

Migration phenology and winter habitat quality are related to circulating androgen in a long-distance migratory bird

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In migratory birds, the timing of departure from wintering grounds is often dependant on the quality of habitat on an individual's territory and may influence individual fitness, resulting in an interaction of life history stages across large geographical distances. American redstart *Setophaga ruticilla* males who overwinter in high quality habitats arrive early to breed and subsequently produce more offspring than late arrivers. Since many migratory species overlap vernal migration with the physiological transition to breeding, we examined if breeding preparation plays a role in this seasonal interaction. We tested the hypothesis that early arriving male redstarts from high quality winter habitats are in superior breeding condition by simultaneously measuring winter habitat quality (stable-carbon isotopes) and breeding preparation (circulating androgen, cloacal protuberance (CP) diameter) upon arrival at breeding grounds. Compared with late arrivers, early arriving males were from higher quality winter habitats and had higher androgen, but smaller CPs. Males arriving with higher androgen were in more advanced physiological migratory condition, as measured by haematocrit. Early arrivers were more likely to successfully breed, but there was no significant relationship between androgen upon arrival and breeding success. One possible explanation for these relationships is that androgen measured during arrival is most relevant in a migratory context, such that birds with high androgen may benefit from effects on migratory condition, positively influencing fitness through earlier arrival.

Migratory animals complete different life history stages in geographically disparate areas. Events that occur in one location can affect events in another location during a subsequent stage. Although there is evidence for the role of habitat quality in these 'seasonal interactions' (Marra et al. 1998, Gunnarsson et al. 2006, Studds et al. 2008, Reudink et al. 2009a), few studies have explored the underlying physiological processes of these patterns (Marra and Holberton 1998). Conditions during winter can determine the timing of migration events, such as departure and arrival dates (Marra et al. 1998, Studds and Marra 2005), and variation in migration phenology has been shown to limit reproductive success (Aebischer et al. 1996, Reudink et al. 2009a). Many birds overlap migration with the physiological transition to breeding (Holberton and Dufty 2005, Ramenofsky and Wingfield 2006). If winter habitat quality also impacts the degree of physiological breeding readiness upon arrival, males from high quality winter habitats could have a reproductive advantage that arises from greater preparation for breeding, as well as that conferred by early arrival time.

The first days after arrival at the breeding grounds are critically important to reproductive success in many migratory animals. Males, in particular, can gain a

reproductive advantage through early arrival (Morbey and Ydenberg 2001). In passerines, studies examining condition during the arrival period have found that early arriving males are generally in superior condition compared with late arrivers (Møller 1994, Marra et al. 1998, Ninni et al. 2004). However, while several studies have compared variation in breeding development during the arrival period between different sub-populations of a species (Raess and Gwinner 2005, Rolshausen et al. 2010), or examined within-population transitions to subsequent breeding stages (Morton 2002, Schwabl et al. 2005), few, if any, have documented individual variation in measures of physiological breeding preparation during the arrival stage.

For most birds, the physiological events that lead to reproduction begin when changes in photoperiod activate the hypothalamic-pituitary-gonadal (HPG) axis, resulting in gonadal steroid and gamete production (Norris 2007). While changes in photoperiod can initially trigger this cascade, non-photic cues, such as food availability, can influence the timing of breeding hormone production and the resulting expression of secondary sex characteristics during the periods leading up to breeding (Deviche and Small 2001, Bauchinger et al. 2009). If variation in the onset and rate of HPG axis development can be influenced

by non-breeding conditions, such as winter habitat quality, males within a breeding population may arrive at the breeding grounds with varying degrees of HPG activity, such as plasma androgen concentration and subsequent androgen-dependent secondary sex characters.

In most songbirds, androgens, such as testosterone, play an important role in the acquisition of high quality breeding territories through regulation of male-male aggression (Moore 1984, Wingfield 1984, Beletsky et al. 1990) and territory boundary advertisement (i.e. singing behaviour; Ketterson et al. 1992, De Riddler et al. 2000; but see Moore et al. 2004a, b, Lynn 2008 for examples of behavioural insensitivity to testosterone in some species). Androgens also play a role in the expression of secondary sex traits and courtship behaviours that influence breeding success (Hegner and Wingfield 1987, Saino and Møller 1995, Blas et al. 2007).

