0.129	0.060	2.2	0.043	0.19	20
2013	-0.016	0.087	-0.2	0.857	0.00	16

Table S2. 2. Relationship between adult age and δ deuterium values from claw tissues, using the full dataset of adult males with known ages and with minimum estimated ages. White-ruffed manakin claw samples were obtained at a mid-elevation site on the Caribbean slope of Costa Rica.

Predictor	DF	Sum Sq	F	P
Age (4+)	4	50.8	0.5	0.726
Year	4	339.2	3.4	0.015
Ordinal Date	1	208.0	8.4	0.006
Year*Ordinal Date	4	144.6	1.5	0.228

The simpler model including adult age only (δD ~ Adult Age) is summarized in the main text ($F_{4,59}$ =1.2, P = 0.326, r^2 = 0.07). The table presents the full model including adult age, ordinal date, year of claw sample, and their interaction (δD ~ Adult Age + Ordinal Date * Year). Full model: $F_{13,50}$ = 2.4, P = 0.015, adjusted R^2 = 0.22.

Table S2. 3. Relationship between display rate (aerial displays per hour) and δ deuterium values from claw tissues. White-ruffed manakin claw samples were obtained at a mid-elevation site on the Caribbean slope of Costa Rica.

Predictor	Estimate	SE	*t*	*P*
Aerial displays per hour	0.074	0.254	0.3	0.772
Ordinal Date	0.107	0.071	1.5	0.139
2010	-32.669	21.159	-1.5	0.128
2011	23.074	15.377	1.5	0.139
2012	-13.049	9.670	-1.3	0.183
2013	13.100	10.423	1.3	0.214
2010*Ordinal Date	0.291	0.204	1.4	0.159
2011*Ordinal Date	-0.274	0.176	-1.6	0.125
2012*Ordinal Date	0.086	0.117	0.7	0.463
2013*Ordinal Date	-0.138	0.127	-1.1	0.284

The simpler model including display rate only (δD ~ Aerial displays per hour) is summarized in the main text ($F_{1,64}$ = 0.1, P = 0.798, r^2= 0.00). The table presents the full model including aerial displays per hour, ordinal date, year of claw sample, and their interaction (δD ~ Aerial displays per hour + Ordinal Date * Year). Full model: $F_{10,55}$ = 2.6, P = 0.011, adjusted R^2 = 0.20.

Table S2. 4. Relationship between plumage classes (HY, SY, TY, and ATY) and δ deuterium values from white-ruffed manakin claw tissue samples. Both models include the full dataset of males with assigned plumage classes. White-ruffed manakin claw samples were obtained at a mid-elevation site on the Caribbean slope of Costa Rica.

Predictor	DF	Sum Sq	*F*	*P*
Plumage Class	3	132.9	1.9	0.138
Year	4	470.7	5.0	0.001
Ordinal Date	1	151.6	6.4	0.013
Year*Ordinal Date	4	85.0	0.9	0.466

The simpler model including plumage class only (δD ~ Plumage Class) is summarized in the main text ($F_{3,103} = 1.8$, $P = 0.143$, $r^2 = 0.05$). The table presents the full model including plumage class, ordinal date, year of claw sample, and their interaction (δD ~ Plumage Class + Ordinal Date * Year). Full model: $F_{12,94} = 3.3$, $P = 0.001$, adjusted $R^2 = 0.21$.

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Chapter 3 - Rainfall Reduces Reproductive Effort at the Wet End of a Species' Hygric Niche

Abstract

Thermal performance curves are common tools for predicting how climate change may affect species' behavior, physiology, and demography. As the unpredictability and severity of weather events increase due to anthropogenic influences, precipitation is emerging as a critical abiotic factor with effects on endotherm behavior and physiology that remain largely unknown. Just as endotherms in arid environments are negatively influenced by drier seasons and respond positively to wetter years, theory predicts that endotherms in wet environments may experience negative effects when exposed to greater-than-normal rainfall. However, mechanisms linking rain to population dynamics are largely unknown. We tested predictions of one hypothesized mechanism linking rain variability to population limitation by determining how the reproductive activities of a small Neotropical bird, white-collared manakins (*Manacus candei*), respond to rainfall at low- and mid-elevation display courts in Costa Rica. First, we predicted that white-collared manakins would decrease the frequency of display behavior in the presence of rainfall. Second, because of increased thermogenic costs of cooler temperatures at higher elevations, we also predicted that display rates would decline under rainy conditions more quickly at mid elevations than at low elevations. We quantified display activity at 13 courts for a total of 683 hours over 287 recording sessions. Using a zero-inflated Bayesian mixture model, we found that displays decreased by 31% during rainfall, consistent with the first prediction. However, display rate declined similarly at both low and mid elevations as rainfall increased, but the decline was slightly steeper at low elevation display courts. Our findings demonstrate how individual-level

behavioral modifications may scale up to population-level processes by limiting reproduction and constraining distributions of taxa living in wet environments.

Keywords: climate change, display behavior, elevation, endotherm physiology, hygric niche, lek mating systems, Pipridae, precipitation, rain, thermogenic costs

Introduction

Understanding how abiotic factors affect animal behavior is a central goal of ecological research (Rahman & Candolin, 2022; Wingfield, 2013). While ecologists have long benefitted from a detailed understanding of animal thermal performance curves, far less is known about how and why precipitation affects animals. Recently, the concept of hygric niches was described as a framework for understanding how and why endotherm populations respond to temporal variation in rainfall (Boyle et al., 2020). Just as animals have optimal temperature ranges, they are expected to exhibit peak performance in the middle of the precipitation conditions they can tolerate (Boyle et al., 2020). In experimental rainfall conditions, colder precipitation increases metabolic costs and likely prompts behavioral changes prior to exhibiting physiological effects (Karr et al., 2025). However, we so far know relatively little about the mechanistic bases of the effects of precipitation, especially in tropical species. A key prediction of hygric niche theory is that in very wet environments, wetter than average conditions or heavy rainfall events will result in negative responses at individual and population levels (Boyle et al., 2020). There are multiple potential mechanisms linking rainfall to those responses, and few empirical studies which test those alternative pathways. As tropical endotherms experience increasing frequency and severity of weather events due to anthropogenic climate change, understanding how and why increased precipitation influences behavior and physiology is urgent (Boyles et al., 2011).

Heavy rainfall can have direct, adverse effects on endotherms via direct wetting and humidity, both of which can increase costs of thermoregulation. Water infiltration into the integument (feathers or fur) drastically reduces heat retention. Furthermore, active plumage or pelage compression to promote water shedding further exacerbates heat loss by reducing the depth of the insulating boundary layer (Karr et al., 2025). In some taxa, direct wetting can result in a 50% reduction in insulative capacity (McCafferty et al., 1997). Additionally, high humidity limits the efficacy of evaporative cooling, further constraining an endotherm's ability to regulate body temperature (Monteith et al., 2013). Even under the relatively mild conditions of low-elevation tropical forest, birds are affected by humidity and temperature (M. T. Freeman et al., 2024). Endotherms may respond to physiological stressors by first modulating their behavior to avoid the stressor itself (Willmer et al., 2004), such as avoiding exposing themselves to the costs of wetting (Foster, 1974). When such behaviors involve forgoing reproduction, they provide a link between individual- and population-level responses to rain.

Rain should affect endotherms differently depending on temperature, and therefore, elevation (M. T. Freeman et al., 2024). This is because temperature decreases with increasing elevation, about 0.6º C per 100 m in humid air, and energetic costs increase as ambient temperatures diverge from internal temperatures (Scholander et al., 1950). Thus, at higher elevations under cooler temperatures, thermogenic costs of rain should be greater than at lower elevations (Karr et al., 2025; Monteith et al., 2013) and the behavioral responses to rain should be more pronounced at higher elevations.

We studied the influence of rain on reproductive behavior in white-collared manakins (*Manacus candei*). These passerines are a member of the Neotropical family of manakins (Pipridae) known for their exuberant male mating displays. In Costa Rica, white-collared

manakins are found in wet forests from sea level to 700 m above sea level (Stiles & Skutch, 1989), with more rain falling at higher elevations. White-collared manakins are members of the well-studied genus *Manacus* (Schlinger et al., 2013), whose loud, mechanical "snap-jump displays" provide an easily quantifiable metric of display rate. Males attend to their display courts and perform breeding displays for females year-round. The small body size of this species (~18.5 g) suggests a limited thermal inertia, meaning metabolic costs and thus, behavioral consequences, of rainfall are likely to be greater than in larger birds (Willmer et al., 2004).

We quantified rainfall and associated display rates in July and August of 2022, using rainfall measurements and audio recordings at each display court microsite. We used behavioral observations to quantify auditory thresholds that represent focal displays at each court. We related display rates to both the *occurrence* and *intensity* of rainfall. We predicted that (1) all manakins would decrease their courtship display rates in the presence of rain. We also predicted that (2) rainfall intensity would be negatively associated with display rates, and that displays would decline more sharply at higher elevations due to the increased thermogenic costs of rain in cooler environments.

Methods

Data Collection

White-collared manakins range from southeastern Mexico to the western edge of Panama. Males are common in low secondary-growth thickets and recently disturbed tracts of land where they create display courts (Schlinger et al., 2013). Generally, display courts are composed of 1-3 cleared areas on the forest floor framed by small vertical saplings; these courts tend to be associated with one main displaying male and intermittent displaying visitors. Their snap-jump displays generally involve intermittent popcorn-like "wingsnap" sounds while jumping between

adjacent saplings, and a longer "rollsnap" sonation elicited by performing multiple wingsnaps in extremely rapid succession (Schlinger, 2023). Although males may display for females in any month, breeding in Costa Rica has been reported between April-August (Stiles & Skutch, 1989) and between March-November (Wolfe et al., 2009).

During four weeks from July – August 2022, we studied manakins at both low and mid elevation display courts along a mountain range in northeastern Costa Rica. We worked at La Selva Biological Station (~41 m asl), a research station encompassing 1,536 ha of lowland tropical rainforest (Matlock Jr. & Hartshorn, 1999), in premontane wet forest near Puesto El Ceibo Guard Station (~534 m asl), and in San Ramón de la Virgen (~426 m asl) just outside the western edge of Braulio Carrillo National Park. We alternated 4–5-day sampling periods between low- and mid-elevation sites near the upper elevational distribution of the species to capture variation in bird behavior under a variety of weather conditions at each site. At La Selva Biological Station, the mean temperature during our field season was 25.1 º C (Organization for Tropical Studies, 2026). Based on the adiabatic lapse rate in humid air, we expected that the mid-elevation sites were cooler than low-elevation sites by ~2-3º C.

We located as many active display courts of white-collared manakins as possible, using a combination of auditory cues and local expertise within edge and secondary growth forest. Where we found a male displaying, we mounted a SONY ICD PX370 Mono Digital Voice Recorder, protected and camouflaged using a Rubbermaid© Brilliance food storage container with holes drilled located near the microphone for sound clarity. We mounted each recorder beneath a pad of upholstery foam to muffle the sounds of rain on the containers that otherwise would interfere with our ability to detect manakin display behavior from audio recordings. We placed recording devices 1.5-5 m from the center of display courts (mean: 2.9 m) and set up two

rain gauges within 0.5-4.5 m (mean: 2.4 m) of one another. We mounted recorders and rain gauges on 0.5-m tall sticks, locating them behind foliage to minimize visual disturbances at courts and avoiding areas obviously sheltered from rain by large leaves.

At each elevation, we recorded at up to six courts per day and aimed to obtain five 2-hour recordings per court. For each recording, we documented start and end times and measured rainfall using two gauges. At all courts, we obtained samples spanning all times of day and weather conditions. After each session, we recorded rain accumulation and emptied both gauges before the next recording. We related display behavior to the mean of the two rain gauge measurements to reduce canopy-related measurement error.

To quantify displays at focal courts from audio recordings, we determined the sound threshold levels representing focal vs non-focal courts via behavioral observations. Throughout the field season, we aimed for 4 hours of observation per court (2 hours each by two separate observers). These observations permitted us to filter out the sounds of non-focal displays in subsequent analyses using court-specific audio threshold calibrations. To determine minimum sound threshold levels, we prioritized observing courts while males were displaying, continuing until we had obtained useful behavioral observations at all courts or until the field season ended.

We expected to encounter one displaying adult male per display court (Stein & Uy, 2006). However, we sometimes observed 1-2 additional males performing at the courts of other males. Likewise, males occasionally flew from their main courts to display at neighbouring courts. Therefore, we treated each focal display court as the unit of replication. Whenever males displayed within earshot of the focal courts during behavioral observations, we recorded the time and distance of those sounds to facilitate later synchronization with acoustic data.

We conducted bioacoustic analyses in Raven Pro 1.6 (The Cornell Lab of Ornithology, 2024). We identified and tallied wingsnaps and rollsnaps (hereafter, "displays") from each recording and calculated total display rate as the sum of displays per hour. For periods with corresponding behavioral observations, we divided recordings into consecutive 5-minute windows and manually quantified displays by matching field notes to specific audio timestamps. To distinguish focal from quieter non-focal displays, we established a decibel cutoff based on spectral amplitude (dB FS/Hz). We measured the mean, minimum, and maximum amplitude for known focal displays; because wingsnaps are quieter than rollsnaps, we used the minimum dB FS/Hz of wingsnaps as the final threshold for inclusion. These values represent relative digital amplitude rather than calibrated sound pressure levels.

Ethical Note

We conducted all fieldwork in Costa Rica at La Selva Biological Station, Puesto El Ceibo Guard Station, and on private land with landowner permission. We obtained research permits from SINAC (Sistema Nacional de Áreas de Conservación; permit SINAC-ACC-PI-re-073-2022) and secured approval from station administration and landowners.

