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Testosterone, Migration Distance, and Migratory Timing in Song Sparrows *Melospiza melodia*

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3 Testosterone, migration distance, and migratory timing in song sparrows *Melospiza melodia*

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16 Abstract

17 In seasonally migratory animals, migration distance often varies substantially within populations
18 such that individuals breeding at the same site may overwinter different distances from the
19 breeding grounds. Shorter migration may allow earlier return to the breeding grounds, which
20 may be particularly advantageous to males competing to acquire a breeding territory. However,
21 little is known about potential mechanisms that may mediate migration distance. We investigated
22 naturally-occurring variation in androgen levels at the time of arrival to the breeding site and its
23 relationship to overwintering latitude in male and female song sparrows (*Melospiza melodia*).
24 We used stable isotope analysis of hydrogen ($\delta^2\text{H}$) in winter-grown claw tissue to infer relative
25 overwintering latitude (migration distance), combined with 14 years of capture records from a
26 long-term study population to infer the arrival timing of males versus females. Relative to
27 females, males had higher circulating androgen levels, migrated shorter distances, and were more
28 likely to be caught early in the breeding season. Males that migrate short distances may benefit
29 from early arrival at the breeding grounds, allowing them to establish a breeding territory. Even
30 after controlling for sex and date, androgen levels were highest in individuals that migrated
31 shorter distances. Our findings indicate that androgens and migration distance are correlated
32 traits within and between sexes that may reflect individual variation within an integrated
33 phenotype in which testosterone has correlated effects on behavioral traits such as migration.

34 Key words

35 Androgens, deuterium, differential migration, isotope ecology, migration, protandry, testosterone

36

37 **Introduction**

38 The arrival of males to the breeding grounds, or emergence from hibernacula, before
39 females is known as protandry (Morbey and Ydenberg, 2001). This pattern is documented in a
40 diverse array of taxa including mammals (*Urocyon richardsonii*: Michener, 1983), fish
41 (*Oncorhynchus* spp.: Morbey, 2000), amphibians (*Notophthalmus viridescens*: Hurlbert, 1969),
42 reptiles (*Thamnophis sirtalis parietalis*: Gregory, 1974), and birds (*Phylloscopus sibilatrix*:
43 Francis and Cooke, 1986). Several, not mutually exclusive, hypotheses have been posited to
44 explain protandry. Because obtaining a breeding territory is essential for reproduction in many
45 species (Cristol et al., 1999), males that arrive earliest may gain the best territories (the *rank*
46 *advantage hypothesis*) and/or gain greater opportunities to mate with females (*mating*
47 *opportunity hypothesis*) resulting in the evolution of migratory protandry as a reproductive
48 strategy (Morbey and Ydenberg, 2001). For example, male wood warblers (*Phylloscopus*
49 *sibilatrix*) arrive to the breeding grounds before females and establish breeding territories
50 (Francis and Cooke, 1986). Similarly, male red-winged blackbirds (*Agelaius phoeniceus*) arrive
51 at and begin competing for breeding territories almost a month before females arrive (Cristol et
52 al., 1999). Conversely, in species of Alaskan shorebirds in which neither sex is territorial,
53 protandry is not observed: males and females arrive simultaneously to the breeding grounds
54 (Connors et al., 1978). In sex-role-reversed spotted sandpipers (*Actitis macularia*), females
55 compete for territories, make shorter migrations, and arrive earlier to the breeding grounds than
56 males (Oring and Lank, 1982). Thus, in species in which one sex (typically males) is territorial,
57 arriving early to the breeding grounds may be advantageous because it provides an advantage in
58 competing for territories and/or mates. For the sex that engages in territory defense, the costs

59 associated with later arrival to the breeding grounds (failure to breed) are presumably greater
60 than they are for the other sex (delay in breeding; Cristol, 1995; Cristol et al., 1999).

61 Migration distances from the breeding grounds may also vary among age- and sex-classes
62 within populations, a phenomenon termed differential migration. The *arrival time hypothesis*
63 (Fudickar et al., 2013) posits that whichever population class establishes and defends breeding
64 territories benefits most from shorter migration distances through increased territory quality and
65 increased breeding opportunities (Ketterson and Nolan, 1985). Although sex differences in avian
66 migration appear to be widespread (Cristol et al., 1999; Fudickar et al., 2013; Ketterson and
67 Nolan, 1976; Muller et al., 2014; Oring and Lank, 1982), candidate mechanisms that may
68 mediate these differences remain poorly understood.

