

## Electronic Supplementary Material for "Lekking birds in a tropical forest forego sex for migration"

### Supplementary Materials and Methods

**Manakin annual cycle and displays** White-ruffed Manakins breed in middle-elevation wet forests from Honduras to Venezuela. Caribbean slope populations in Costa Rica initiate breeding displays between late Feb–Mar with females nesting between Mar–Jun and young fledging Jun–Jul. From Jul through to late as Dec, individuals in this population migrate down to lowland forests, remaining there until the following Feb or Mar [1, 2]. Birds stressed by parasites or moult are more likely to migrate, and more adult (>3<sup>rd</sup> yr) males migrate than females or sub-adult males [3, 4].

Mark-recapture data provide concrete evidence that this species is indeed partially migratory; some individuals have been recaptured at breeding sites throughout the year and other individuals have been recaptured at lower elevations during the non-breeding season than where they were first marked [3]. Additionally, migrants appear to vary in the elevations they migrate to because as far as we know, White-ruffed Manakin non-breeding range extends from breeding elevations down to sea level without elevational gaps in their distribution. However, distinguishing whether mid-elevation captures represent individuals that remain there for the duration of the non-breeding season or individuals in transit to lower elevations is challenging. Mark-recapture data and capture rates at different elevations also provide evidence that once individuals have made the decision to migrate, they remain at low elevations for the remainder of the non-breeding season. We have recaptured individuals repeatedly at low elevations over several months and capture rates gradually increase at low elevations and gradually decrease at breeding elevations until the spring uphill migration period [1].

Leks in this species have been described most frequently as solitary dispersed leks [5], but also as solitary aggregated leks [6], or leks involving coordinated displays by multiple males [7] on fallen mossy logs on or near the forest floor. We use the word "lek" in this study to refer to single display logs and their immediate environs. The alpha may be the only male attending a lek in a season or he may be joined regularly by one-to-several other males that congregate and display together during the breeding season. Subordinate males form social hierarchies, and are tolerated on or in close proximity to the display log by the alpha male. Activity levels (e.g., number of attending males, call frequency, display frequency) varies dramatically among leks. During the non-breeding season, males rarely vocalize at leks or land on logs but individuals of both sexes and all ages visit leks throughout the year.

Displays (listed in increasing order of intensity) consist of rapid flights between low perches, log landings, throat flagging (crouching low to the log while puffing out throat feathers), aerial flaps (high-speed aerial dives from above the canopy suddenly stalled mid-dive, accompanied by mechanical sounds), butterfly flights (slow, upright, bobbing flights), and the "flap-chee-wah" which starts with an ascent to above the canopy, circling ~10 m above the canopy in a butterfly-like flight while making "seeuw" calls, then a high-speed dive to the log followed immediately by a small leap [6](ESM video 1). If a female visits a lek, lands on the log, and appears receptive, the alpha male will do the flap-chee-wah display over her, then immediately mount her, and copulate (ESM video 2).

**Study site, lek searching, and manakin sampling** We studied White-ruffed Manakins breeding at Rara Avis and the adjoining Selva Tica (collectively "Rara Avis"; RA) reserves in NE Costa Rica (10°17.3" N, 84°03.1" W, ~500–850 m) during 2008 and 2009 (mid Feb–early May). Mean annual rainfall exceeds 8200 mm, and although less rain falls during the manakin breeding season than in other months, mean monthly rainfall always exceeds 500 mm. Heavy rains in combination with steep topography result in epiphyte-laden trees, frequent branch and tree falls, a rich understory, and broken canopy.

We searched for leks using two approaches. We established 2.25 km of research trails in a grid-like pattern with trails located ~50 m apart from each other and covering ~10 ha. We intensively searched for leks on this plot by slowly walking along trails and listening for advertisement trills and displays. If we detected repeated advertisement trills or any displays, we circled the area, searching for potential leks, and sat quietly until we confirmed a display log by seeing a manakin perform the flap-chee-wah display on the log. Advertisement trills can be easily heard > 25 m away so we are confident we found most, if not all, active leks in this area each year. Additionally, we opportunistically walked trails over much of the reserve, listening and looking for manakins, adopting the same protocols as on the plot when we detected a potential lek. We captured manakins in mist nets both at fixed net locations on the study plot as well as in the understory of forest and along trails near to manakin leks. We placed one numbered aluminium and two coloured bands in unique combinations on each individual, aged and sexed birds using plumage and reproductive characteristics, and clipped the distal 1.5 mm from the central claw on each foot. We measured claws before and after clipping, and upon recapture to estimate claw growth rates, focusing capture efforts early in each season to obtain claw samples as soon as possible after return from migration.