We used non-invasive observational methods only at a total of 16 observed display courts, which were generally each associated with one focal male. We did not capture, handle, mark, or experimentally manipulate animals. As such, we did not seek Institutional Animal Care and Use Committee (IACUC) approval because the research was classified as a non-invasive field study exempt from IACUC review under the U.S. Animal Welfare Act. We conducted observations conducted at a minimum distance of approximately 5 m, usually with a single observer present and occasionally with two observers present during training sessions. Birds at most display courts appeared habituated to observer presence, often even continuing to display

during our equipment manipulations. We observed no evidence of court abandonment associated with observations. One male appeared more sensitive to nearby observers and displayed infrequently during observation periods; this individual is discussed in the manuscript. All procedures complied with the ethical guidelines for the treatment of animals in behavioral research and teaching published by the Association for the Study of Animal Behavior and the Animal Behavior Society (Association for the Study of Animal Behavior & Animal Behavior Society, 2020).

Statistical Analyses and Model Structure

We performed all analyses in R (version 4.4.2) using the *rjags* and *coda* packages (Plummer et al., 2006, 2025; R Core Team, 2024). We modeled the number of displays recorded during each audio recording using a Bayesian zero-inflated Poisson (ZIP) mixture model. Because recording duration varied among observations, we included the log of recording duration as an offset so that expected counts represent display rates. Zero inflation is a common feature in behavioral datasets due to frequent observations of zero displays that may occur due to the absence of birds from their courts.

We treated each focal court observation, y_i, as arising from a mixture of two processes defined by a latent binary variable z_i:

$$y_i \mid z_i = \begin{cases} 0, & z_i = 0 \\ \text{Poisson}(\mu_i), & z_i = 1 \end{cases}$$

When $z_i = 0$, observations are structural zeros, representing recordings in which a bird was absent from the display court and could not have displayed. When $z_i = 1$, counts arise from a Poisson process representing recordings in which a bird was present and could produce zero or

more displays. The Poisson process may therefore reflect the presence of sampling zeros or non-zero display counts, both of which arise from behavioral states of individual birds.

We modeled the expected count for recording i as:

$$\log(\mu_i) = \log(t_i) + \mathrm{X}_i\,\beta$$

where t_i is recording duration in hours, X_i is the design matrix of covariates, and β is a vector of regression coefficients.

We assigned a flat prior to the zero-inflation probability $p = \Pr(z_i = 1)$:

$$p \sim \mathrm{Beta}(1,1)$$

We implemented the model in JAGS using the "zeros trick" (Shao, 2022), which separates zero counts from positive counts to calculate the log-likelihood manually.

To test our predictions, we examined the posterior distributions of the regression coefficients. To test our first prediction regarding the occurrence of rain (Model 1), we evaluated β_1. Because the model uses a log link, exponentiated coefficients (exp(β)) represent changes in expected display rate on the response scale. We inferred support for an effect when the 95% credible interval for β_1 did not include zero. We included rainfall as a binary variable (0 = no rain, 1 = rain), giving the expected count:

$$\log(\mu_i) = \log(t_i) + \beta_0 + \beta_1\,Rain_i$$

To test our second prediction (Model 2), we assessed an interaction between rainfall and elevation to assess whether the effect of rainfall differed between elevations. We estimated the effect of rainfall (mm/hr) on display rate, including elevation category (0 = low; 1 = mid) and a rainfall × elevation interaction to assess whether the effect of rainfall differed by elevation:

$$\log(\mu_i) = \log(t_i) + \beta_0 + \beta_1 Elevation_i + \beta_2 Rainfall_i + \beta_3(Elevation_i * Rainfall_i)$$

For prediction 2, because low elevation was the reference category, the rainfall slope at low elevation equals β_2, and the rainfall slope at mid elevation equals $\beta_2 + \beta_3$. Thus, the interaction coefficient β_3 directly represents the difference in rainfall slopes between elevations. We evaluated the support for elevation-specific rainfall effects by evaluating whether the 95% credible interval for β_3 excluded zero**.**

During exploratory analyses, we identified four observations with high bivariate leverage and removed them prior to model fitting to best represent typical display behavior. We applied a consistent exclusion across all four observations rather than selecting individual points (see Supplementary Material for a detailed explanation of the analytical approach).

We assigned normal priors centered at zero with large variance to regression coefficients to allow effect sizes to vary broadly while avoiding extreme or unstable estimates. We assigned weakly informative priors for regression coefficients:

$$\beta \sim \mathrm{MVN}(0, 10^6 \mathrm{I})$$

We report results using this single, justified prior specification, to avoid presenting multiple priors as interchangeable (Gelman et al., 2013; Hooten & Hobbs, 2015; Lemoine, 2019). However, sensitivity analyses with biologically informed narrower and wider priors produced highly consistent results.

We fitted models using Markov Chain Monte Carlo (MCMC) sampling and assessed convergence using trace plots and the potential scale reduction factor $\hat{R}$ (Gelman et al., 2013). We evaluated posterior distributions to confirm adequate model fit. We summarized the posterior distributions for the structural zero probability (p) and regression coefficients (β) using posterior means and 95% credible intervals. We fit all Bayesian models with 3 chains, with each chain run for 5,000 iterations, discarding the first 1,000 as burn-in.

Results

We collected audio recording data at 16 display courts (nine at low elevation and seven at mid elevation) between 20 July and 16 August 2022. We analyzed audio data from 13 of these courts (eight at low elevation and five at mid elevation), excluding three courts where proximity to adjacent active courts prevented reliable assignment of focal vs. non-focal court displays. Across these 13 courts, we obtained 291 recording sessions over the course of 26 days. After excluding four recording sessions identified as having disproportionately high leverage in the rainfall–display relationship, we retained 287 recording sessions for the main analysis. Recording sessions captured manakin display behavior between 06:18 and 15:08 and totaled 682.9 hours. Sessions lasted a mean of 142.8 minutes (median = 127, range = 43–406). On average, we completed 22.1 recording sessions per display area (median = 26, range = 11–29). Across all recording sessions, mean rainfall was 0.9 mm per session (median = 0, range = 0–20). We conducted 35 behavioral observations lasting a mean of 101.0 minutes (± 21.5) between 07:39 and 16:18. We analyzed an average of 2.7 behavioral observations per court (range: 1–7), for a mean total of 272 minutes (± 140.0) per court. Our total behavioral sampling effort across all included courts was 58.9 hours**.**

At one of the 13 courts, no males displayed during behavioral observation periods, although displays were present in the unobserved recording sessions. We thus obtained paired behavioral observation and acoustic data for 12 of the 13 courts. To estimate the cutoff for the 13th court, we regressed threshold sound intensity against the measured distance between each recording device and its associated display court. We then used the known distance between the recording device and the 13th display court to predict its corresponding sound intensity cutoff

from a linear regression, using the predicted spectral amplitude (dB FS/Hz) as the minimum volume threshold for identifying focal displays at that court.

Statistical Analysis

Birds decreased their display rates in the presence of rainfall as predicted (Fig. 3.1, Table 3.1). Under dry conditions, the posterior mean display rate was 45.64 displays per hour (95% credible interval: 44.94-46.38). Rain of any intensity reduced display rates by an estimated 31% (95% credible interval: 28.1–33.4%), corresponding to an expected display rate of 31.5 displays per hour. The zero-inflation component of Model 1 provided an estimate that approximately 60% of observations arose from the Poisson (count) process ($z_i = 1$; posterior mean p = 0.60, 95% credible interval: 0.54–0.65), representing situations in which a bird was present at the court and could have displayed (including both observed displays and sampling zeros). The remaining 40% of observations were attributed to the structural zero process ($z_i = 0$), reflecting likely absence from the court. These results indicate substantial zero inflation while suggesting that most recording sessions involved birds that were present and able to display.

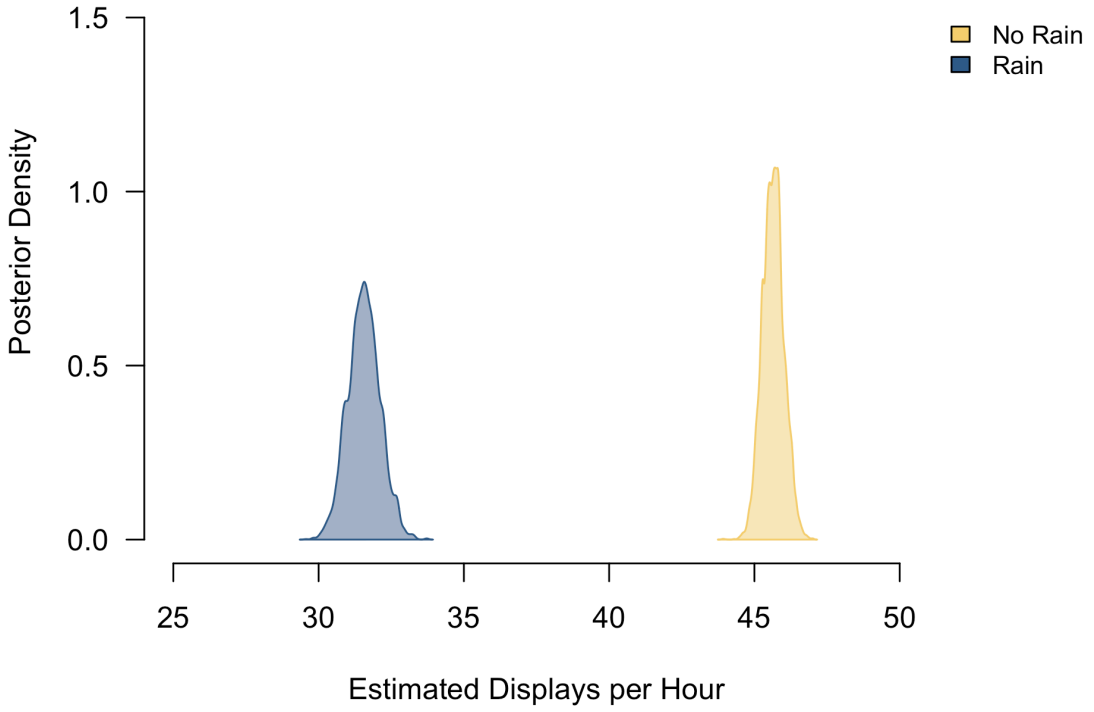


Figure 3.1. Visualizations of posterior distributions for estimated mean display rates under rainfall (blue) and no-rain (gold) conditions (Model 1; $n = 287$ recording sessions). The horizontal axis denotes estimated displays per hour, and the vertical axis represents posterior density.

Table 3.1. Posterior mean estimates and 95% credible intervals for the zero-inflated Poisson (ZIP) model of manakin display rates as a function of the occurrence of rain (Model 1; n = 287 recording sessions).

Parameter	Estimate (Link)	95% Credible Interval	Response Scale	95% Credible Interval
		Count Process (Log Link)		
Intercept (No Rain)	**3.82**	**[3.81, 3.84]**	**45.64**	**[44.94, 46.38]**
Rain	**-0.37**	**[-0.41, -0.33]**	**0.69**	**[0.67, 0.72]**
		Zero-Inflation Probability		
Probability of Poisson Process ($z_i = 1$)	**0.60**	**[0.54, 0.65]**	—	—

Results of model 2 revealed that rainfall reduced display rates at low elevation by an estimated 42% for each 1 mm/hr increase in rainfall (95% credible interval: 39–46%), corresponding to a factor of 0.58 (95% credible interval: 0.54–0.61); on the log scale, this effect was $\beta_2 = -0.55$ (95% credible interval: −0.61 to −0.49). At zero rainfall, mid-elevation males displayed more frequently than low-elevation males, with display rates estimated to be 28% greater (95% credible interval: 24–32%); on the log scale, this corresponded to $\beta_1 = 0.25$ (95% credible interval: 0.21–0.28).

The interaction between elevation and rainfall was positive but modest, indicating that rainfall reduced display rates at both elevations (Fig. 3.2, Table 3.2). However, the decline with increasing rainfall was less steep at mid elevation than at low elevation, with an interaction factor of 1.17 (95% credible interval: 1.09–1.26); on the log scale, this interaction term was $\beta_3 = 0.15$

(95% credible interval: 0.08–0.23). This corresponds to an estimated 42% reduction in display rate per 1 mm/hr increase in rainfall at low elevation, compared with an estimated 33% reduction at mid elevation. Posterior visualizations of this interaction show that display rates decreased with increasing rainfall at both elevations, with a steeper decline at low elevation than at mid elevation.

The zero-inflation component of Model 2 again indicated that approximately 60% of observations arose from the Poisson (count) process ($z_i = 1$; posterior mean p = 0.60, 95% credible interval: 0.54–0.65), representing situations in which a bird was present at the court and could have displayed (including both observed displays and sampling zeros). The remaining 40% of observations were attributed to the structural zero process ($z_i = 0$), reflecting likely absence from the court. The consistency of the zero-inflation parameter across models reflects that the probability of bird presence was modeled as constant and not influenced by rainfall or elevation.