69 Hormones are often thought to act as mediators of trade-offs between life-history traits
70 such as the allocation of energetic resources to growth, fat storage, and reproduction (Cerasale et
71 al., 2011; Love et al., 2014; Love et al., 2004; Wingfield, 1984). Gonadal sex steroid hormones
72 are particularly important for the transition from non-breeding to a breeding state (Ramenofsky
73 and Wingfield, 2007), a metabolically and physically demanding process. Migratory individuals
74 must allocate resources for fat deposition and muscle development during migration but soon
75 afterwards, must invest in gonadal growth and production of gametes for breeding (Tonra et al.,
76 2011a; Wingfield, 1984). Increases in daylength (photoperiod) regulate androgen levels in the
77 spring and allows for the stimulation of sperm production and enlargement of the cloacal
78 protuberance that is needed for reproduction (Wingfield, 1984). Furthermore, elevation of
79 testosterone well above the levels induced by the photoperiod is experienced during male-to-
80 male competition, such as for territory establishment and subsequent boundary advertisement at
81 the breeding grounds (Hunt et al., 1997; Wingfield and Hahn, 1994). Thus, gonadal androgens

82 may play a role in regulating both the timing of arrival to the breeding grounds and preparing for
83 reproduction and territorial interactions.

84 Experimental evidence supports a link between androgens and spring migration in
85 songbirds. Male dark-eyed juncos (*Junco hyemalis*) with experimentally elevated testosterone
86 prior to migration advanced migratory preparation and reached peak migratory condition more
87 rapidly in comparison to controls without elevated testosterone (Tonra et al., 2013). Androgen
88 levels increased in several species of Nearctic-Neotropical migratory songbirds during spring
89 migration (Covino et al., 2015). Thus although the correlations and functional relationships
90 between androgens and spring migration are not clear, further research is warranted.

91 In the current study we investigate sex differences in spring arrival at the breeding
92 grounds, within-species variation in androgen levels, and variation in migration distance
93 (overwintering latitude). Our objectives were to 1) evaluate sex differences (protandry) in spring
94 arrival to the breeding grounds, 2) assess sex differences in migration distance, and 3) investigate
95 whether androgens are related to within-species variation in migration. We used a long-term
96 study population of differentially-migrant eastern song sparrows (*Melospiza melodia melodia*).
97 Prior band-recapture data indicate that this subspecies has a wide geographic wintering range
98 (Davis and Arcese, 1999, S.A. MacDougall-Shackleton unpublished data). In spring, males
99 compete intensely for breeding territories and are highly philopatric between breeding seasons
100 (Potvin et al., 2015).

101 We hypothesized that regulation of androgens may be functionally related to migration
102 and result in i) sex differences in arrival at the breeding ground (protandry), ii) males wintering
103 closer to the breeding ground than females, and iii) a correlation between pre-breeding levels of
104 androgens and latitude of the wintering grounds. To test these predictions, we measured

105 androgen levels shortly after birds arrived at the breeding site, and measured stable hydrogen
106 isotope ratios in claw tissue grown on wintering grounds to infer wintering latitude (Hobson and
107 Wassenaar, 2008).

108

109 **Materials and Methods**

110 *Study site and sample collection*

111 We conducted our research on land owned by Queen's University Biological Station near
112 Newboro, Ontario (44.66 °N/76.22 °W) on a population of song sparrows (*Melospiza melodia*
113 *melodia*) that has been studied each breeding season since 2002. During the spring field seasons
114 of 2014 and 2015, each morning (0630 –1030 EST) we set seed-baited Potter traps, arranged
115 along a trap-line, and checked them at least every 60 minutes for the presence of song sparrows.
116 For this study, we had 288 captures of 214 unique individuals (2012: 47, 2013: 56, 2014: 69,
117 2015: 116; numbers include 42 song sparrows caught in multiple years) trapped shortly after the
118 arrival to the breeding site (capture dates 12 April through 9 May each year) and from which we
119 collected blood and claw samples. We determined the sex of each bird (2014: n = 27 females, n
120 = 42 males; 2015: n = 41 females, n = 75 males) based on the presence (male) or absence
121 (female) of a cloacal protuberance, observed singing behavior (males), as well as presence of a
122 brood patch (females).