**Observations and video recordings** Observers watched leks for 3-hr periods between 0600 and 1700. We observed leks at a mean distance of 8.8 m (5.6–15 m) from the log from inside small blinds constructed of pvc and green shade cloth camouflaged *in situ* with vegetation. Observers recorded the identity (band combination) of any manakin seen, duration of visit to the lek, log landings, number and type of vocalizations, and number and type of all display behaviours. We determined the ranking of individual males in the social hierarchy during each observation based on their frequency of vocalization and display, interactions with females and other males, and perching distance from the display log. In analyses exploring the relationship between lek quality and  $\delta D$  of the alpha male, we chose the male in each season most frequently characterized as the alpha in observations at that lek (when different individuals were identified as alphas at the same lek during different observations).

Because we were unsure if the presence of observers in blinds affected male display rate and (especially) propensity of females to visit leks, we used video recordings as an independent measure of display frequency and female visitation. We made two ~5.5 hr video recordings each day at different leks from between 0500–1200 and 1100–1700 using a Sony Handycam HDR-SR12 housed inside a waterproof case and mounted ~1 m above the ground on a tripod. We located the camera an average of 5.5 m (4.1–7.1 m) from the display log and camouflaged it with green shade cloth and vegetation. We minimized human disturbance in the area during the recordings.

In 2008, we apportioned observer effort roughly equally among all leks while continuing to search for new leks. Thus, we observed leks discovered earlier in the season more frequently than those discovered later in the season. In 2009, we observed all leks (other than those

discovered toward the end of the field season)  $\geq 3$  times, and we observed most leks 5–6 times. Additionally, we chose 10 focal leks at which we recorded video data and conducted additional observations (6–8 observations total). We chose focal leks that spanned the full range of activity levels (based on 2008 data). Three trained observers watched videos in real-time, noting time, duration, and behaviours of any manakin visible in the video frame. Due to differences in field of view at each lek recorded, we were limited to quantifying behaviours in close association with the display log.

**Stable isotope data** Because water molecules containing deuterium (D or  $^2\text{H}$ ) precipitate out of clouds more rapidly than those containing protium ( $^1\text{H}$ ), the ratio of D:H (expressed as  $\delta\text{D}$  as parts per thousand (‰) deviation from the Vienna Standard Mean Ocean Water, V-SMOW where  $\delta\text{D} = [(\text{Ratio}_{\text{sample}}/\text{Ratio}_{\text{standard}}) - 1 \times 1000]$ ) in precipitation decreases with increasing elevation, especially on windward mountain slopes near oceans.  $\delta\text{D}$  of precipitation also decreases with increasing amount of precipitation [8]. This elevational gradient in precipitation isotope ratios is transferred up foodwebs to consumers and so can be used to infer movement patterns along altitudinal gradients [9]. In our study region, differences among sites in precipitation  $\delta\text{D}$  due to elevational are likely enhanced by differences in precipitation amount. Manakin breeding areas receive over ~8 m rain annually compared to ~4 m at the base of the mountain which would serve to magnify the elevation effect.

Avian claws grow continuously and turn over completely in ~3–5 mo in small passerines [10]. Due to the conical structure of avian claws, the distal tip combines the oldest tissues with younger interior keratin layers. Therefore, isotope ratios in claw samples integrate spatial information over temporal scales that depend upon claw growth rates [11]. Thus, our index of migratoriness (i.e. claw  $\delta\text{D}$ ) is a continuous measure that incorporates both temporal and spatial information. In this short-distance, facultative migration system, a continuous measure of migratoriness accurately represents the biological reality of migration because different individuals depart their breeding grounds for very different lengths of time (departing as early as Jul and as late as Dec but returning together late Feb or early Mar). Because of these large temporal differences and the relatively small spatial (elevational) differences over which individuals spend the non-breeding season, it is likely that most of the variation in claw  $\delta\text{D}$  reflects this temporal component of the migration patterns. Although it is possible these two variables could confound one another to some degree, such confounding would only introduce unexplained error into our results and reduce, not increase, our ability to detect associations between variables of interest.