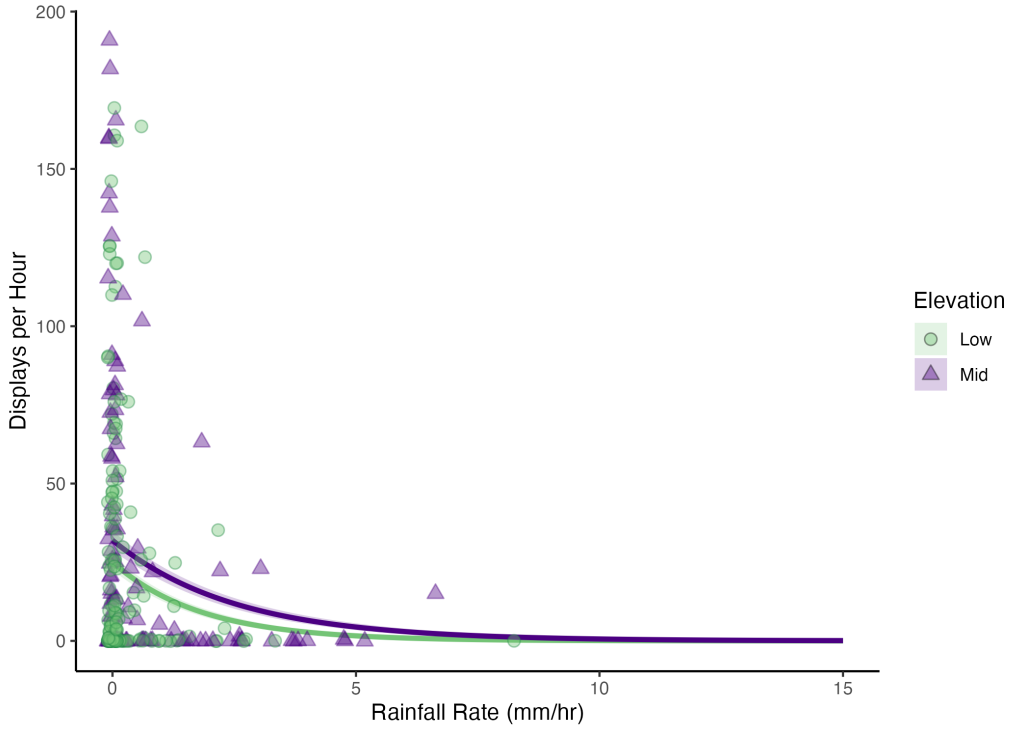


Figure 3.2. Start Posterior distributions for estimated mean display rates as a function of rainfall intensity (mm/hr) across low- and mid-elevation display courts (Model 2; n = 287 recording sessions). The slopes of the lines are derived from the interaction between elevation and rainfall intensity (Table 3.2), showing how display rates change with increasing rainfall intensity at each elevation. Lines represent the median expected display rates for low-elevation (green circle) and mid-elevation (purple triangle) sites. Shaded ribbons indicate 95% credible intervals representing the uncertainty in the expected mean (i.e., credible intervals for the expected mean, not posterior predictive intervals).

Table 3.2. Posterior mean estimates and 95% credible intervals for the zero-inflated Poisson (ZIP) model of manakin display frequency addressing elevation and rainfall interactions (Model 2; n = 287 recording sessions).

Parameter	**Estimate (Link)**	**95% Credible Interval**	**Response Scale**	**95% Credible Interval**
		Count Process (Log Link)		
Intercept	**3.74**	**[3.72, 3.76]**	**41.99**	**[41.15, 42.86]**
Elevation (Mid vs Low)	**0.25**	**[0.21, 0.28]**	**1.28**	**[1.24, 1.32]**
Rainfall Rate (mm/hr)	**-0.55**	**[-0.61, -0.49]**	**0.58**	**[0.54, 0.61]**
Elevation x Rainfall	**0.15**	**[0.08, 0.23]**	**1.17**	**[1.09, 1.26]**
		Zero-Inflation Probability		
Probability of Poisson Process ($z_i = 1$)	**0.6**	**[0.54, 0.65]**	—	—

Discussion

The results of this study demonstrate that rainfall strongly inhibits display behavior of white-collared manakins. Manakins reduced their display behavior by 31% when rain was falling. Furthermore, at both low- and mid-elevation display courts, increasing rates of rainfall were associated with exponential decreases in display rates, with a slightly steeper drop-off at low elevations as compared to mid elevations. While our findings are consistent with our first prediction, display rates did not decline more dramatically under rainy conditions at the higher-elevation site.

The fact that display rates did not decline more quickly at mid elevation sites than in the lowlands was unexpected. Many of the potential mechanisms linking rain to endotherm behavior are temperature-dependent, with greater investment in thermoregulation required in colder rather than warmer rainfall (Karr et al., 2025). Prior to exhibiting physiological responses, birds exposed to thermal stressors often engage in behavioral mechanisms such as feather fluffing or altered microhabitat selection; thus, a compromised ability to thermoregulate in rainfall could more strongly affect birds at cooler mid-elevation display sites (Zhou et al., 2017). It is possible that the elevational difference between our lowland and mid-elevation sites were insufficiently different in temperature (~3*C) to lead to such costs. However, we sampled very near to the upper elevational limit for this species in the region; thus, we had limited scope to detect additional costs under cooler temperatures. Alternatively, the behavioral changes we detected may not reflect direct thermogenic mechanisms. It is possible that males cease displaying if females avoid flying between leks during rain, similar to the reductions in foraging flights documented in both birds and bats (Erickson & West, 2002; Foster, 1974; LaVal & Lawton, 2021). This data point reflected a verified, rare biological event rather than a measurement

artifact, but was an extreme outlier resulting in poor model fit. We strongly suspect a female visited the court and multiple males displayed in response to her presence. Because this event may be informative about the upper bounds of performance or behavioral flexibility, we retained this observation and the other three outliers in the Supplementary Material to represent the full range of biological variation (Fig. S3.1, Fig. S3.2, Table S3.1, Table S3.2). It is still possible that this kind of event may occur more frequently under warmer temperatures, but our dataset was inadequate for assessing that possibility.

Our findings join a broader literature in demonstrating how that rainfall shapes reproductive timing and investment in Neotropical birds. Many tropical species time their breeding to coincide with peaks in precipitation, and rainfall can influence nest initiation, clutch size, and long-term reproductive activity (Díaz et al., 2024; Moreno-Palacios et al., 2025). Because our study demonstrates the effects of precipitation of courtship behaviors, we suggest that costs of display in rainfall could be a potential mechanism by which breeding seasons are constrained. Put in the context of broader phenological findings in the Neotropics, our results suggest a possible trade-off between resource abundance and display activity. Rainfall can have opposing effects on reproduction depending on timing: while precipitation during breeding can directly inhibit reproductive behavior, rainfall prior to breeding often enhances food availability, creating resource pulses that birds track when initiating reproduction (Boyle et al., 2020; Silber & Boyle, 2026). Together, these patterns suggest that breeding timing may be shaped by a trade-off between resource availability and the environmental constraints on reproductive behavior.

Because reproductive behavior represents a major component of fitness, constraints on display can scale up to influence population dynamics and distributions. Rainfall gradients are associated with variation in breeding activity and local distributions, with wetter conditions often

reducing breeding effort or altering its timing (Díaz et al., 2024; Moreno-Palacios et al., 2025). In Neotropical birds, variation in rainfall has also been linked to both range shifts and population declines, suggesting that precipitation can act as a key ecological constraint (B. G. Freeman et al., 2024).

Rainfall may influence mating opportunities and reproductive success by constraining the timing and duration of courtship, resulting in consequences for demographic and potentially evolutionary processes. More broadly, species' life-history traits mediate their ability to track changing climatic conditions, influencing whether populations persist, shift, or decline under environmental change (Jiguet et al., 2007; Pearson & Dawson, 2003). In the context of climate change, shifts in precipitation may disproportionately affect species with narrow rainfall niches, contributing to population declines and range contractions (B. G. Freeman et al., 2024). Integrating behavioral mechanisms with life-history and climate-niche approaches. will be essential to predict how tropical species respond to changing rainfall patterns.

Supplementary Information

Supplementary Text

Model Description

We modeled display counts using a zero-inflated Poisson mixture model with a log link, including log (recording duration) as an offset to account for variation in recording session duration and to model display rates. Model 1 included the occurrence of rainfall as a predictor, and Model 2 included elevation, rainfall rate, and their interaction. The zero-inflation parameter (p) represents the probability that an observation arises from the Poisson sampling process ($z_i = 1$) rather than the structural zero process ($z_i = 0$). We confirmed model convergence through visual inspection of trace plots and by ensuring the Gelman-Rubin statistic was approximately 1.00 for all parameters.

Outlier Identification and Treatment

Out of 291 recording sessions, we identified four observations as high-leverage outliers with strong bivariate influence. To maintain model stability and identify the broader ecological trend, we excluded these four high-influence points from our main analyses (n =287 recording sessions). The supplementary figures and tables that follow include model results and visualizations when instead including these four outliers (n = 291 recording sessions) (Fig. S3.1, Fig. S3.2, Table S3.1, Table S3.2)**.**

The interaction effect in Model 2 was highly sensitive to the inclusion of four outliers, particularly based on a single recording session associated with high bivariate leverage. Direct field observations confirmed this unusual session, which involved a rare multi-male display during a heavy rainstorm—a biological anomaly that disproportionately influenced the model's

outcome. Upon reviewing previous field notes during analysis, we had noted the multi-male display via direct field observation while incidentally passing the display court in question. We can thus confirm that the extreme display count was a genuine biological event rather than a recording artifact or measurement error. Based on our experience with white-collared manakins and other manakin species, we suggest this event may have represented multiple birds converging opportunistically to gain the attention of a passing female. However, its extreme influence on the rainfall-elevation interaction would have obscured the broader landscape-level patterns we aimed to characterize.

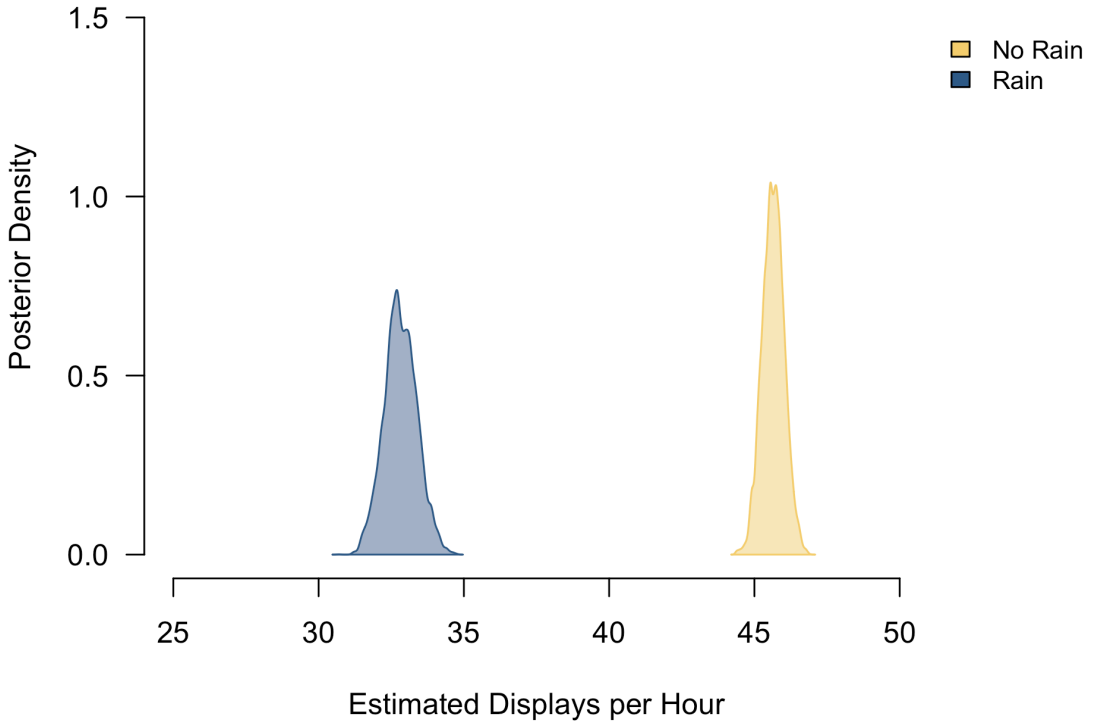


Figure S3. 1. Visualizations of posterior distributions for estimated mean display rates under rainfall (blue) and no-rain (gold) conditions (Model 1; n = 291 recording sessions). The horizontal axis denotes estimated displays per hour, and the vertical axis represents posterior density. Results presented here include the larger dataset (n = 291), including four observations identified as outliers via high bivariate leverage.

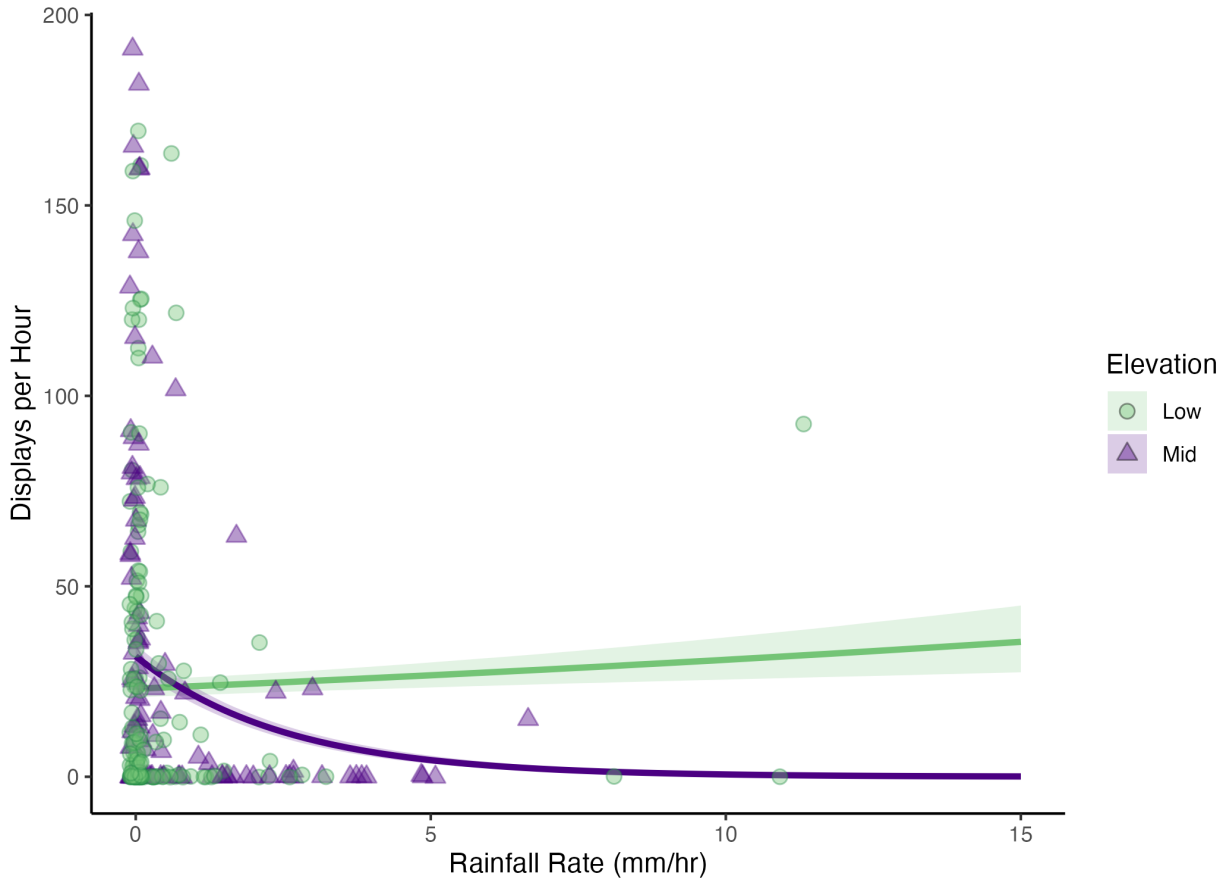


Figure S3. 2. Visualizations of posterior distributions for estimated mean display rates as a function of rainfall intensity (mm/hr) across low- and mid-elevation display courts (Model 2; n = 291 recording sessions). The slopes of the lines are derived from the interaction between elevation and rainfall intensity (Table S3.2), demonstrating how display rates change with increasing rainfall intensity at each elevation. Lines represent the median expected display rates for low-elevation (green circle) and mid-elevation (purple triangle) sites. Shaded ribbons indicate 95% credible intervals representing the uncertainty in the estimated mean (i.e., not posterior predictive intervals). Results presented here include the larger dataset (n = 291), including four observations identified as outliers via high bivariate leverage.