123 To measure androgen levels we collected a blood sample (approx. 200 µL) within two to
124 four minutes of approaching a trap via brachial venipuncture. Blood was kept on ice for no more
125 than three to four hours before being centrifuged for ten minutes in a micro-hematocrit centrifuge
126 at 13,000 g. We collected plasma using a Hamilton syringe and stored this plasma at -20 °C, in

127 labeled microcentrifuge tubes containing O-rings to prevent evaporation until we conducted
128 androgen assays (see below).

129 To quantify stable isotope composition of tissues grown on the wintering grounds, we
130 clipped approximately 2.5 mm of claw from both hallux toes of each bird. We stored claw
131 samples in 0.6 mL microcentrifuge tubes at room temperature until stable isotope analysis was
132 performed. All procedures were approved by the Animal Use Subcommittee at the University of
133 Western Ontario and were carried out under permission of Environment Canada.

134 *Sex differences in arrival time*

135 We gathered capture record data from field notebooks and banding records from 2002–
136 2015 to determine if males in this population of song sparrows arrive to the breeding grounds
137 before females (protandry). We only used birds caught in seed-baited Potter traps for further
138 analysis. Only the first capture date of the year was used for each individual. We also excluded
139 captures after 5 May, as females in this population begin incubation around this time (personal
140 observation, E. A. MacDougall-Shackleton and S. A. MacDougall-Shackleton) reducing the
141 likelihood of them entering traps. Birds caught at this study site are assumed to be local breeding
142 individuals based on repeat observations using colored leg bands for identification (personal
143 observation, E. A. MacDougall-Shackleton and S. A. MacDougall-Shackleton) and recaptures
144 throughout the season and in following years.

145 *Stable hydrogen isotope analysis*

146 We used stable hydrogen isotope (deuterium, $\delta^2\text{H}$) measurements of claw tissue to
147 estimate the wintering latitude of song sparrows caught on the breeding grounds. Isotope data
148 reported here were also used in a parallel study (Kelly et al., in revision). Pilot studies indicated
149 that the distal 2.5 mm of claw tissue in song sparrows is produced about 3 months earlier (Kelly

150 et al., in revision), thus this tissue would contain the isotopic values indicative of food consumed
151 on the wintering grounds prior to our collection of tissue in April and May. We took advantage
152 of the latitudinal gradient in $\delta^2\text{H}$ in growing-season precipitation to estimate the latitude at which
153 metabolically inert tissues were grown and thus migration distance can be inferred (Hobson et
154 al., 2014; Woodworth et al., 2016).

155 We cleaned claw samples from each song sparrow in 1.6 mL of 2:1 chloroform-methanol
156 solution to remove any dirt and/or oil and removed the remaining solution via pipette. The
157 samples were left overnight in a fume hood to dry. We weighed nail samples to $350 \mu\text{g} \pm 10 \mu\text{g}$;
158 larger samples were shaved from the proximal end using an X-acto knife blade and did not
159 include samples weighing less than $350 \mu\text{g}$ (2012: n=8; 2013: n=1; 2014: n=9; 2015: n=4). We
160 crushed the samples (2012: n = 39; 2013: n = 55; 2014: n = 60; 2015: n = 112) in silver capsules
161 and stored them in a sterile 96-well microplate at room temperature.