Using a continuous isotopic measure of migratoriness is also appropriate given the costs and benefits of variation in migratory behaviour we propose. The proposed benefits of migrating involves escape from heavy rains which serves to minimize adverse weather-related effects on condition and ultimately, likely increases survival probability. Rainfall (and temperature) data that we and others have collected at breeding, lowland, and intermediate elevations (200m, 300 m, and 500 m) on this gradient confirm that on daily bases as well as seasonal and annual time scales, climatic conditions vary roughly linearly over the gradient that manakins migrate (meteorological data from Rara Avis and La Selva biological stations, the Volcán Barva TEAM project, [1]). Thus, if variation in distance migrated accounts for some of the variation in  $\delta\text{D}$  in claws, that variation should reflect differences in the weather-related benefits of migrating to different elevations.

The proposed costs of migration also should vary continuously depending on the amount of time that a male spends away from his lek. Individuals may spend between ~2 and 7 months away from breeding areas. We suspect that the longer a male is absent, the greater his chances of losing status among other males and the less likely he will be to regain control of his lek the following breeding season. Furthermore, if reproductive costs are incurred via resident females monitoring male presence and assessing male quality based on their ability to withstand adverse climatic conditions, the longer males are gone, the more likely females will be to detect their absence.

We used three approaches to verify that differences detected in the  $\delta D$  of manakin claws early in the breeding season reflected differences in non-breeding elevation. First, we calculated the relationship between elevation and  $\delta D$  using the mean monthly rainwater values obtained from Costa Rican Caribbean-slope meteorological stations that are part of the Global Network of Isotopes in Precipitation (GNIP) program [12] (ESM figure 2). Second, we collected claws during Nov–Dec 2008 from resident and migrant White-ruffed Manakins at both breeding (RA) and non-breeding elevations (at La Selva Biological Station at ~30–100 m elevation, LS). Third, we collected claws during Nov–Dec 2008 from four other (non-migratory) frugivorous bird species whose elevational range spans breeding and non-breeding elevations of White-ruffed Manakins: White-collared Manakin (*Manacus candei*, resident at both elevations), Red-capped Manakin (*Pipra mentalis*, resident at LS), Tawny-capped Euphonia (*Euphonia annae*, resident at RA), and Olive-backed Euphonia (*Euphonia gouldi*, resident at LS). We followed the same capture and claw collection protocols as during the breeding season. We calculated differences in claw  $\delta D$  among individual White-ruffed Manakins wintering at both breeding and low elevations (ESM figure 3a), between individual White-collared Manakins resident at both elevations (ESM figure 3b), and (least square mean  $\delta D$ ) between claws grown at both elevations in all four non-migratory frugivorous species (accounting for species-level differences; ESM figure 3c).

While we expected consistent differences among species in the magnitude of the  $\delta D$  elevation effect, we did not expect species to necessarily be similar in the absolute values of  $\delta D$  within an elevation. Species-level differences in body size and physiological processes (especially BMR, [13]) affect fractionation within a bird as foraging substrate isotopic signatures are incorporated into tissues [14], and dietary differences (i.e., species of fruits consumed and the relative contribution of arthropod prey in diets) affect fractionation prior to consumption [15]. Mean body mass of the six species for which we compared  $\delta D$  range from 11.63 g (White-ruffed Manakins) to 17.33 g (White-collared Manakins). Although all of these species are primarily frugivorous, they do also differ in the proportion of arthropod prey in their diets and the species of fruit on which they forage [16]. Differences in manakin  $\delta D$  in the non-breeding season are not likely to be due to changes in diets because detailed dietary data from this species collected from individuals at three non-breeding elevations during the non-breeding season revealed remarkable consistency in the genera of plants species whose fruits manakins consumed, as well as a consistently low contribution of arthropod prey in male diets [1]. Similarly, body size of male manakins during the non-breeding season varies little (mean mass 11.19 g  $\pm$  1 SD 0.70, N = 81).