Table S3. 1. Posterior mean estimates and 95% credible intervals for the zero-inflated Poisson (ZIP) model of manakin display rates as a function of the occurrence of rain (Model 1; n = 291 recording sessions). Results presented here include the larger dataset, including four observations identified as outliers via high bivariate leverage.

Parameter	**Estimate (Link)**	**95% Credible Interval**	**Response Scale**	**95% Credible Interval**
		Count Process (Log Link)		
Intercept (No Rain)	**3.82**	**[3.81, 3.84]**	**45.65**	**[44.91, 46.39]**
Rain	**-0.33**	**[-0.37, -0.29]**	**0.72**	**[0.69, 0.75]**
		Zero-Inflation Probability		
Probability of Poisson Process ($z_i = 1$)	**0.59**	**[0.53, 0.65]**	—	—

Table S3. 2. Posterior mean estimates and 95% credible intervals for the zero-inflated Poisson (ZIP) model of manakin display frequency addressing elevation and rainfall interactions (Model 2; n = 291 recording sessions). Results presented here include the larger dataset, including four observations identified as outliers via high bivariate leverage.

Parameter	**Estimate (Link)**	**95% Credible Interval**	**Response Scale**	**95% Credible Interval**
		Count Process (Log Link)		
Intercept	**3.67**	[**3.65, 3.69**]	**39.18**	[**38.45, 39.95**]
Elevation (Mid vs Low)	**0.31**	[**0.28, 0.34**]	**1.37**	[**1.33, 1.41**]
Rainfall Rate (mm/hr)	**0.03**	[**0.01, 0.04**]	**1.03**	[**1.01, 1.05**]
Elevation x Rainfall	**-0.42**	[**-0.47, -0.38**]	**0.65**	[**0.62, 0.69**]
		Zero-Inflation Probability		
Probability of Poisson Process ($z_i = 1$)	**0.59**	[**0.53, 0.65**]	—	—

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Chapter 4 - Population Genetic Structure is a Key Factor When Interpreting Sex Chromosome-to-Autosomal Diversity Ratios

Abstract

Genomes contain patterns that reflect signatures of evolution. One intriguing signature across animal taxa is the sex chromosome-to-autosomal diversity ratio, which reflects differences in effective population size between sex chromosomes and autosomes. This metric can be shaped by sex-specific demography and selection. In birds where males are the homogametic (ZZ) sex, a Z-to-autosomal (Z/A) diversity ratio of 0.75 (the neutral expectation based on three Z chromosomes for every four autosomal copies in a population) is theoretically expected under equal reproductive contributions of males and females. In sexually selected species where reproductive success is concentrated among relatively few males (high reproductive skew), reduced male reproductive contributions can theoretically drive these ratios toward 0.5.

To empirically investigate the drivers of Z/A diversity ratios, we studied Neotropical white-ruffed manakins (*Corapipo altera*), which have a lek mating system involving strong sexual selection through female mate choice. Within their wet premontane habitats, these birds may also experience natural selection associated with precipitation. We performed whole-genome sequencing on 150 white-ruffed manakin blood samples collected from 15 sites across Costa Rica and Panama. We tested six *a priori* directional predictions for climatic effects on Z/A based on the hypothesis that climate-associated changes in dominant male survival and tenure could alter reproductive skew. Alternatively, we tested whether Z/A diversity was more strongly associated with population genetic structure, genetic differentiation (F_{ST}), and/or absolute sequence divergence (d_{XY}).

Population structure analyses via ADMIXTURE identified five genetic clusters across the sampled range. After accounting for population genetic structure, Z/A diversity ratios were negatively associated with interannual variability in wettest-quarter rainfall, contrary to our expectations. However, we found no evidence of an association between Z/A ratios and five of the six proposed climatic variables. Genetic cluster was strongly associated with Z/A ratios across climatic models, and genetic differentiation was associated with differences in Z/A among genetic clusters while absolute sequence divergence was not.

The Costa Rica–Pacific cluster had the lowest Z/A among the five genetic clusters, reflecting a disproportionately strong reduction in Z-linked relative to autosomal diversity. Together, our results suggest that demographic processes associated with population differentiation and genetic drift may contribute substantially to spatial variation in Z/A diversity, in addition to the effects of reproductive skew predicted by theory.

Introduction

Selective and demographic processes can leave informative signatures of evolution across a genome. Because sex chromosomes and autosomes differ in their effective population sizes and relative exposure to selection, these processes can differentially affect patterns of diversity across the two chromosome types. One complex signature of evolution is therefore the ratio between sex chromosome and autosomal diversity. Sex chromosome-to-autosomal diversity ratios have been used to investigate sex-biased demography and selection in a variety of mammals and birds, including humans (Ellegren, 2009; Wilson Sayres, 2018). Although some studies have used these ratios as a metric of reproductive skew (Corl & Ellegren, 2012; Huang & Rabosky, 2015), emerging evidence shows that such diversity metrics can be influenced by overlapping processes such as selection, population bottlenecks, and speciation (Balakrishnan et al., 2026; Payseur et

al., 2018; Wright et al., 2015). Evolutionary theory offers competing predictions about these ratios that can be tested through the study of wild animals (Irwin, 2018; Wilson Sayres, 2018). However, to our knowledge, published empirical studies have rarely tested predictions regarding how direct abiotic pressures may influence such genetic diversity ratios.

Birds have a ZW sex chromosome system in which males are homogametic (ZZ) and females are heterogametic (ZW). Assuming an equal adult sex ratio in a given population, three Z chromosomes occur for every four copies of each autosome. When there is then an equal reproductive contribution by monogamously breeding males and females, a neutral expectation of 0.75 for the ratio of Z-linked to autosomal nucleotide diversity (Z/A) should be present in the population (Ellegren, 2011; Irwin, 2018). Deviations from breeding monogamy can shift the Z/A ratio, particularly in sexually selected species (Irwin, 2018; Wilson Sayres, 2018). In lek mating systems, males aggregate at display sites and females choose males as reproductive partners. Females then tend to provide all parental care, while remarkably few males account for a disproportionate share of male reproductive success (Höglund & Alatalo, 1995). When there is high variance in male reproductive success (i.e., strong reproductive skew), the relative contribution of the Z chromosome is lower than that of the autosomes, which is expected to reduce Z/A diversity ratios (Mank et al., 2010; Wilson Sayres, 2018). Extreme reproductive skew can therefore drive Z/A ratios toward 0.5 as male reproductive success becomes increasingly concentrated among fewer individuals (Wright et al., 2015).

While high reproductive skew alone can thus strongly depress Z/A diversity ratios relative to the neutral expectation, low Z/A ratios may also reflect the consequences of environmental pressures or of population genetic structure (Vicoso & Charlesworth, 2009; Anderson et al., 2026). Processes beyond reproductive skew may therefore influence Z/A

diversity in lekking species, potentially accounting for Z/A ratios that fall below theoretical expectations even under extreme reproductive skew (Oyler-McCance et al., 2015). Ecological drivers may have a particularly strong influence on Z/A diversity in lekking species exposed to environmental selective pressures; if abiotic forces interact with sexual selection to influence the proportion of siring males contributing to subsequent generations, climatic variation may be a strong driver influencing variation in Z/A diversity (Shogren, 2020; Shogren et al., 2021). On the other hand, long-term processes affecting genetic structure (e.g., changes in population size over time, gene flow, and genetic drift), may also contribute strongly to variation in Z/A diversity ratios; population genetic structure is itself a potential source of Z/A variation.

By examining Z/A variation within species exposed to strong natural and sexual selective pressures, we can minimize species-level differences in mating patterns and evolutionary history while disentangling how this metric is associated with environmental conditions and population genetic structure. White-ruffed manakins (*Corapipo altera*) provide such a system: these Neotropical birds have a lek mating system and experience strong climatic pressures in their wet premontane habitats (Shogren et al., 2021). As in other manakins across their clade (Family: Pipridae), white-ruffed manakin males perform elaborate displays, and females choose mates without receiving assistance with parental care, food resources, or territory acquisition (DuVal & Kempenaers, 2008). For these birds, substantial tropical rainfall can negatively influence foraging and courtship opportunities and the persistence of occupied display logs across years (Boyle et al., 2010, 2011; Shogren et al., 2021). When exposed to consistent annual rainfall, increased seasonal precipitation, interannual rainfall variability, and relatively cool temperatures, these birds may experience limited foraging opportunities and increased thermogenic stress, conditions hypothesized to increase mortality risk (Boyle, 2008; Boyle et al., 2020).These effects

provide a potential pathway through which climatic conditions could alter the relative strength of natural and sexual selection.

White-ruffed manakin populations are distributed from eastern Honduras through northwestern Colombia in Central America (Stiles & Skutch, 1989); across this range, inhospitable mountain barriers separate geographic regions whose climatic regimes are shaped by long-standing Caribbean and Pacific oceanographic influences (Obrist-Farner et al., 2023). These regional barriers may also contribute to genetic differentiation among populations (Shogren, 2020). For example, Costa Rican and Panamanian populations occur on either side of the continental divide and have historically been assigned to Caribbean and Pacific subspecies based on plumage and behavioral differences (Kirwan & Green, 2012; Shogren et al., manuscript in preparation). We performed whole-genome sequencing for 150 white-ruffed manakins from 15 sampling sites across Costa Rica and Panamá to test how climatic factors and population genetic structure each contribute to Z/A ratios. We selected sampling sites to span a range of precipitation regimes and maximize geographic coverage across the geographic barrier formed by the continental divide. While population structure itself is central to understanding our hypothesis regarding population history, it is also recognized as a major confounding factor when testing associations between climate and genomic signatures. This challenge has motivated approaches that account for underlying genetic structure when evaluating environmental relationships (e.g., Frichot et al. 2013; Rellstab et al. 2015). As such, we used population structure analysis to define clusters among our sampling sites and establish a basis for disentangling climatic pressures from population genetic structure.

After characterizing genetic population structure, we estimated Z-linked versus autosomal nucleotide diversity at two grouping levels: first, at each of our 15 sampling sites, and

second, for the five genetic clusters identified through structure analysis. Using reciprocal conditional analyses on the sampling site dataset, we tested whether a) site-level Z/A ratios were associated with six separate *a priori* climatic metrics while accounting for broad genetic cluster membership, and b) sampling site genetic cluster membership was associated with Z/A while accounting for each climatic metric. We specifically calculated partial R^2 for both terms in each model, allowing us to separately quantify the variation in Z/A uniquely associated with climate after accounting for genetic cluster and the variation uniquely associated with genetic cluster after accounting for climate. Finally, we tested whether absolute differences in Z/A diversity among the five genetic clusters increased with cluster-based pairwise estimates of autosomal genetic differentiation (F_{ST}) and absolute sequence divergence (d_{XY}). These metrics provide information about population structure and history; specifically, F_{ST} measures the relationship of among-population genetic differentiation to within-population diversity among paired populations, whereas d_{XY} measures average sequence divergence between paired populations.

Methods

Ethical Approval and Permits

Capture and sampling procedures were approved by the Kansas State University Institutional Animal Care and Use Committee (IACUC; Protocols #3256, #3728, #4254, and #4813). Research permits were issued by the Costa Rican Sistema Nacional de Áreas de Conservación (SINAC-SE-CUS-PI-R-065-2016, SINAC-SE-CUS-PI-R-032-2016, SINAC-SE-CUS-PI-R-050-2017, SINAC-SE-CUS-PI-R-064-2016, M-P-SINAC-PNI-ACAT-052-2018) and through Panamanian Ministry of Environment (MiAmbiente) (ARG-008-2023). Permits for collecting genetic material were issued by the Costa Rican Ministerio de Ambiente y Energía Comision

Nacional para la Gestion de la Biodiversidad (R-024-2016-OT-CONAGEBIO, R-010-2017-OT-CONAGEBIO, R-025-2017-OT-CONAGEBIO, R-024-2017-OT-CONAGEBIO, R-022-2016-OT-CONAGEBIO, R-020-2016-OT-CONAGEBIO).