162 Samples were analyzed at the Stable Isotope Laboratory of Environment Canada, in
163 Saskatoon, Canada, by one of the authors (KH). Online continuous-flow isotope mass
164 spectrometry (CF-IRMS) was performed on a Micromass Isoprime mass spectrometer (Micro-
165 mass UK, Manchester, UK) interfaced with a Eurovector (Milan, Italy) elemental analyzer to
166 obtain the amount of nonexchangeable hydrogen in each sample. Using the comparative
167 equilibrium method, three keratin standards (Caribou Hoof Standard (CBS): -197‰, Commercial
168 Keratin (SPK): -121.6 ‰, and Kudu Horn Standard (KHS): -54.1 ‰) were used to correct for the
169 effects of hydrogen exchange with ambient laboratory water vapor (Wassenaar and Hobson,
170 2003) and to provide estimates of the $\delta^2\text{H}$ value of the non-exchangeable claw H. Hydrogen
171 isotopic measurements were performed on H_2 gas derived from high-temperature (1350 °C) flash
172 pyrolysis of claw samples and keratin standards. All keratin $\delta^2\text{H}$ results are reported in units of

173 per-mille (‰) and normalized on the Vienna Standard Mean Ocean Water–Standard Light
174 Antarctic Precipitation (VSMOW-SLAP) standard scale. Based on within-run replicate (n = 5)
175 analyses of five of each keratin standard, the analytical precision was estimated to be ± 2 ‰.
176 Three samples in 2015 were lost during analysis.

177 *Androgen assay*

178 We quantified plasma androgen levels for both male (2014: n = 42; 2015: n = 74) and
179 female (2014: n = 25; 2015: n = 41) song sparrows using a commercially available enzyme
180 immunoassay kit (1-2403, Salimetrics, State College, PA, U.S.A.). This kit was previously used
181 to measure plasma testosterone levels in a variety of birds (Washburn et al., 2007) and has been
182 validated for use in song sparrows (Schmidt et al., 2014). Since the kit cross-reacts with
183 dihydrotestosterone, hormone levels are referred to as androgen rather than testosterone levels.
184 We conducted this assay according to the manufacturer’s instructions except that we first diluted
185 plasma samples 1:5 with assay buffer and analyzed the sample in duplicate by adding 25 μ L of
186 diluted plasma to each well. Mean sensitivity for all plates (both 2014 and 2015) was 3.10
187 pg/mL, determined as two standard deviations above values of the zero wells. No sample values
188 were below this detectability level. If samples yielded concentrations over 600 pg/mL (the
189 highest concentration on the standard curve) we re-ran the sample diluted 1:20 (2014: n = 8;
190 2015: n = 23). The inter-assay coefficient of variation was 9.6% for 4 plates processed in 2014
191 and 13.2% for 3 plates processed in 2015. The intra-assay coefficient of variation was 3.08%.
192 Androgen concentrations for each sample were adjusted according to the appropriate dilution
193 factor (5 or 20).

194 *Statistical analyses*

195 *Protandry.* To assess sex differences in arrival time we could not directly quantify arrival
196 day because birds may have been present at the field site for an unknown number of days prior to
197 capture. Thus, to assess protandry we calculated the proportion of birds captured that were male
198 on each day of the year prior to 5 May (n = 412 males, n = 266 females) combining data from
199 fourteen consecutive field seasons (2002–2015). We then conducted a linear regression between
200 date of capture and the proportion of males caught for each date.

201 *Sex differences in migration distance.* To assess sex differences in migration distance we
202 compared male and female $\delta^2\text{H}$ values using an independent samples t-test, assuming equal
203 variances (Levene's test for equality of variances: $F = 2.029$, $p = 0.156$). For birds caught in
204 multiple years (2013 n = 11 [6 males and 5 females], 2014 n = 13 [11 males and 2 females],
205 2015 n = 31 [26 males and 5 females]), were included only the $\delta^2\text{H}$ value for the first year of
206 capture. We think this is unlikely to bias the results as previous findings from this study
207 population indicate that individuals are consistent in $\delta^2\text{H}$ over multiple years (Kelly et al., in
208 revision).

209 *Androgens and migration distance.* To test whether prebreeding androgen levels were
210 related to migration distance we used a linear mixed models with androgen level as the
211 dependent variable. Predictor variables in the initial model included main effects of sex, age,
212 date and $\delta^2\text{H}$, and interactive effects of $\delta^2\text{H} \times \text{age}$, $\delta^2\text{H} \times \text{sex}$, and $\text{sex} \times \text{date}$, plus random
213 effects of bird ID and of year. Following West et al. (2015), we used likelihood ratio tests to
214 exclude uninformative predictors and create the most parsimonious final model. Significance of
215 factors in the final model was tested with Type III tests of fixed effects, and effect sizes were

216 estimated by using Pearson's correlations to generate Fisher's Z. All analyses were run with
217 SPSS v. 23.