We surface sterilized claws using a 2:1 chloroform:methanol solution, air-dried under a fume hood, and then weighed out 350  $\mu$ g of claw tissue from each individual into silver capsules. In order to determine the  $\delta D$  of non-exchangeable H in claws, we used the comparative equilibration method of Wassenaar and Hobson [17] which uses keratinous lab standards that

have previously been calibrated for  $\delta D$  of non-exchangeable H. Stable hydrogen isotope measurements of samples and standards were performed on hydrogen derived from high-temperature (1350°C) flash pyrolysis of claw tissue and continuous flow-isotope ratio mass spectrometry (CF-IRMS). Pure H was used as the sample analysis gas and the isotopic reference gas. A Hekatech HTO with an autosampler was used to automatically pyrolyse claw tissue samples to a single pulse of H gas (and N<sub>2</sub> and CO gases). The resolved H sample pulse was then introduced to the isotope-ratio mass spectrometer (Elementar Isoprime, New Jersey) via an open split capillary. All isotope assays were conducted at Environment Canada's Stable Isotope Hydrology and Ecology Research Laboratory.

**Analyses** We assigned males the highest status achieved each year (i.e., alpha, beta, gamma, other male visitor, or captured but not seen at a lek). Because few leks had an individual clearly distinguished as third or "gamma" in the hierarchy, we combined gammas with other male visitors. For analyses exploring the relationship between lek quality and  $\delta D$  of the alpha male, we chose the male in each season most frequently characterized as the alpha male when different individuals were identified as alphas at the same lek during different observations.

We characterized alpha male attentiveness in two ways: (i) by calculating the proportion of minutes of observations during which he was within 15 m of his log, and (ii) using the number of advertisement trills/hr he made during observations. We estimated the total size of the subordinate male hierarchy at a lek by summing the number of distinct individuals observed throughout the season at that lek.

We categorized the change in each individual's status as: (i) birds maintained a "same-high" status if they remained an alpha or beta both years; (ii) birds "increased" if they went up at least one rank from being not seen → regular visitors → betas → alphas; (iii) birds "decreased" if they moved down through these rankings; or (iv) birds maintained a "same-low" status if they were not seen, or were infrequent or low-status visitors in both years.

Because claws grow continuously, lowland isotopic signatures would be expected to degrade as a function of time since return to breeding elevations. In both years, mean  $\delta D$  increased as the season progressed, consistent with more negative  $\delta D$  values in claw tissues grown in the lowlands being replaced with locally-grown tissue. However, slopes of the date relationship differed slightly between years with a stronger date effect in 2008 than 2009. Thus, for each year separately, we used the regression equation to standardize  $\delta D$  to the median date of claw sample collection. We used these adjusted  $\delta D$  claw values in all subsequent analyses. We also explored the possibility that small differences in the elevation of capture (between 650 m and 800 m) within our study site might influence  $\delta D$ . The slope of the relationship between capture elevation and  $\delta D$  was indistinguishable from zero (slope = 0.003,  $P = 0.816$ ) so we did not include capture elevation in our statistical models.

### References for Electronic Supplemental Material

1. Boyle, W. A. 2010 Does food abundance explain altitudinal migration in a tropical frugivorous bird? *Can. J. Zool.-Rev. Can. Zool.* **88**, 204–213. (DOI 10.1139/Z09-133)
2. Rosselli, L. 1994 The annual cycle of the White-ruffed Manakin, *Corapipo leucorrhoa*, a tropical frugivorous altitudinal migrant, and its food plants. *Bird Conserv. Intl.* **4**, 143-160.
3. Boyle, W. A. 2008 Partial migration in birds: tests of three hypotheses in a tropical lekking frugivore. *J. Anim. Ecol.* **77**, 1122-1128. (DOI 10.1111/j.1365-2656.2008.01451.x)