Study Sites and Sample Collection

During the white-ruffed manakin 2016-2018 and 2023-2024 breeding seasons, we collected blood samples from 150 white-ruffed manakins (*Corapipo altera*) at 15 sampling sites across Costa Rica and Panamá (Fig. 4.1A). We selected sampling sites to represent broad climatic variation and maximize geographic coverage across the continental divide. On the Caribbean slope of Costa Rica, white-ruffed manakin breeding occurs between 400 and 900 meters above sea level (m.a.s.l.), while on the Pacific slope populations breed at 1000-1500 m.a.s.l. (Garrigues & Dean, 2014; Stiles & Skutch, 1989). In Panamá, white-ruffed manakins occupy the foothills and lower highlands of both slopes, generally ranging from 450 to 1500 m.a.s.l. (Angehr & Dean, 2010; Ridgely, 1976). Across Costa Rica, the continental divide follows the high-elevation spine of the Cordillera de Talamanca and divides white-ruffed manakins across an inhospitable barrier, with the elevation of this ridge generally decreasing as it enters the west-central portion of Panamá.

We sampled birds at six Costa Rican sampling sites: Parque Nacional Volcán Tenorio (VT), the Rara Avis Biological Reserve adjacent to Parque Nacional Braulio Carrillo (RA), Reserva Biológica El Copal (EC), Parque Nacional La Cangreja (CG), the private reserve La Chaqueta (LQ), and the Organization for Tropical Studies Las Cruces Biological Station (LC). We sampled birds at nine Panamanian study sites; in western Panamá, we sampled birds near the Changuinola Reservoir in Bocas del Toro Province (BT) and on either side of the continental divide in Fortuna (FC and FP, for Fortuna Caribbean and Fortuna Pacific, respectively). Across

central Panamá, we sampled birds on either side of the continental divide in Santa Fé National Park (SC and SP) and along the continental divide itself in Altos del María (AP and AC). Finally, we sampled eastern Panamá sites at Cerro Azul (CA) and Reserva Natural Cerro Chucantí (CH). Locations of the 15 sampling sites are shown on a map in Figure 4.1A.

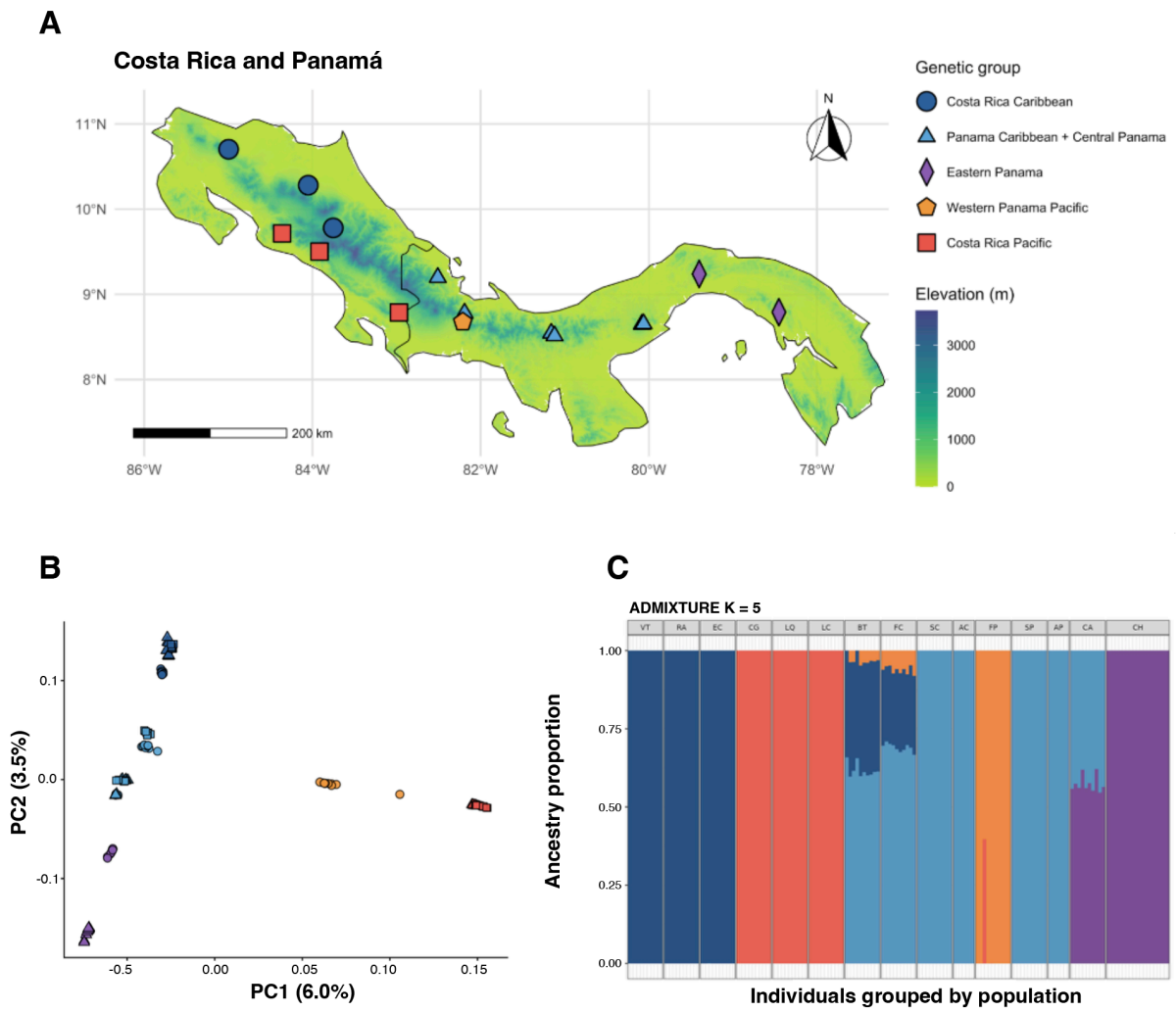


Figure 4.1 Population genetic structure of 150 white-ruffed manakins across Costa Rica and Panamá. Colors and symbols are consistent across panels and indicate the five genetic clusters identified by ADMIXTURE. **A**. Sampling sites of 150 individuals across Costa Rica and Panamá (n = 15 sampling sites). **B**. Principal component analysis (PCA) of whole-genome SNP variation for all 150 birds, with individuals colored and shaped according to their ADMIXTURE genetic cluster. **C**. ADMIXTURE ancestry proportions for K = 5, the value of K with the lowest cross-validation error. Individuals were assigned to genetic clusters based on their highest inferred ancestry proportion.

Although we managed to sample white-ruffed manakins on either side of the continental divide in Panamá, the topographical separation between sampling sites decreased substantially among paired-slope sampling sites heading eastward across Panamá. At Fortuna in western Panamá, populations on the Caribbean and Pacific slopes were clearly separated by an intervening windswept ridge-top dwarf cloud forest unsuitable for white-ruffed manakins. This ridge declined in elevation when moving eastward throughout central Panamá, particularly at our planned sampling sites on the opposite slopes (SC/SP and AC/AP). At those paired sites around, the very ridge itself was ideal for the species' selected elevation and primary forest habitat, and the limited overall area of suitable habitat at this elevation had the effect of concentrating white-ruffed manakins more closely together across a much more hospitable portion of the continental divide.

We captured birds with 12-m mist nets of 38-mm mesh (Ecotone, Gydnia, Poland) placed in intact forest understory where we detected white-ruffed manakin display logs. We aged male birds as hatch-year (HY), second-year (SY), third-year (TY), or after-third-year (ATY, adult) by plumage (Jones et al., 2014). We did not assign age classes to females because females retain green plumage throughout life. Because HY and SY males may closely resemble females, we conservatively classified some of the most ambiguous green birds as sex unknown. We sequenced 10 birds per site at 12 of our 15 sites. At the most closely adjacent pair of sites near Altos del María (AC and AP), where we considered genetic overlap extremely likely, we sampled only six individuals per site. At the easternmost Panamanian location, Cerro Chucantí (CH), we sequenced 18 birds because genomic sampling from this portion of the species' range is limited.

For each captured bird, we collected 50–70 μL of whole blood via brachial venipuncture and immediately transferred blood samples into Queen's lysis buffer. We marked sampled birds with plastic color bands to avoid double-sampling of individual birds. We kept samples cool in field accommodations before transportation to Kansas State University where we froze blood samples at -80°C until DNA extraction. We chose samples for sequencing based on DNA purity and prioritized male DNA samples whenever possible.

DNA Extraction, Whole-Genome Sequencing, and Alignment

We extracted genomic DNA from preserved blood samples using Qiagen DNeasy Blood and Tissue Kits (QIAGEN Inc., USA) following manufacturer protocols. We assessed DNA concentration using a NanoDrop spectrophotometer and gel electrophoresis. We stored DNA extracted from samples collected in 2016–2018 at −20°C, and stored DNA extracted from samples collected in 2023–2024 at −80°C.

We sent DNA samples to Novogene for whole-genome sequencing. Novogene performed DNA quality control and prepared libraries using the plant and animal whole-genome workflow. Novogene then sequenced samples on an Illumina NovaSeq X Plus platform to generate 150-bp paired-end reads (PE150), targeting approximately 18x genome-wide coverage per individual (Illumina, 2025).

We processed raw sequencing reads by first removing adapter contamination and low-quality bases from paired-end reads using Galore. We used the --nextseq 20 option to remove poly-G artifacts associated with Illumina NovaSeq two-color sequencing chemistry, retain unpaired reads, and apply a stringency threshold of 5 bp of overlap between the read and adapter sequence for adapter trimming. We used Trim Galore to generate FastQC reports for each trimmed read file (Andrews, 2010; Krueger, 2019), evaluating read quality and summarizing

quality-control metrics across all samples using MultiQC (Ewels et al., 2016). We then aligned quality-filtered reads to the chromosome-level *Chiroxiphia lanceolata* reference genome (Rhie et al., 2021) using BWA-MEM (Li & Durbin, 2009). This reference provided the most complete and contiguous assembly and comprehensive genome annotation among alternative manakin reference genomes available (bChiLan1.pri; RefSeq accession GCF_009829145.1). We converted SAM output to coordinate-sorted BAM files using SAMtools (Li et al., 2009).

We added read-group information to each BAM file using GATK/Picard AddOrReplaceReadGroups, including sample ID, library, sequencing platform, and flow cell/lane identifiers (McKenna et al., 2010). We identified and marked PCR duplicate reads using Picard MarkDuplicates. For individuals sequenced across multiple lanes, we provided all lane-specific BAM files for that individual as inputs to MarkDuplicates, producing a single duplicate-marked BAM file per individual.

Variant Calling, All-Sites Generation, and Filtering

We identified sites for each bird using GATK HaplotypeCaller in GVCF mode against the *Chiroxiphia lanceolata* RefSeq genome, producing one gVCF per individual. We ran HaplotypeCaller using the default diploid setting. We then used GATK CombineGVCFs with chromosome-specific intervals to merge individual gVCFs into cohort gVCFs for each chromosome, including the autosomes and sex chromosomes. We jointly genotyped chromosome-specific cohort gVCFs using GATK GenotypeGVCFs to produce multisample all-sites VCFs for each chromosome. We filtered sites according to GATK best-practice recommendations for non-model organisms by flagging sites with QD < 2.0, SOR > 3.0, FS > 60.0, MQ < 40.0, MQRankSum < −12.5, or ReadPosRankSum < −8.0.

For downstream analyses, we generated chromosome-specific filtered VCFs from the flagged all-sites datasets. We retained sites that passed all site-level filters and applied an additional mean depth filter of 10–40 reads across individuals. We retained all-sites datasets for estimation of pi, F_{ST}, and d_{XY}. For the Z chromosome, we additionally flagged sites at which any female had a putative heterozygous genotype using a chromosome-specific BED file during GATK VariantFiltration. We subsequently recoded these sites as missing for both sexes prior to downstream analyses. We inferred sex from relative sequencing coverage of the Z and W chromosomes to clarify sex of the few birds whose sex was uncertain. Specifically, birds with <16X Z coverage and >11X W coverage were identified as females.

Population Structure Analyses

For population structure analyses, we generated a variant-site dataset from the filtered autosomal VCFs. We retained only biallelic SNPs with a minor allele frequency ≥0.01 using VCFtools (Danecek et al., 2011). We then identified approximately independent SNPs by linkage disequilibrium pruning in PLINK 1.9 using the --indep-pairwise 50 5 0.5 algorithm, corresponding to a 50-SNP window, a five-SNP step size, and an r^2 threshold of 0.5 (Chang et al., 2015). We performed principal component analysis (PCA) and ADMIXTURE on the resulting LD-pruned autosomal SNP dataset. We conducted PCA using SNPRelate in R (Zheng et al., 2012). We recoded RefSeq chromosome names to integer chromosome codes for ADMIXTURE compatibility and ran ADMIXTURE v1.3.0 with cross-validation for $K = 1–10$ using four threads (Alexander et al., 2009). We then compared cross-validation (CV) error among K values to compare support for alternative numbers of genetic clusters.

Genetic Diversity and Divergence Analyses

For genetic differentiation and ancestral divergence analyses, we used the quality- and depth-filtered all-sites VCFs as input to pixy (Korunes & Samuk, 2021). We estimated nucleotide diversity (π), F_{ST}, and d_{XY} in non-overlapping 50-kb windows. Because multiple sampling sites belonged to the same ancestral genetic clusters, we calculated Z/A at two scales to evaluate the influence of broader population structure on estimates of relative Z-linked diversity. We estimated π separately for the autosomes and the Z chromosome using our two distinct population-grouping schemes: the 15 sampling sites and the five genetic clusters generated from ADMIXTURE analysis. For both the sampling-site-level and cluster-level grouping schemes, we divided mean Z-linked π by 1.1 to account for the larger Z-linked mutation rate associated with male mutation bias (Chase et al., 2025; Schield et al., 2021). We also calculated Z/A as mutation-corrected mean Z-linked π divided by mean π across all autosomes, following Chase et al., 2025 and Schield et al., 2021.