Androgens can also interact with other hormones to influence pre-migratory hyperphagia and fat deposition (Deviche 1995) as well as physiological aspects of condition. Testosterone, for instance, is erythropoietic, stimulating red blood cell synthesis (Domm and Taber 1946, Kern et al. 1972, Nirmalan and Robinson 1972). Although some studies have found that haematocrit, the ratio of packed red blood cells to total blood volume, is positively associated with measures of energetic condition (Brown 1996, Potti et al. 1999), others have raised concerns about this use of haematocrit, which can be influenced by multiple factors (Fair et al. 2007). However, researchers have repeatedly found that changes in haematocrit are a physiological component of the migratory state, often independent of energetic condition. Haematocrit increases in migratory birds around the time of migration (Wingfield and Farner 1980, Bairlein and Totzke 1992, Morton 1994, Piersma et al. 1996), decreases following arrival at breeding areas in males (Saino et al. 1997), and has been positively associated with early arrival (Ninni et al. 2004, Hatch et al. 2010). Androgen-induced erythrocyte synthesis could result in greater oxygen carrying capacity during migration in birds, increasing flight performance. Thus, elevating androgen in preparation for migration could enhance an individual's ability to not only arrive early at the breeding grounds (arrival time), but also to meet the demands of breeding (arrival condition), collectively maximizing breeding success.

American redstarts *Setophaga ruticilla* overwinter throughout the Caribbean, eastern Central, and northern South America. Both sexes defend territories throughout the winter period (Sherry and Holmes 1997), and birds that over-winter in wet evergreen habitats are able to maintain superior physical condition (Marra and Holberton 1998, Studds and Marra 2007), have higher annual survival (Johnson et al. 2006, Angelier et al. 2009), and depart earlier for spring migration (Marra et al. 1998, Studds and Marra 2005). Male redstarts from high quality winter habitats arrive earlier at the breeding grounds and ultimately sire more offspring than those from low quality habitats (Marra et al. 1998, Reudink et al. 2009a). The redstart study system provides an ideal opportunity to

examine the physiological underpinnings of carry-over effects in seasonal interactions.

We tested the hypothesis that circulating androgen mediates the carry-over effects between winter habitat quality and breeding success in male redstarts through its effects on migratory condition and breeding preparation. We first tested two predictions to confirm that previously observed relationships between winter habitat, arrival date, and breeding success (Marra et al. 1998, Reudink et al. 2009a) occurred in our population. We predicted that early arriving individuals: 1) would be from higher quality winter habitats, as indexed by stable-carbon isotope ratios in claws ($\delta^{13}\text{C}$; Marra et al. 1998, Bearhop et al. 2003) and 2) would have higher reproductive success. We then made five novel predictions: 1) early males would arrive in more advanced breeding condition, as measured by circulating androgen and cloacal protuberance diameter (CP; an additional measure of androgen activity; Wingfield 1984), 2) males in more advanced breeding condition would be from higher quality winter habitats, 3) males with higher androgen would be in superior migratory condition, as measured by size-corrected mass and haematocrit, 4) males with high androgen upon arrival would be more likely to successfully breed, and 5) as a mechanism for the effects of androgen upon arrival on breeding success, males arriving with higher androgen would be more aggressive, as measured by latency of response to a simulated territorial intrusion (STI).

Material and methods

Field data collection

We conducted this study during the 2008 and 2009 breeding seasons on two plots in the Hubbard Brook Experimental Forest, North Woodstock, NH, USA (43°56'N, 71°45'W). The vegetation of this forest has been described by Holmes and Sherry (2001).

To determine arrival dates for male redstarts in both years, we systematically surveyed all plots, both by ear and visually, for males every 1-2 d beginning on 3 May. We standardized arrival dates for the two years by designating the first day on which we detected a redstart as day 0 and then numbered days sequentially for the remainder of the arrival period. First and median arrival dates varied by one day between the two years. We used conspecific playback and stuffed redstart decoys to lure birds into mist nests within two days of arrival. To control for any possible effects of playback duration on androgen levels (Goymann 2009), only birds that were captured within 3 min of playback were used in this study. In order to control for any effects of time of day we only captured birds between 10:00 and 14:00 h. To measure aggression, we recorded the latency of response to territorial intrusion as the amount of time between the start of playback and when the bird was first seen moving towards the speaker (2009 only).