218 Most birds caught at this study site are confirmed to be breeding in the local area, based
219 on repeated captures throughout the season and in following years, together with behavioral
220 observations on color-banded individuals. However, of the 167 birds used in the current study,
221 52 were not recaptured or resighted following the initial capture, raising the possibility that these
222 individuals might have been captured in the process of migrating farther north. Accordingly, as a
223 complementary analysis we repeated the above models including only the subset of individuals
224 that were positively identified as local breeders (territorial males, $n = 88$ and nesting females, $n =$
225 27).

226

227 **Results**

228 *Sex differences in arrival time*

229 The proportion of captured birds that were male decreased significantly throughout the
230 season ($r^2 = 0.491$, $F_{1,24} = 23.12$, $p < 0.001$, Fisher's $Z_r = 0.867$). Males were more likely to be
231 caught than females early in the breeding season, and the proportion of males captured decreased
232 to approximately 0.5 by the onset of incubation (Fig. 1).

233 *Sex differences in migration distance*

234 Female song sparrows had significantly more positive values of claw $\delta^2\text{H}$ than did males
235 ($t = -2.30$, $n = 208$, $p = 0.022$, Cohen's $d = -0.322$; female = -63.51 ± 1.73 [mean \pm SEM], $n =$
236 88; male = -68.29 ± 1.24 , $n = 122$), interpreted as females having overwintered farther south and
237 migrated longer distances (Fig. 2).

238 *Testosterone and migration distance*

239 When all individuals were included in the model, the final minimally parsimonious linear
240 mixed model included main effects of sex, date, migration distance, and a random effect of Bird
241 ID as predictors of plasma androgen levels. As expected, androgen levels were higher in males
242 than in females ($F_{1,134.9} = 14.77$, $p < 0.001$, Cohen's $d = 0.622$; male = 1032.67 ± 144.89 pg/mL,
243 $n = 108$; female = 472.99 ± 71.77 pg/mL, $n = 59$). Androgen levels also increased with capture
244 date ($F_{1,123.5} = 9.83$, $p = 0.002$, Fisher's $Z_r = 0.174$), and decreased with migration distance
245 ($F_{1,143.3} = 11.79$, $p = 0.001$, Fisher's $Z_r = -0.277$). Figure 3a illustrates the relationship between
246 androgen levels and migration distance (δ^2H) for males and females.

247 When only those individuals that were confirmed as local breeders were included in the
248 model, the final minimally parsimonious linear mixed model included only the main effect of
249 migration distance and a random effect of bird ID as predictors of plasma androgen levels.
250 Androgen levels decreased with increasing migration distance (Fig. 3b; $F_{1,93.6} = 22.85$, $p < 0.001$,
251 Fisher's $Z_r = -0.414$).

252 **Discussion**

253 Our study provides further evidence for protandry in a songbird species in which males
254 defend breeding territories. Further, our results indicate a sex difference in overwintering
255 latitude, and a relationship between migration distance and pre-breeding levels of androgens.
256 Using the compiled data from 2002-2015 we found the proportion of birds caught that were
257 males was greatest at the beginning of the breeding season. As the season progressed, this
258 proportion decreased to about 0.5 by the onset of breeding and incubation (Fig. 1). This supports
259 our prediction that males arrive before females in this population of song sparrows, i.e., that
260 protandry occurs. Although we observed substantial variation among individuals in migration

261 distance as inferred from $\delta^2\text{H}$ of winter-grown claw tissue, on average males migrated shorter
262 distances than did females (Fig. 2). Males also had higher concentrations of circulating
263 androgens than did females (Fig. 3a, Fig. 3b). In both sexes, however, androgen concentrations
264 decreased with increasing migration distance (Fig. 3a, Fig. 3b), supporting our hypothesis that
265 androgens such as testosterone may be related to migration distance in this species.