4. Boyle, W. A., Norris, D. R. & Guglielmo, C. G. 2010 Storms drive altitudinal migration in a tropical bird. *Proc. Roy. Soc. B* **277**, 2511-2519. (DOI doi:10.1098/rspb.2010.0344)
5. Prum, R. O. 1994 Phylogenetic analysis of the evolution of alternative social behavior in the manakins (Aves: Pipridae). *Evolution* **48**, 1657-1675.
6. Rosselli, L., Vasquez, P. & Ayub, I. 2002 The courtship displays and social system of the White-ruffed Manakin in Costa Rica. *Wilson Bull.* **114**, 165-178.
7. Slud, P. 1964 The birds of Costa Rica: distribution and ecology. *Bull. Am. Mus. Nat. Hist.* **128**, 1-430.
8. Bowen, G. J. 2010 Isoscapes: Spatial pattern in isotopic biogeochemistry. *Annu. Rev. Earth Pl. Sci.* **38**, 161-187. (DOI 10.1146/annurev-earth-040809-152429)
9. Bowen, G. J., Wassenaar, L. I. & Hobson, K. A. 2005 Global application of stable hydrogen and oxygen isotopes to wildlife forensics. *Oecologia* **143**, 337-348. (DOI 10.1007/s00442-004-1813-y)
10. Bearhop, S., Furness, R. W., Hilton, G. M., Votier, S. C. & Waldrons, S. 2003 A forensic approach to understanding diet and habitat use from stable isotope analysis of (avian) claw material. *Funct. Ecol.* **17**, 270-275.
11. Mazerolle, D. F. & Hobson, K. A. 2005 Estimating origins of short-distance migrant songbirds in North America: Contrasting inferences from hydrogen isotope measurements of feathers, claws, and blood. *Condor* **107**, 280-288.
12. IAEA/WMO 2006. Global Network of Isotopes in Precipitation. The GNIP Database. <http://www.iaea.org/water>.
13. Wiersma, P., Muñoz-Garcia, A., Walker, A. & Williams, J. B. 2007 Tropical birds have a slow pace of life. *Proceedings of the National Academy of Science USA* **104**, 9340-9345. (DOI doi:10.1073/pnas.0702212104)
14. Betini, G. S., Hobson, K. A., Wassenaar, L. I. & Norris, D. R. 2009 Stable hydrogen isotope ( $\delta D$ ) values in songbird nestlings: effects of diet, temperature, and body size. *Can. J. Zool.-Rev. Can. Zool.* **87**, 767-772. (DOI 10.1139/Z09-069)
15. Herrera, L. G., Hobson, K. A., Rodriguez, M. & Hernandez, P. 2003 Trophic partitioning in tropical rain forest birds: insights from stable isotope analysis. *Oecologia* **136**, 439-444. (DOI 10.1007/s00442-003-1271-y)
16. Boyle, W. A., Conway, C. J. & Bronstein, J. L. 2011 Why do some, but not all, tropical birds migrate? A comparative study of diet breadth and preference *Evol. Ecol.* **25**, 219-236. (DOI 10.1007/s10682-010-9403-4)
17. Wassenaar, L. I. & Hobson, K. A. 2003 Comparative equilibration and online technique for determination of non-exchangeable hydrogen of keratins for use in animal migration studies. *Isot. Environ. Health S.* **39**, 211-217. (DOI 10.1080/1025601031000096781)
18. Lachniet, M. S. & Patterson, W. P. 2009 Oxygen isotope values of precipitation and surface waters in northern Central America (Belize and Guatemala) are dominated by temperature and amount effects. *Earth Planet. Sci. Lett.* **284**, 435-446. (DOI 10.1016/j.epsl.2009.05.010)
19. Hobson, K. A., Wassenaar, L. I., Milá, B., Lovette, I., Dingle, C. & Smith, T. B. 2003 Stable isotopes as indicators of altitudinal distributions and movements in an Ecuadorean hummingbird community. *Oecologia* **136**, 302-308. (DOI 10:1007/s00442-003-1271-y)
20. Poage, M. A. & Chamberlain, C. P. 2001 Empirical relationships between elevation and the stable isotope composition of precipitation and surface waters: Considerations for studies of paleoelevation change. *Am. J. Sci.* **301**, 1-15.