We used the five genetic clusters to define the appropriate population units for pairwise analyses of genetic differentiation and divergence. We calculated genetic differentiation (F_{ST}) and absolute sequence divergence (d_{XY}) among the five genetic clusters, yielding 10 pairwise estimates for each metric. To evaluate whether differentiation and divergence differed between the Z chromosome and autosomes, we compared Z-linked and autosomal F_{ST} and d_{XY} estimates for each pairwise genetic-cluster comparison.

Climate Metrics and Analyses

We extracted precipitation data for 1981–2024 for each of the 15 sampling sites from the Climate Hazards InfraRed Precipitation with Station data (CHIRPS; Funk et al., 2015; González-Méndez et al., 2025), a gridded precipitation dataset. We initially selected eight *a priori* climate

metrics representing precipitation and temperature: within-year rainfall variability, interannual variability in wettest-quarter rainfall, interannual variability in driest-quarter rainfall, wettest-month rainfall, wettest-quarter rainfall, driest-month rainfall, driest-quarter rainfall, and elevation as a proxy for temperature. We calculated within-year rainfall variability as the coefficient of variation (CV) among monthly mean rainfall values, to reflect how consistently manakins experience rainfall across the year. We calculated interannual variability in wettest- and driest-quarter rainfall as the CV of precipitation among years for each quarter. We assessed collinearity among the eight original climate metrics using pairwise correlations and variance inflation factors (VIFs). We considered variables with $|r| \geq 0.7$ or $VIF \geq 5$ to be highly collinear. Because wettest-month and wettest-quarter rainfall were highly correlated, as were driest-month and driest-quarter rainfall, we retained only the wettest and driest quarter measures and ultimately retained six climatic metrics (Dormann et al., 2013; Zuur, 2009).

Using all 15 sampling sites, we fit six separate linear models to evaluate the conditional associations of climate and population genetic structure with Z/A. Each model included one of the six climate variables and genetic cluster ($K = 5$) as fixed effects. For each model, we evaluated the contribution of each term by comparison with the corresponding reduced model, allowing us to assess the association of climate with Z/A after accounting for genetic cluster and, reciprocally, the association of genetic cluster with Z/A after accounting for climate. We treated genetic cluster as a fixed rather than random effect because we sought to evaluate differences among the five inferred clusters rather than estimate among-cluster variance (Gomes, 2022). To evaluate whether differences in Z/A were associated with population differentiation or divergence, we used linear models relating absolute pairwise differences in cluster-level Z/A to pairwise autosomal F_{ST} and d_{XY}, respectively.

Statistical Analyses

We interpreted statistical results using $\alpha = 0.05$ while avoiding strictly dichotomous interpretations based on statistical significance alone. When discussing biological associations, we considered effect estimates in the context of their uncertainty and avoided strong biological inference when model support was limited. Given the small sample sizes in both cluster- and site-level analyses, we did not exclude observations as statistical outliers.

Results

Statistical Genome Resequencing and Variant Calling

Sequencing generated 194.18 ± 17.92 SD million paired-end reads per individual, with 98.71 ± 0.76% mapping to the *Chiroxiphia lanceolata* reference genome and mean sequencing depth of 19.97 ± 1.86X. Following joint genotyping and quality filtering, the autosomal variant-site VCF contained 43,897,247 total variant records, including 37,941,220 SNPs, while the Z-linked all-sites VCF contained 75,361,589 total records, including 4,026,237 SNPs. After filtering for population-structure analyses, the LD-pruned autosomal dataset contained 15,108,887 SNPs for PCA and ADMIXTURE. We retained all 150 birds for downstream analyses. Sequencing results demonstrated that 135 birds were males and 15 were females.

Population Structure Analyses

Our clustering analyses showed clear geographically associated population structure among the 15 sampling sites (Fig. 4.1A). Principal component analysis separated birds into groups broadly consistent with geographic regions, with PC1 and PC2 explaining 6.0% and 3.5% of genomic variation, respectively (Fig. 4.1B). Cross-validation error from ADMIXTURE analyses of K = 1–10 was minimized at $K = 5$ (Fig. S4.1), identifying five genetic clusters distributed across

Costa Rica and Panamá (Fig. 4.1A, C). Two genetic clusters were divided clearly within Costa Rica by the Cordillera de Talamanca (n = 3 sampling sites per cluster on each slope). One single-cluster site (n = 1) was located on the Pacific side of the Cordillera as it spread into western Panamá before declining in elevation eastward. Meanwhile, a much larger cluster of sites (n = 6) was revealed throughout much of Central Panama, including two sets of adjacent paired sites that bridged the low continental divide. Finally, the easternmost Panamanian cluster was found to include two sampling sites (n = 2). Respectively, these genetic clusters are referred to throughout the text as Costa Rica Caribbean, Costa Rica Pacific, Caribbean + Central Panama, Western Panama Pacific, and Eastern Panama.

Z/A Diversity Ratios

Across the 15 sampling sites, mutation-corrected Z/A nucleotide diversity ratios averaged 0.456 ± 0.035 SD and ranged from 0.387 to 0.487 (Table 4.1). Autosomal and Z-linked nucleotide diversity covaried across sites, such that sites with relatively high autosomal diversity generally also exhibited high Z-linked diversity. Across the five genetic clusters, mutation-corrected Z/A ratios ranged from 0.406 in Costa Rica Pacific to 0.497 in Caribbean + Central Panamá (Table 4.2). These two genetic clusters also had the lowest and highest nucleotide diversity, respectively, with autosomal π ranging from 0.00344 to 0.00411 and mutation-corrected Z-linked π from 0.00140 to 0.00205. The genetic cluster with the highest mean Z/A ratio was the genetic cluster having the most sampling sites (n = 6). The lower Z/A ratio of the Costa Rica Pacific cluster relative to Costa Rica Caribbean cluster (with a subspecies separated by the Cordillera de Talamanca) is reflective of previous pilot study data that showed decreased Z/A diversity at the same Costa Rica Pacific sites (Shogren et al., manuscript in preparation).

Climatic Variation and Conditional Associations with Z/A

The 15 sampling sites encompassed substantial climatic variation. Within-year rainfall variability ranged from 0.393 to 0.808, interannual variability in wettest-quarter rainfall from 0.085 to 0.199, and interannual variability in driest-quarter rainfall from 0.269 to 0.960. Wettest-quarter rainfall ranged from 943 to 1,747 mm, driest-quarter rainfall from 28 to 537 mm. Elevation of sampling sites ranged from 640 to 1,335 m.

Table 4.1 Mean autosomal nucleotide diversity (πA), mutation-corrected Z-linked nucleotide diversity (πZ), Z/A diversity ratios (πZ/πA), standard errors (SE), and numbers of analyzed windows for each of 15 sampling sites. Genetic cluster assignments correspond to K = 5 ADMIXTURE groups. Z-linked nucleotide diversity and Z/A diversity ratios were corrected for male-biased mutation rate by dividing πZ by 1.1.

Sampling Site	Genetic Cluster (K = 5)	πA	SE	πZ	SE	πZ/πA	Windows (A)	Windows (Z)
FP	Western Panama Pacific	0.00385	1.27 × 10^{-5}	0.00179	2.63 × 10^{-5}	0.466	19,821	1,404
EC	Costa Rica Caribbean	0.00391	1.30 × 10^{-5}	0.00181	2.68 × 10^{-5}	0.464	19,821	1,404
RA	Costa Rica Caribbean	0.00378	1.28 × 10^{-5}	0.00168	2.59 × 10^{-5}	0.444	19,821	1,404
VT	Costa Rica Caribbean	0.00373	1.27 × 10^{-5}	0.00162	2.56 × 10^{-5}	0.436	19,821	1,404
AC	Panama Caribbean + Central Panama	0.00387	1.27 × 10^{-5}	0.00187	2.69 × 10^{-5}	0.484	19,821	1,404
AP	Panama Caribbean + Central Panama	0.00393	1.32 × 10^{-5}	0.00186	2.67 × 10^{-5}	0.472	19,821	1,404
BT	Panama Caribbean + Central Panama	0.00403	1.33 × 10^{-5}	0.00196	2.75 × 10^{-5}	0.487	19,821	1,404
FC	Panama Caribbean + Central Panama	0.00409	1.35 × 10^{-5}	0.00197	2.84 × 10^{-5}	0.481	19,821	1,404
SC	Panama Caribbean + Central Panama	0.00402	1.33 × 10^{-5}	0.00195	2.82 × 10^{-5}	0.483	19,821	1,404
SP	Panama Caribbean + Central Panama	0.00399	1.32 × 10^{-5}	0.00194	2.77 × 10^{-5}	0.487	19,821	1,404
CA	Eastern Panama	0.00407	1.33 × 10^{-5}	0.00197	2.75 × 10^{-5}	0.483	19,821	1,404
CH	Eastern Panama	0.00380	1.26 × 10^{-5}	0.00179	2.65 × 10^{-5}	0.472	19,821	1,404
CG	Costa Rica Pacific	0.00336	1.27 × 10^{-5}	0.00131	2.37 × 10^{-5}	0.391	19,821	1,404
LC	Costa Rica Pacific	0.00341	1.29 × 10^{-5}	0.00132	2.43 × 10^{-5}	0.387	19,821	1,404
LQ	Costa Rica Pacific	0.00337	1.27 × 10^{-5}	0.00137	2.46 × 10^{-5}	0.407	19,821	1,404

Table 4.2 Mean autosomal nucleotide diversity (πA), mutation-corrected Z-linked nucleotide diversity (πZ), Z/A diversity ratios (πZ/πA), standard errors (SE), and numbers of analyzed windows for each of five K = 5 genetic clusters. Z-linked nucleotide diversity and Z/A diversity ratios were corrected for male-biased mutation rate by dividing πZ by 1.1.

Genetic Cluster (K = 5)	πA	SE	πZ	SE	πZ/πA	Windows (A)	Windows (Z)
Costa Rica Caribbean	0.00387	1.30×10^{-5}	0.00176	2.64×10^{-5}	0.455	19,821	1,404
Panama Caribbean + Central Panama	0.00411	1.34×10^{-5}	0.00205	2.87×10^{-5}	0.497	19,821	1,404
Eastern Panama	0.00400	1.31×10^{-5}	0.00195	2.78×10^{-5}	0.489	19,821	1,404
Western Panama Pacific	0.00385	1.27×10^{-5}	0.00179	2.63×10^{-5}	0.466	19,821	1,404
Costa Rica Pacific	0.00344	1.29×10^{-5}	0.00140	2.49×10^{-5}	0.406	19,821	1,404

In conditional analyses assessing the effect of climate after accounting for genetic cluster, Z/A diversity ratios were negatively associated with interannual variability in wettest-quarter rainfall ($\beta = -0.190 \pm 0.083$ SE, 95% CI = −0.379 to −0.001, $t = -2.28$, $P = 0.049$, partial $R^2 = 0.366$; Fig. 4.2; Table 4.3). We found no evidence of relationships between Z/A diversity ratios and within-year rainfall variability (CV among monthly mean rainfall; $\beta = -0.017 \pm 0.032$, $P = 0.600$), interannual variability in driest-quarter rainfall ($\beta = -0.004 \pm 0.030$, $P = 0.887$), wettest-quarter rainfall ($\beta = -7.60 \times 10^{-6} \pm 1.65 \times 10^{-5}$, $P = 0.657$), driest-quarter rainfall ($\beta = 9.57 \times 10^{-7} \pm 2.31 \times 10^{-5}$, $P = 0.968$), or elevation as a proxy for temperature ($\beta = 5.42 \times 10^{-6} \pm 1.87 \times 10^{-5}$, $P = 0.779$). In reciprocal conditional analyses, genetic cluster was strongly associated with Z/A across all six conditional climate models, with all five clusters exhibiting a similar pattern when controlling for the climatic factor in question ($F = 25.05$–65.03, $P < 0.001$, partial $R^2 = 0.918$–0.967; Fig. 4.3; Table 4.3). Differences in predicted Z/A among genetic clusters were

qualitatively consistent across all six climate models, with the same clusters exhibiting higher or lower predicted Z/A diversity after accounting for each climate variable.

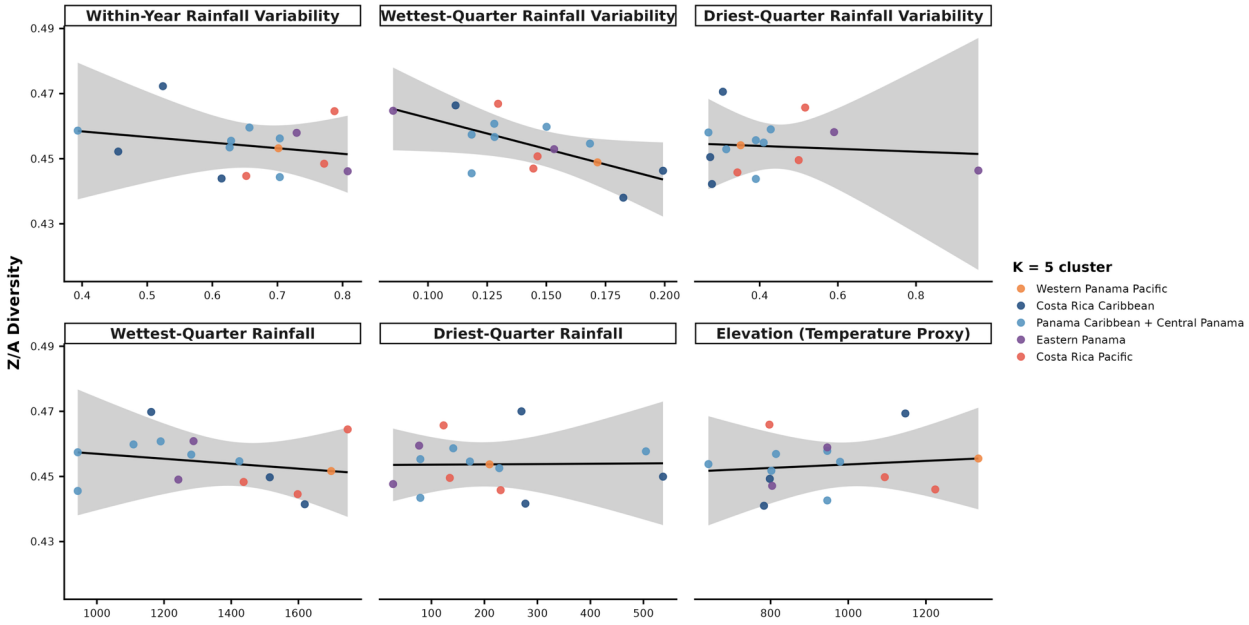


Figure 4.2 Relationships between genetic cluster (K = 5) and mutation-corrected Z/A nucleotide diversity ratios across 15 white-ruffed manakin sampling sites. Points show estimates from linear models fit to all 15 sampling sites and including each of six *a priori* climate metrics as a fixed effect; genetic clusters are indicated by color and error bars indicate 95% confidence intervals.