266 We assumed in our study that both males and females are equally likely to venture into
267 the traps and thus that date of first capture is reliably associated with arrival date. Because males
268 and females were equally likely to enter traps by the onset of breeding and incubation (Fig. 1) it
269 seems reasonable to assume that the male bias in trapping earlier in the season reflects sex
270 differences in the number of birds present at the breeding site, rather than sex differences in
271 trapping success. Prior research evaluating protandry have used similar trap records of various
272 species of birds (Saino et al., 2010).

273 Several ultimate causes of protandry have been considered (Morbey and Ydenberg,
274 2001), however they need not be mutually exclusive. Recently, the *mate opportunity hypothesis*
275 which proposes that early-arriving males maximize mating opportunities has gathered much
276 support (Kokko et al., 2006; Schmaljohann et al., 2015). However, song sparrows are socially
277 monogamous and have low rates of extra-pair paternity (Arcese, 1989; Major and Barber, 2004),
278 including in our study population (E. MacDougall-Shackleton, unpublished data). Thus, it is
279 unlikely that extra mating opportunities have driven the evolution of protandry in song sparrows.
280 The *rank advantage hypothesis* (Morbey and Ydenberg, 2001) posits that one sex may arrive
281 earlier from migration in order to acquire a breeding territory (Oring and Lank, 1982; Francis
282 and Cooke, 1986). In song sparrows males sing and aggressively defend territories. In our study
283 population males with larger song repertoires tend to have nests earlier in the breeding season,

284 higher reproductive success, and are more likely to breed at the same territory across years
285 (Potvin et al., 2015). Thus, competition for breeding territories between males may drive the
286 evolution of protandry in this species.

287 Not only did male song sparrows arrive before females, but we also found that males
288 completed shorter migrations on average than females (Fig. 2). Our study further demonstrates
289 that $\delta^2\text{H}$ measurements in metabolically inactive tissue such as feathers and claws provides a
290 valuable tool for assessing overwintering latitude (see also Woodworth et al., 2016). Because
291 song sparrows do not normally molt any feathers during winter we used the distal portion of claw
292 tissue to infer the $\delta^2\text{H}$ value of tissue deposited several months earlier. As predicted we found
293 sex differences in $\delta^2\text{H}$. Sex differences in migration distance have been documented in other bird
294 species (*Plectrophenax nivalis*: Banks et al., 2009; *Agelaius phoeniceus*: Cristol et al., 1999;
295 *Falco sparverius*: Heath et al., 2012; *Passerculus sandwichensis*: Woodworth et al., 2016) and
296 this has been suggested as a proximate mechanism for protandry, allowing males to return before
297 females without necessarily increasing migration speed (Schmaljohann et al., 2015). Wintering at
298 higher latitudes (short migration distance) may allow males to better time their arrival to the
299 breeding grounds in order to be able to establish a territory. Differences between sexes or age
300 classes in migration distance may reflect differences in social dominance or optimal arrival date
301 (Ketterson and Nolan, 1987). Harsher environments (cold, snow) are present at higher latitudes
302 and may limit access to resources, mainly food. Subordinate birds may thus be outcompeted by
303 dominant individuals and forced to winter further south. For male song sparrows, the benefit of
304 earlier arrival than females may outweigh the costs associated with overwintering in a harsher
305 environment.

306 We also present the first evidence that regardless of sex, individuals exhibiting higher
307 levels of androgens at the breeding grounds also tend to have relatively short migration distances
308 (Fig. 3). This relationship could arise in several ways. First, higher androgen levels on the
309 breeding grounds may result from shorter migration distance. This could occur if less energy is
310 expended during migration, or the photoperiod experienced at a higher latitude could affect
311 migration (Coppack et al. 2008) as well as androgen levels. Second, if higher androgen levels
312 during the early breeding season reflect higher androgen levels year-round, then androgens may
313 directly mediate migration behavior and affect overwintering latitude. Finally, pre-breeding
314 androgens and migration distance may reflect two aspects of an integrated phenotype. In this
315 case birds that produce high levels of androgens may also possess traits that lead them to migrate
316 a shorter distance (boldness and aggression). Experimental manipulations would be required to
317 distinguish amongst these alternatives.