## **Supplementary video legends**

### **Supplementary Video 1**

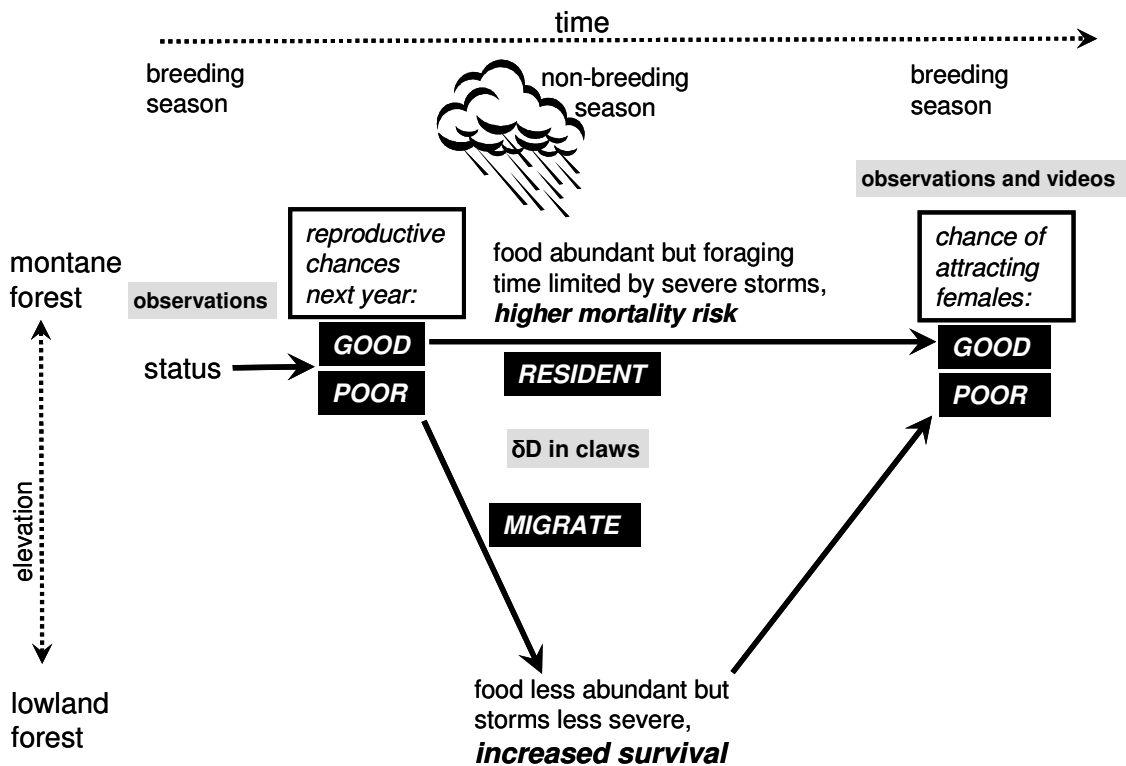
Clip showing an alpha-beta pair of adult males displaying together at a single lek. At 12 seconds, one male does the diving "flap-chee-wah" display over the second male who is already perched on the log. Over the next minute, both males perform several butterfly flights to and from the log and throat flag (both individually and together) before a second flap-chee-wah in the final seconds of the clip.

### **Supplementary Video 2**

Clip showing the final sequence of events leading up to a copulation. Three seconds into the recording, a female flies from the right onto the log near the worn 'landing pad'. The alpha male (who is not joined by other males in this clip) does bobbing, butterfly flights around her twice, lands and inflates his ruff in a throat-flag while crouching on the log beside her, then butterflies off at 19 sec to fly up above the canopy. The female looks up at the circling male high above her ~30-40 secs, and at 47 seconds, he dives into a flap-chee-wah followed immediately by copulation. As far as we know, this is the first video of matings in this species to be made publicly available.