Population Genetic Structure and Conditional Associations with Z/A

Genetic differentiation (F_{ST}) varied among pairwise comparisons of the five genetic clusters. Mean autosomal F_{ST} ranged from 0.05630 to 0.23097, whereas mean Z-linked F_{ST} ranged from 0.12648 to 0.39942. Z-linked F_{ST} exceeded autosomal F_{ST} for all 10 pairwise comparisons (Table 4.4). Absolute sequence divergence (d_{XY}) varied over a comparatively narrow range; mean autosomal d_{XY} ranged from 0.00396 to 0.00473, and mean Z-linked d_{XY} ranged from 0.00206 to 0.00299 (Table 4.5). In contrast to F_{ST} values, Z-linked d_{XY} was lower than autosomal d_{XY} for all 10 pairwise comparisons, with both autosomal and Z-linked d_{XY} at their lowest when comparing

paired geographically proximate clusters. Absolute differences in Z/A diversity ratios increased with mean autosomal F_{ST} among the ten pairwise comparisons of the clusters ($\beta = 0.304 \pm 0.123$ SE, 95% CI = 0.020–0.588, $t = 2.47$, $P = 0.039$, $R^2 = 0.432$; Fig. 4.4A). Absolute differences in Z/A diversity ratios were not significantly associated with mean autosomal d_{XY} ($\beta = 45.6 \pm 42.9$ SE, 95% CI = −53.4–145, $t = 1.06$, $P = 0.319$, $R^2 = 0.124$; Fig. 4.4B).

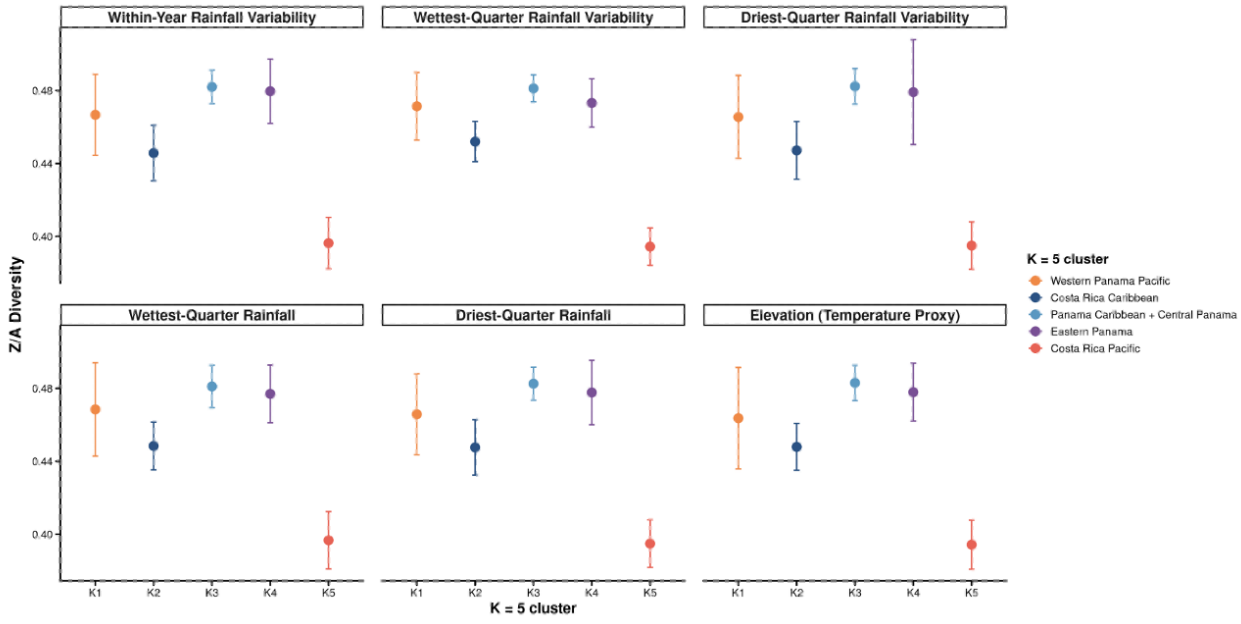


Figure 4.3 Relationships between genetic cluster (*K* = *5*) and mutation-corrected Z/A nucleotide diversity ratios across 15 white-ruffed manakin sampling sites. Points show estimates from six linear models fit to all 15 sampling sites, each including genetic cluster (*K* = 5) and one of six *a priori* climate metrics as fixed effects; genetic clusters are indicated by color and error bars indicate 95% confidence intervals.

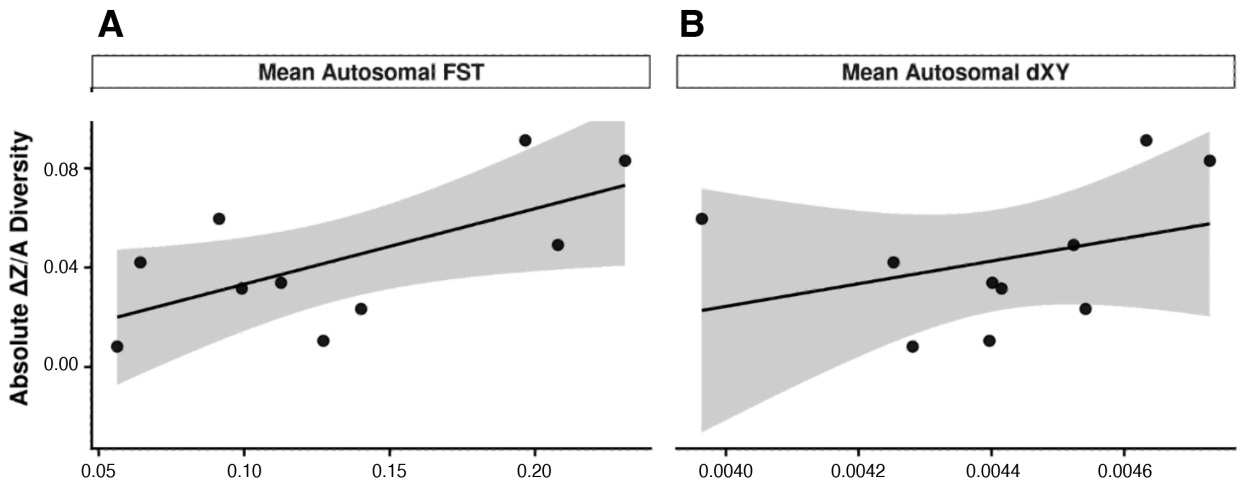


Figure 4.4 Relationships between autosomal genetic differentiation and ancestral divergence as compared to absolute differences in Z/A diversity ratios among the five K = 5 genetic clusters. A. Relationship between mean autosomal genetic differentiation (F_{ST}) and absolute differences in Z/A diversity ratios. B. Relationship between mean autosomal genetic divergence (d_{XY}) and absolute differences in Z/A diversity ratios. Black lines show fitted linear regressions, and gray shading represents 95% confidence intervals.

Table 4.3 Results from linear models examining relationships between six *a priori* climate metrics and mutation-corrected Z/A nucleotide diversity ratios across 15 white-ruffed manakin sampling sites, with genetic cluster ($K = 5$) included as a fixed effect. Partial R^2 values indicate the variance attributable to each climate metric and genetic cluster after accounting for the other predictor. Genetic-cluster F statistics have 4 and 9 numerator and residual degrees of freedom, respectively.

Climate Variable	Estimate	SE	95% CI Lower	95% CI Upper	Climate P	Climate Partial R^2	Genetic Cluster F	Genetic Cluster P	Genetic Cluster Partial R^2	Full Model R^2	Full Model Adjusted R^2	Full Model P
Within-Year Rainfall Variability	-0.017	0.032	-0.089	0.054	0.600	0.032	41.978	7.963×10^{-6}	0.949	0.952	0.925	1.129×10^{-5}
Wettest-Quarter Rainfall Variability	-0.190	0.083	-0.379	-0.001	0.049	0.366	65.029	1.224×10^{-6}	0.967	0.969	0.951	1.716×10^{-6}
Driest-Quarter Rainfall Variability	-0.004	0.030	-0.073	0.064	0.887	0.002	43.226	7.034×10^{-6}	0.951	0.951	0.923	1.290×10^{-5}
Elevation (Temperature Proxy)	5.419×10^{-6}	1.871×10^{-5}	-3.690×10^{-5}	4.774×10^{-5}	0.779	0.009	39.507	1.029×10^{-5}	0.946	0.951	0.924	1.251×10^{-5}
Wettest-Quarter Rainfall	-7.602×10^{-6}	1.653×10^{-5}	-4.500×10^{-5}	2.980×10^{-5}	0.657	0.023	25.054	6.789×10^{-5}	0.918	0.952	0.925	1.176×10^{-5}
Driest-Quarter Rainfall	9.575×10^{-7}	2.306×10^{-5}	-5.121×10^{-5}	5.313×10^{-5}	0.968	1.915×10^{-4}	43.096	7.125×10^{-6}	0.950	0.950	0.923	1.302×10^{-5}

Table 4.4 Mean pairwise autosomal ($F_{ST,A}$) and Z-linked ($F_{ST,Z}$) genetic differentiation, standard errors (SE), and numbers of analyzed windows for all ten pairwise comparisons among the five K = 5 genetic clusters.

Pairwise Comparison	$F_{ST,A}$	SE	$F_{ST,Z}$	SE	Windows (A)	Windows (Z)
Costa Rica Caribbean vs Panama Caribbean + Central	0.06429	2.22×10^{-4}	0.13008	1.62×10^{-3}	19,814	1,404
Costa Rica Caribbean vs Eastern Panama	0.11267	3.38×10^{-4}	0.22374	2.32×10^{-3}	19,814	1,404
Costa Rica Caribbean vs Western Panama Pacific	0.12722	4.19×10^{-4}	0.28636	3.33×10^{-3}	19,813	1,404
Costa Rica Caribbean vs Costa Rica Pacific	0.20788	6.78×10^{-4}	0.39942	3.89×10^{-3}	19,814	1,404
Panama Caribbean + Central vs Eastern Panama	0.05630	2.04×10^{-4}	0.12648	1.67×10^{-3}	19,815	1,404
Panama Caribbean + Central vs Western Panama Pacific	0.09918	3.87×10^{-4}	0.21977	2.96×10^{-3}	19,815	1,404
Panama Caribbean + Central vs Costa Rica Pacific	0.19666	6.44×10^{-4}	0.35646	3.35×10^{-3}	19,815	1,404
Eastern Panama vs Western Panama Pacific	0.14020	4.18×10^{-4}	0.27313	2.81×10^{-3}	19,815	1,404
Eastern Panama vs Costa Rica Pacific	0.23097	6.71×10^{-4}	0.39837	3.43×10^{-3}	19,814	1,404
Western Panama Pacific vs Costa Rica Pacific	0.09136	4.04×10^{-4}	0.15366	2.22×10^{-3}	19,815	1,404

Table 4.5 Mean pairwise autosomal ($d_{XY,A}$) and Z-linked ($d_{XY,Z}$) absolute sequence divergence, standard errors (SE), and numbers of analyzed windows for all ten pairwise comparisons among the five K = 5 genetic clusters.

Pairwise Comparison	$d_{XY,A}$	SE	$d_{XY,Z}$	SE	Windows (A)	Windows (Z)
Costa Rica Caribbean vs Panama Caribbean + Central	0.00425	1.37×10^{-5}	0.00240	3.27×10^{-5}	19,821	1,404
Costa Rica Caribbean vs Eastern Panama	0.00440	1.40×10^{-5}	0.00260	3.38×10^{-5}	19,821	1,404
Costa Rica Caribbean vs Western Panama Pacific	0.00440	1.39×10^{-5}	0.00270	3.33×10^{-5}	19,821	1,404
Costa Rica Caribbean vs Costa Rica Pacific	0.00452	1.42×10^{-5}	0.00284	3.47×10^{-5}	19,821	1,404
Panama Caribbean + Central vs Eastern Panama	0.00428	1.37×10^{-5}	0.00250	3.27×10^{-5}	19,821	1,404
Panama Caribbean + Central vs Western Panama Pacific	0.00442	1.39×10^{-5}	0.00271	3.33×10^{-5}	19,821	1,404
Panama Caribbean + Central vs Costa Rica Pacific	0.00463	1.43×10^{-5}	0.00294	3.53×10^{-5}	19,821	1,404
Eastern Panama vs Western Panama Pacific	0.00454	1.42×10^{-5}	0.00282	3.44×10^{-5}	19,821	1,404
Eastern Panama vs Costa Rica Pacific	0.00473	1.45×10^{-5}	0.00299	3.62×10^{-5}	19,821	1,404
Western Panama Pacific vs Costa Rica Pacific	0.00396	1.34×10^{-5}	0.00206	3.19×10^{-5}	19,821	1,404

Discussion

We studied patterns of sex chromosome to autosomal diversity within a single sexually-selected bird species across a range of climatic regimes. Mutation-corrected Z/A ratios were uniformly low, averaging 0.456 across 15 sampling sites, with a range from 0.406 - 0.497 among the five genetic clusters. Similar to other studies on lekking species, these values were lower than the theoretical expectation based on reproductive skew alone and varied systematically with genome-wide population structure (Anderson et al., 2026; Oyler-McCance et al., 2015). We tested two alternative hypotheses regarding the drivers of Z/A diversity. First, we asked whether variation in Z/A ratios among white-ruffed manakin populations was correlated with climatic factors that we predicted may alter the effect of natural selection on reproductive hierarchies. Specifically, we hypothesized that precipitation and temperature conditions imposing stronger natural-selection pressures could alter differences in siring success among males and thereby affect reproductive skew and Z/A diversity. We tested this hypothesis against the competing prediction that population genetic structure and history, measured through population structure analyses, genetic differentiation, and absolute sequence divergence, play a significant role in driving variation in Z/A diversity ratios. To directly evaluate the relative contributions of climate and population structure, we used conditional models to quantify the variation uniquely attributable to each after accounting for the other.