We weighed each individual to the nearest 0.1 g, collected morphological data (wing, tail, and tarsus length), and measured the diameter of the CP to the nearest 0.01 mm with digital calipers. Male redstarts were aged as second

year (SY) or after second year (ASY) using plumage characteristics (Pyle 1997). Since most SY males do not acquire mates or maintain season-long territories (Tonra unpubl.) we only included ASY males in our sample. We banded each redstart with a unique combination of an aluminum United States Geological Survey (USGS) band and two plastic color bands. To create a size-corrected index of condition, we performed a principle component analysis on wing, tail, and tarsus lengths. We then regressed the first principle component, which accounted for 52% of the variation in body size, with mass and hereafter refer to the residuals of the linear relationship observed as size-corrected mass (Albrecht et al. 2005). To determine territory boundaries, we visited all territories every 1-2 d and mapped singing locations for each male using a hand-held global positioning system (GPS) unit. We measured breeding success for each male on a territory basis, considering a male to be successful if his territory fledged at least one offspring and unsuccessful if all attempts on his territory failed. Redstart pairs do not double brood in a breeding season (Sherry and Holmes 1997). When possible, we determined clutch completion date of the first clutch and fledge date for each territorial male. We used food delivery by parents to determine nest fate for nests placed over 15 m high.

Determining winter habitat quality

Due to variable water-use efficiency of plants in wet vs dry tropical habitats, $\delta^{13}\text{C}$ is negatively associated with habitat wetness, and this difference is reflected in the tissues of birds using such habitats (Marra et al. 1998, Reudink et al. 2009b). Bird claws are suited for measuring past habitat quality because of low turnover rates (several weeks) and minimal disturbance to the bird (Bearhop et al. 2003, Reudink et al. 2009a). To determine relative winter habitat quality (i.e. wetness) experienced by individuals arriving at the breeding grounds, we collected 2-3 mm of claw from the middle digit of each foot for $\delta^{13}\text{C}$ analysis. We conducted all isotope analyses at the Smithsonian Inst. Stable Isotope Mass Spectrometry Facility in Suitland, MD, USA.

Measuring haematocrit and circulating androgen

We collected 50-70 μl of blood from the brachial vein of each bird within 2 min of capture for hormone analysis. We maintained the sample on wet ice in a small cooler until it was centrifuged, within 4 h, to separate plasma, which was then stored at -20°C . Prior to separation, we measured haematocrit (the ratio of packed cells to total sample volume in the first capillary tube collected from each bird) with digital calipers to the nearest 0.01 mm. To determine plasma androgen concentration, we used a testosterone radioimmunoassay following Wingfield et al. (1992). The testosterone antibody (T antibody T3-125, Endocrine Sciences/Esoterix, Calabasas, CA) has high specificity for testosterone but also at least 44% cross reactivity with dihydrotestosterone (DHT), which parallels patterns of seasonal change in testosterone (Wingfield and Farner 1978). Therefore, we report our results as

'total androgen'. We ran separate assays for each year. The inter-assay percent coefficient of variation (CV), based on commercial standard, was 10%. For both assays, the within-assay percent CV, based on a commercial standard, was 1%, and the sensitivity of the standard curve was 1.7 pg ml^{-1} .

Statistical analysis

All statistical analyses were run using SPSS 17.0 (SPSS 2008). We ran multiple regressions for each prediction where we included plot and year as covariates. There was no significant effect of either variable on arrival date when included with androgen (year: $t_{37} = -1.05$, $p = 0.30$; plot: $t_{37} = 0.87$, $p = 0.39$), androgen and size-corrected mass (year: $t_{34} = 0.41$, $p = 0.68$; plot: $t_{34} = -1.32$, $p = 0.20$), or on haematocrit upon arrival when include with androgen (year: $t_{34} = 0.54$, $p = 0.59$; plot: $t_{34} = 0.38$, $p = 0.71$). Therefore, we combined all years and plots and used linear regression to examine these univariate relationships. We did however observe a significant year effect on $\delta^{13}\text{C}$ (2009 values were more depleted; $t_{33} = 6.75$, $p < 0.001$); therefore we used multiple regression to examine the relationships with $\delta^{13}\text{C}$, which included year and the year by $\delta^{13}\text{C}$ interaction as additional independent variables. If individuals were sampled in multiple years ($n = 4$), we randomly selected one of these sampling events to avoid lack of independence in the data. We also used linear regression to examine the relationship between latency of response to playback and arrival androgen (only sampled in 2009). Due to a significant inter-observer effect for CP measurements, we limited our analysis of this variable to those CP measurements taken by a single observer (CMT) who measured 75% of the birds. For analysis of breeding success we used logistic regression to examine if arrival date, androgen upon arrival, and CP upon arrival were significant predictors of the binary response: successful or unsuccessful. We only ran univariate regressions due to a correlation between the independent variables (Results). Total androgen concentrations and haematocrit were square-root transformed to meet the assumptions of all analyses. We found no relationship between time from arrival to capture and androgen ($F_{2,36} = 0.46$, $p = 0.64$) or haematocrit ($F_{2,36} = 1.25$, $p = 0.30$). All figures show untransformed values and regression lines, while all statistics are derived from transformed variables. Three extreme statistical outliers ($T > 10\,000 \text{ pg ml}^{-1}$, $n = 2$; and $\delta^{13}\text{C} < -25$, $n = 1$) were removed ($|R \text{ Student}| > 2$, Cook's $D > 0.5$).