Supplementary figures and legends

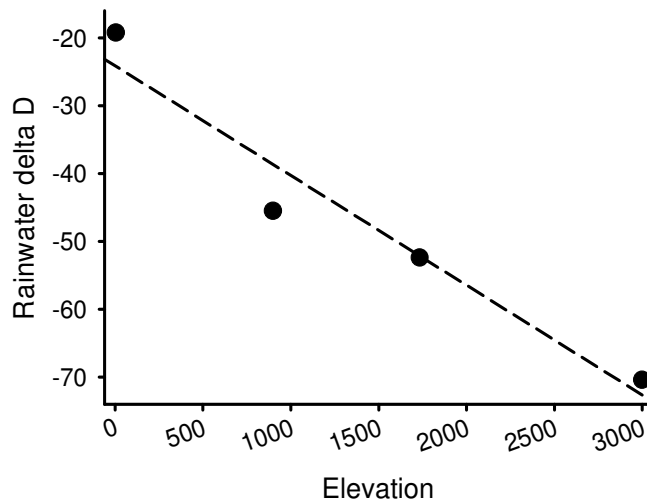
Figure 1



Schematic of factors proposed to influence migratory decisions of male White-ruffed Manakins (*Corapipo altera*). Boxes shaded grey indicate the types of data used in this study with results of this study shaded black. As the breeding season ends, birds move down slope in asynchronous movements. Migratory decisions are influenced by an individual's prospects for achieving a high-ranking status on a lek the following year (results of this study) and his current condition [4]. High-ranking males may have compromised their condition via aerobic displays at their leks to the point where they can no longer withstand the stressors of the wet-season storms. Likewise, males who have little or no prospects for mating the following year likely migrate to maximize their chances of survival. Forests at the base of the mountains produce less food for manakins during the non-breeding season [1] but receive half the rainfall. Manakins respond dramatically to heavy rains, both behaviourally and physiologically, with residents more adversely affected than migrants [4]. However, if they survive, residents are more likely to maintain a previously-high status, or increase in status than migrants (figure 2). Furthermore, among alpha males, resident individuals are more attentive (figure 1b) and attract more females to their lek (figure 1a, ESM figure 4), thereby increasing their chances for reproduction.