Overall, we found little support for our hypothesis that climate regimes influence Z/A ratios. We did not detect an association of Z/A ratios with within-year rainfall variability, wettest or driest quarter rainfall, interannual driest-quarter rainfall variability across years, or elevation as a proxy for temperature. Z/A ratios did decrease as interannual variability in wettest quarter rainfall increased, but this occurred in the opposite direction to our prediction. We had predicted

that specific climatic conditions could destabilize reproductive hierarchies and reduce alpha-male monopolization of reproductive opportunities (Shogren et al., 2021). There is strong evidence that rainfall imposes physiological and energetic costs on white-ruffed manakins and that many individuals migrate altitudinally in response to rain (Boyle et al., 2010; Shogren et al., 2019). However, rainfall-associated mortality has not been demonstrated specifically for males or alpha males, and apparent survival of white-ruffed manakins was not associated with interannual variation in rainfall (Shogren et al., 2019). Moreover, although alpha-male turnover (i.e., replacement of alpha male status at a continuously occupied display log between years) was high and could be associated with mortality of males, turnover also did not differ between populations experiencing contrasting rainfall regimes and was not associated with rainfall across years (Shogren et al., 2021). Our observed relationship between climate and Z/A therefore provides no support for the proposed mechanism of male hierarchy destabilization, as the climatic factors we tested did not translate into Z/A diversity ratios in the predicted directions. Meanwhile, population genetic history, as measured by genetic cluster and genetic differentiation, was strongly associated with variation in Z/A ratios. In models containing both climate and genetic cluster, partial R^2 values allowed us to quantify their relative unique contributions to variation in Z/A across all sampling sites. Genetic cluster consistently explained a large proportion of variation after accounting for climate, whereas five of the six climatic metrics explained little variation after accounting for genetic cluster. Of course, these conditional effects do not quantify variation *shared* between climate and population structure, an issue we return to below.

We also found a significant positive relationship between pairwise autosomal F_{ST} and absolute Z/A differences among pairwise clusters. Since F_{ST} and Z/A are both influenced by within-population diversity, the relationship between them is not entirely independent but may

still provide insight into how changes in within-population diversity contribute to both genetic differentiation and Z/A. Because Z-linked diversity is expected to be more strongly affected by genetic drift than autosomal diversity, demographic events such as population bottlenecks may disproportionately reduce πZ relative to πA and thereby reduce Z/A (Irwin, 2018). Consistent with this expectation and the possibility of such population processes, we found that the Costa Rica Pacific cluster had a particularly strong reduction in Z-linked diversity relative to autosomal diversity, with this region having the lowest Z/A among the five genetic clusters. More broadly, if differences in Z/A among the five regional clusters reflect differences in their demographic histories, the disproportionate contribution of Z-linked diversity is consistent with theoretical expectations; historical demographic processes are expected to leave stronger signatures on sex chromosomes than on autosomes (Irwin, 2018).

Pairwise d_{XY} provides additional context for the history of genetic structuring: despite more than four-fold variation in F_{ST} among genetic clusters, absolute sequence divergence varied comparatively little. Greater variation in d_{XY} might be expected if strong variation in F_{ST} primarily reflected substantially different timescales of ancestral divergence. Instead, the much greater variation in F_{ST} than d_{XY} is consistent with differences in within-population diversity and the partitioning of genetic variation contributing substantially to the observed F_{ST} and Z/A patterns. Although these patterns are consistent with demographic processes such as bottlenecks, founder events, and drift, our current analyses cannot confidently distinguish which demographic mechanisms produced them.

An important limitation of ecological studies of landscape genomics studies is that sampling site-level genomic metrics such as Z/A diversity will necessarily be related to population genetic structure and geography. We addressed this issue using reciprocal conditional

analyses to quantify the unique contributions of climate and population structure: we asked whether climate was associated with Z/A after accounting for genetic cluster, and whether genetic cluster was associated with Z/A after accounting for climate. These analyses therefore estimate the variation uniquely attributable to each predictor, but they do not characterize variation shared between climate and population structure.

A related issue arises from the geographic distribution of our sampling sites: sites within the same genetic cluster may also experience more similar environmental conditions. If geographic region defines both climatic regimes and affects birds' opportunities for genetic differentiation and dispersal, accounting for genetic cluster may also account for variation associated with climate. Thus, some climatic signal could be "absorbed" by population structure in the conditional models, potentially making climatic effects more difficult to detect. One approach to further separate these effects would be to evaluate climate–Z/A relationships within genetic clusters. However, the validity of such analyses depends on sufficient climatic variation and replication within clusters; either restricted climatic variation or low replication may bias analyses *against* detecting an existing climatic relationship (Miciak et al., 2016; Lemoine & Chaves, 2016). Thus, prior to interpreting within-cluster analyses, it would be important to evaluate whether the observed climatic range and replication within clusters provide sufficient power to detect an effect of biologically plausible magnitude. Given these limitations, partial redundancy analysis and variance partitioning may provide an alternative approach for quantifying the unique and shared contributions of climate and population genetic structure to Z/A variation across the full dataset (Rellstab et al. 2015; Frichot et al. 2013).

We attempted to disentangle how abiotic influences and population genetic structure affect sex chromosome to autosomal ratios. By investigating the possible interaction of natural

selection, sexual selection, and population structure, we find further empirical evidence suggesting that Z/A is a multidimensional metric shaped by population genetic history in addition to processes associated with reproductive skew. In particular, sex chromosome-to-autosomal diversity ratios may be used in conjunction with other metrics of evolutionary history to generate hypotheses about historical demography and test theoretical expectations about sex chromosome evolution. We thus suggest that population genetic history is an important source of background variation to be considered carefully when interpreting sex chromosome-to-autosomal diversity across populations and taxa.

Supplementary Information

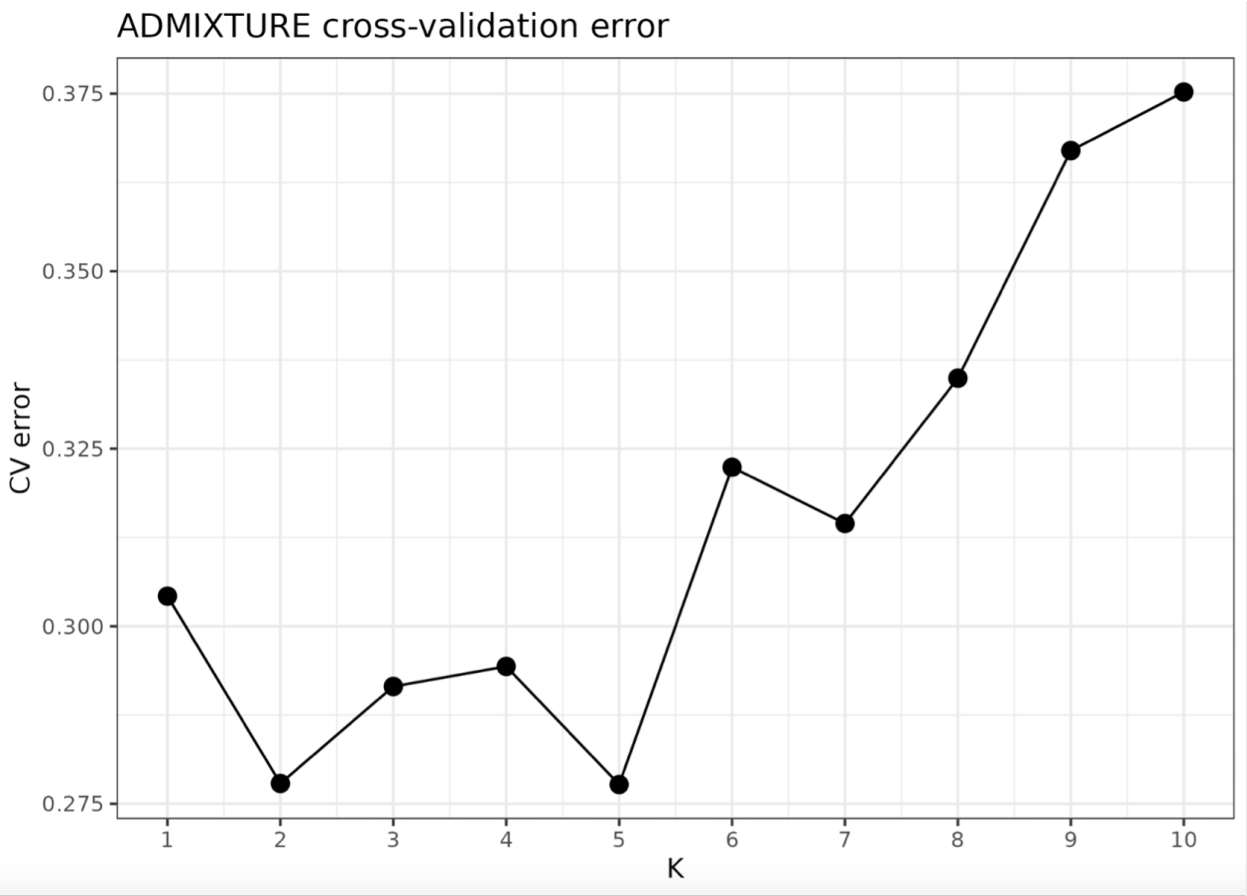


Figure S4. 1 Cross-validation error for ADMIXTURE analyses from K = 1–10. K = 5 had the lowest cross-validation error and was therefore used to define genetic clusters in subsequent analyses.

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Chapter 5 - Conclusions & broader implications

Understanding how abiotic factors affect animals is a central goal of ecological research. In this dissertation, I studied how natural selection and climatic forces interact with sexual selection to affect Neotropical birds' behavior and evolution. Across three empirical chapters, I worked with two Neotropical manakin species to understand the effects of precipitation and elevation on their life history strategies, reproductive effort, and genomic signatures. Broadly speaking, my results emphasize the importance of developing studies that can distinguish subtle biological signals from null results against the background variation inherent in complex ecological systems. Together, these results show pathways by which climatic conditions may shape life history strategies and behavior, while emphasizing that such effects are often subtle and strongly dependent on ecological and evolutionary context.

Even as climatic effects can be difficult to disentangle from longer-term population genetic structure, my research illustrates multiple ways in which precipitation may shape life-history strategies and reproductive effort. While I provided little support for the hypotheses that residual reproductive value or quality drive altitudinal migratory behavior in white-ruffed manakins, I found that condition-related carryover effects may influence migratory decision-making at the individual level. I also found that rainfall affects display behavior strongly, as expected, but does so similarly regardless of the elevation at which it is experienced. This latter finding represents an interesting departure from one of the theoretical predictions of hygric niche theory. Finally, I found little evidence that climatic variables predict Z/A diversity ratios after accounting for broader population genetic structure. My findings demonstrate the value of novel empirical tests of ecological theory, while advancing our understanding of how tropical species respond to climatic variation.

Theoretical links between climatic variation and biological responses generate compelling predictions, but rigorously evaluating these predictions requires careful planning and interpretation. Natural variability in climatic and biological data, interacting drivers, and lagged responses can all complicate attribution of biological responses to climate (Harris et al., 2020; McCain et al., 2016). One important goal in ecological research is ensuring that study design provides a meaningful test of biological effects. Negative results are most informative when studies are designed *a priori* to distinguish biologically meaningful effects from effects too small or context-dependent to resolve. Because features of both the study design and the data can limit our ability to detect and interpret climatic relationships, these challenges should be considered explicitly during study design. For example, prospective analyses conducted prior to fieldwork can help identify the sampling effort needed to detect climatic effects, while predefined criteria for evaluating influential or extreme observations can reduce ambiguity in interpreting results. However, meaningful tests also require falsifiable theoretical expectations. While tests of climatic hypotheses must therefore be designed *a priori* to detect climatic effects when they occur, we should also explicitly state what outcomes would reduce support for our expectations. The mixture of supported and unsupported predictions across this dissertation highlights the importance of both considerations.

Since climatic and biological processes may operate differently depending on the scale of evaluation, testing theoretical expectations at multiple biological scales can improve our ability to detect climatic signals. This dissertation illustrates the value of this approach, with climatic relationships differing across behavioral, life-history, and genomic responses. Together, my findings broaden our understanding of how precipitation shapes the behavior and evolution of tropical birds, from immediate effects on life history strategies and reproductive behavior to

relationships between climate and population genetic structure across a species' range. By identifying both predicted relationships that received empirical support and those that did not, this dissertation helps refine our understanding of when and how climatic conditions affect tropical species.

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