318 Some evidence is consistent with the hypothesis that androgens may be directly affecting
319 migration behavior. Manipulation of testosterone affects migratory restlessness in captive birds
320 (Tonra et al., 2011b; Ramenofsky and Németh, 2014). As well, androgens are linked to
321 courtship displays in the prebreeding season (Ball et al., 2002). Early arrival has been correlated
322 with both greater reproductive success (Savi's warbler *Locustella luscinioides*: Aebisher et al.,
323 1996; dark-eyed juncos *Junco hyemalis*: Tonra et al., 2011b) and heightened sexual displays
324 (willow warblers *Phylloscopus trochilus*, Arvidsson and Neergard, 1991; pied flycatchers
325 *Ficedula hypoleuca*, Slagsvold and Lifjeld, 1988). Our results are consistent with the hypothesis
326 that testosterone may be a mediator of differential migration. If testosterone does affect
327 migration distance, then selection acting on other benefits or costs of testosterone (e.g. sperm
328 competition, sexual selection; Garamszegi et al., 2005) may have a correlated effect on migration

329 distance. Future work manipulating testosterone levels prior to migration are required to test this
330 idea.

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336 Animal Care and approved by the University of Western Ontario Animal Care Committee.

337

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481 **Figure 1.** The proportion of song sparrows that were male captured at breeding grounds near
482 Newboro, ON from 2002–2015, as a function of day of year. The proportion of males captured
483 decreased with day of the year ($r^2 = 0.491$, $p < 0.001$). Line represents the best fit regression line.

484

485 **Figure 2.** Mean \pm SEM stable hydrogen isotope ratios ($\delta^2\text{H}$, migration distance) of male ($n =$
486 122) and female ($n = 88$) song sparrows in 2014 and 2015. Females made significantly longer
487 migrations than males ($t = -2.30$, $p = 0.22$).

488

489 **Figure 3a.** Androgen concentration (pg/ mL) as a function of migration distances (inferred from
490 $\delta^2\text{H}$) of male ($n = 108$) and female song sparrows ($n = 59$) captured near Newboro, ON. Males
491 had greater androgen concentrations than females ($F_{1,134.9} = 14.77$, $p < 0.001$, Cohen's $d = 0.622$)
492 and androgen concentrations decreased with migration distance for both sexes ($F_{1,143.3} = 11.79$, p
493 $= 0.001$, Fisher's $Z_r = -0.277$). Lines represent the best fit regression lines.

494

495 **Figure 3b.** Androgen concentration (pg/ mL) as a function of migration distances (inferred from
496 $\delta^2\text{H}$) of male ($n = 88$) and female song sparrows ($n = 27$) confirmed as local breeders captured
497 near Newboro, ON. Androgen concentrations decreased with migration distance for both sexes
498 ($F_{1,93.6} = 22.85$, $p < 0.001$, Fisher's $Z_r = -0.414$). Lines represent the best fit regression lines.