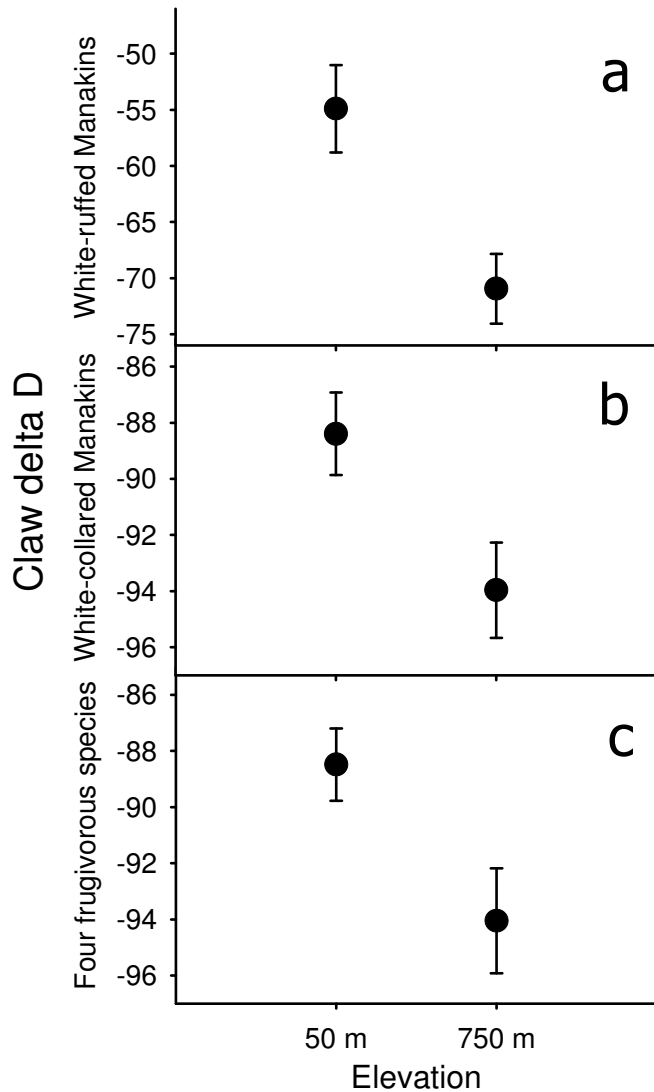


**Figure 2**



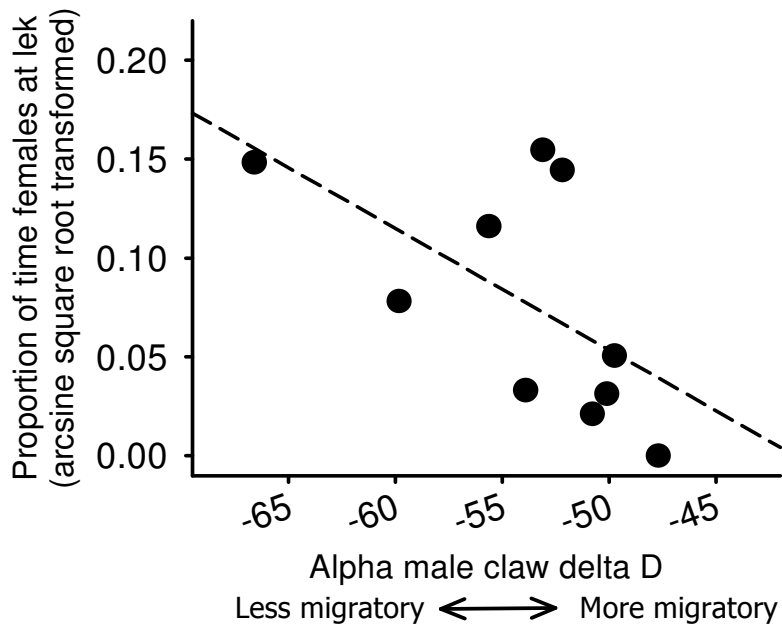
Relationship between elevation and mean monthly  $\delta D$  in precipitation from the four Costa Rican Caribbean slope meteorological stations that contributed data to the Global Network of Isotopes in Precipitation (GNIP) program [12]. Points represent mean  $\delta D$  of all monthly precipitation data available for each station. We found no difference in relationships between the precipitation  $\delta D$  and elevation after using a precipitation amount-weighted average for data available at three of the four meteorological stations. Based on this relationship, the predicted difference in  $\delta D$  between the ~750 m breeding elevation of White-ruffed Manakins and the base of the mountains is 11.7‰, a value intermediate between values calculated based on resident and migrant White-ruffed Manakins (ESM figure 3a) and resident species captured at both high- and low-elevation study sites (ESM figure 3b and 3c). The relationship between elevation and isotopic enrichment in these data closely matches the relationships between elevation and isotopic enrichment in groundwater and precipitation data from nearby countries [18, 19] as well as in data from mountains around the world [20]. These data provide one of several pieces of evidence supporting the interpretation of stable isotope variation in claw tissue as reflecting differences in location of White-ruffed Manakins during the non-breeding season.

**Figure 3**



Mean differences in  $\delta D$  in claws of birds captured during Nov–Dec 2008 at low elevations (50 m) at the base of the mountains where many altitudinal migrants spend the non-breeding season and breeding elevations of White-ruffed Manakins (750 m). Panels depict means ( $\pm$  s.e.m.) of (a) migrant and resident White-ruffed Manakins, (b) resident White-collared Manakins living at both elevations, and (c) least square means (accounting for species-level differences) in four species of resident frugivorous species living at both elevations. Mean differences range from 16.0‰ (White-ruffed Manakins) to 5.6‰ (White-collared Manakins). Differences in absolute values of  $\delta D$  in claws of different species likely represents differences in body size, diet, and/or metabolic processes among species (see ESM Materials and Methods).

**Figure 4**



The relationship between the proportion of time during video recordings that females spent in close proximity to each of the ten focal leks and the  $\delta D$  of the claws of the alpha male at those leks ( $F_{1,8} = 4.2$ ,  $P = 0.074$ ,  $R^2 = 0.35$ ). The slope and intercept of this relationship closely matches that of the same relationship derived from observation data (figure 1a):  $\text{slope}_{\text{obs}} = -0.258 \pm 0.131$ ,  $\text{slope}_{\text{video}} = -0.254 \pm 0.162$ ,  $\text{intercept}_{\text{obs}} = -0.006 \pm 0.002$ ,  $\text{intercept}_{\text{video}} = -0.006 \pm 0.003$ .