Results

Early arriving male redstarts were more likely to be from wetter winter habitats, as indicated by $\delta^{13}\text{C}$ values in claws, independent of year (Table 1a). This is consistent with a study at the same site 10 yr previous to ours, which utilized muscle tissues for isotope analyses (Marra et al. 1998). Earlier arriving males had higher total androgen than later males (Fig. 1a). The overall model for relating androgen upon arrival to $\delta^{13}\text{C}$, with year and year by $\delta^{13}\text{C}$ as additional explanatory variables was not significant

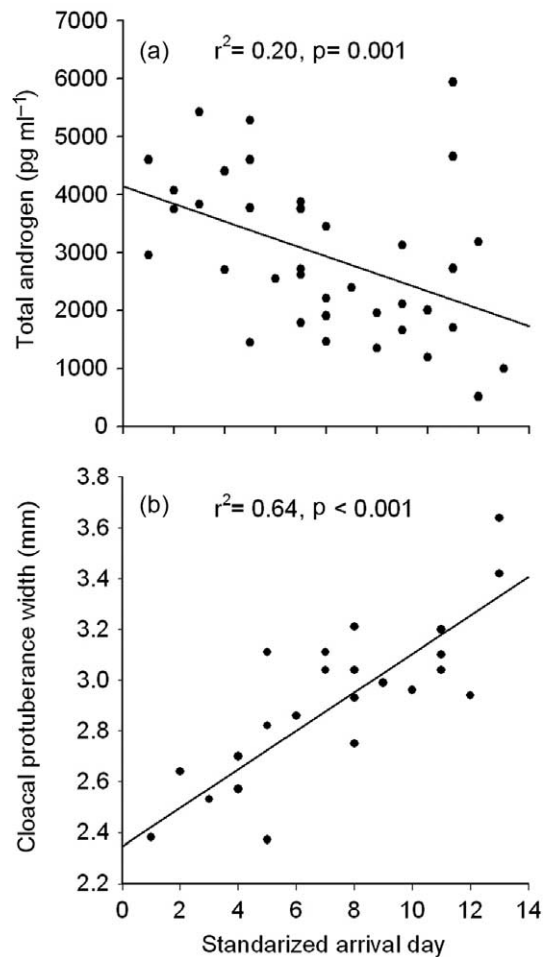


Figure 1. Linear relationships between male redstart arrival date and (a) total androgen ($n = 39$), and (b) cloacal protuberance (CP) diameter upon arrival ($n = 25$) to breeding territories at Hubbard Brook Experimental Forest, North Woodstock, NH, USA in 2008 and 2009.

(Table 1b). However, since there was a significant coefficient for $\delta^{13}\text{C}$, we performed a post hoc analysis of a more parsimonious model, where we removed the non-significant interaction term, and found that males arriving with higher total androgen were more likely to be from wetter winter habitats, independent of year (overall model: $n = 35$, $r^2 = 0.19$, $p = 0.03$; $\delta^{13}\text{C}$: $\beta = -6.76$, $t_{33} = 2.708$, $p = 0.01$; year: $\beta = 14.45$, $t_{33} = 2.195$, $p = 0.04$). $\delta^{13}\text{C}$ was not related to measures of migratory condition (Table 1d, e). Contrary to our predictions, the diameter of the CP was larger in late arriving males (Fig. 1b) and there was no relationship between CP and winter habitat quality (Table 1c). There was no relationship between size-corrected mass and arrival date ($n = 36$, $r^2 = 0.01$, $p = 0.80$) or androgen ($n = 36$, $r^2 = 0.01$, $p = 0.60$). Early arriving males ($n = 36$, $r^2 = 0.14$, $p = 0.03$) and those with high androgen upon arrival (Fig. 2) had higher haematocrit than late arrivers and those with low androgen. Size-corrected mass and haematocrit were not related ($n = 36$, $r^2 = 0.01$, $p = 0.50$).