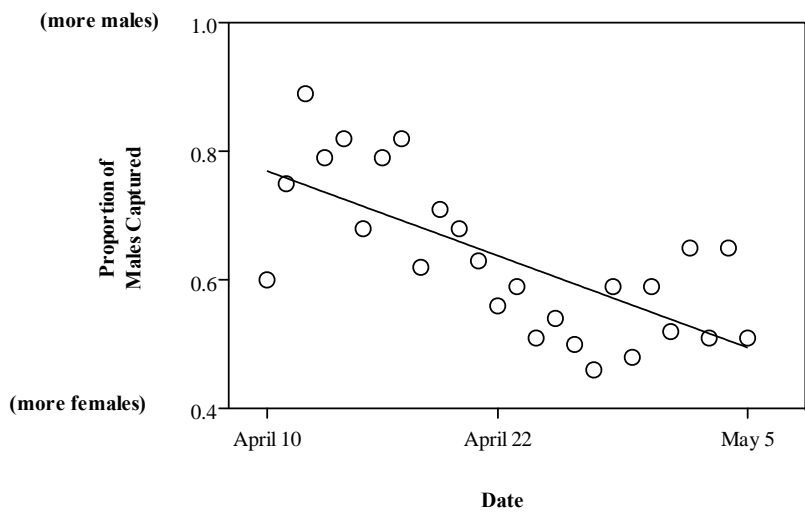


Figure 1

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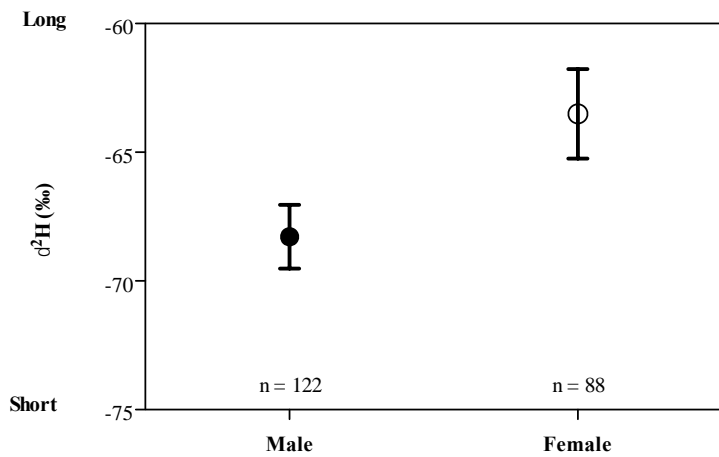


Figure 2

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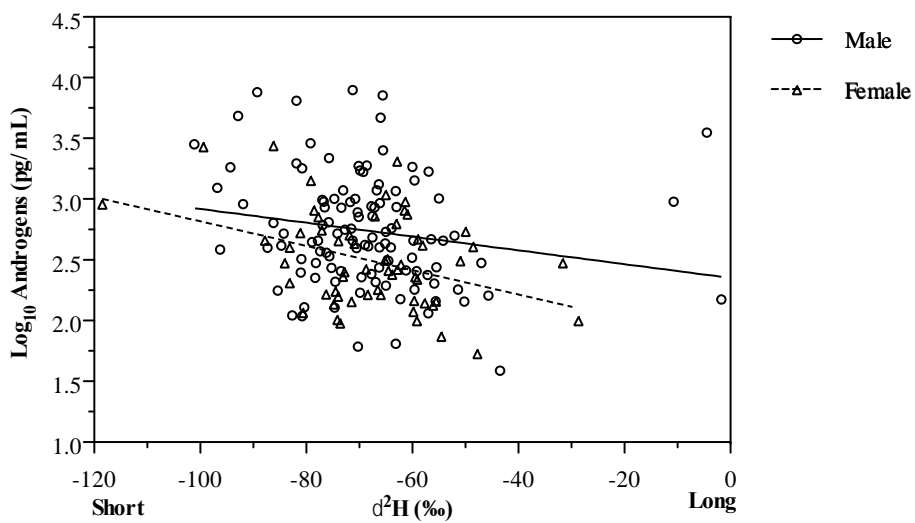


Figure 3a

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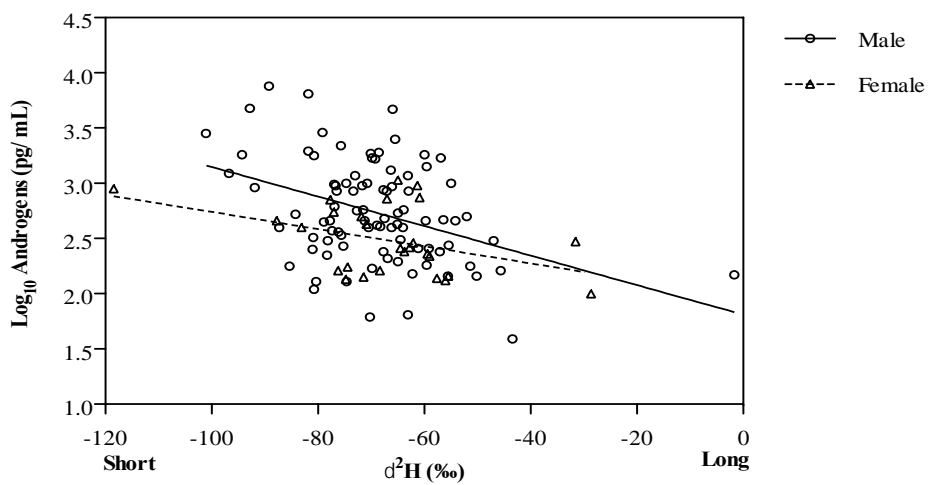


Figure 3b

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