Arrival time ($n = 34$, Wald $\chi^2 = 4.71$, $p = 0.03$), but not androgen level upon arrival ($n = 28$, Wald $\chi^2 = 0.06$, $p = 0.80$), was a predictor of nest success. Successful males

arrived, on average, 3.5 days earlier than unsuccessful males, such that for every day a male arrived after the first male he was 11% less likely to fledge a nest (odds ratios = 0.89). Males arriving with smaller CPs were more likely to successfully breed ($n = 17$, Wald $\chi^2 = 3.76$, $p = 0.05$). Early arriving males also had earlier clutch completion dates for the first clutch laid on their territory ($n = 23$, $r^2 = 0.23$, $p = 0.03$), and earlier fledging dates ($n = 18$, $r^2 = 0.43$, $p = 0.003$) than late arriving males. There was no relationship between androgen upon arrival and first clutch completion date ($n = 17$, $r^2 = 0.20$, $p = 0.08$), fledging date ($n = 11$, $r^2 = 0.02$, $p = 0.71$), or latency of response to playback ($n = 19$, $r^2 = 0.03$, $p = 0.73$). Males arriving with smaller CPs had earlier first clutch completion dates ($n = 11$, $r^2 = 0.40$, $p = 0.04$), however we lacked sufficient sample size to compare CP with fledging dates.

Discussion

This is the first study to simultaneously measure an index of winter habitat quality and breeding preparation during the breeding ground arrival period, and relate them to breeding success in a migratory species. Our results are consistent with earlier studies in which winter habitat quality, through food availability (Studds and Marra 2005), can influence spring departure time (Marra et al. 1998), migratory condition (Bearhop et al. 2004), and breeding success (Reudink et al. 2009a). In a study of captive birds, Bauchinger et al. (2009) demonstrated that experimentally increasing food-availability during migration can result in elevated breeding hormones and increased testis size in garden warblers *Sylvia borin*. We were able to link winter habitat quality, not only with breeding ground arrival time, but also with preparation for breeding upon arrival. However, our measures of breeding readiness at the time of arrival were not strong predictors of subsequent breeding success. It may be that androgen supports greater breeding success by facilitating improved physiological migratory condition, through increased oxygen carrying capacity (haematocrit), needed to arrive at the breeding grounds as early as possible. In this way, winter habitat quality may incur androgen-mediated carry-over effects on breeding success by influencing arrival time, which then facilitates breeding success.

Early arriving males had higher plasma androgen levels than later arriving ones, but early males had smaller CPs upon arrival. One might expect to see initial CP development track plasma androgen levels (Wingfield 1984). However, our results are similar to those from a study of European blackcaps *Sylvia articapilla* arriving to breeding areas (Rolshausen et al. 2010): later arriving males had significantly larger CPs than early males. One possibility for the conflicting relationships of androgen and CP with arrival date could be that high androgen upon arrival serves to activate CP development. However, previous studies have found androgen and CP size in breeding birds to be high concurrently during CP development (Morton et al. 1990), and, similar to our study, Wingfield (1984) found that androgen is at high levels during territory establishment when testis mass and CP size is low. While the CP serves as a sperm storage organ, full CP development may be delayed

Table 1. Parameter estimates (β), standard errors (SE), upper and lower 95% confidence intervals (LCI, UCI), test statistics (t), and p-values for multiple regression of a) arrival date (overall model: $n = 35$, $r^2 = 0.41$, $p < 0.001$), b) androgen upon arrival (overall model: $n = 35$, $r^2 = 0.18$, $p = 0.10$), c) CP upon arrival (overall model: $n = 24$, $r^2 = 0.08$, $p = 0.64$), d) size-corrected mass (overall model: $n = 34$, $r^2 = 0.12$, $p = 0.28$), and e) haematocrit ($n = 28$, $r^2 = 0.14$, $p = 0.29$) with the independent variables stable carbon isotope ratio ($\delta^{13}\text{C}$), year, and their interaction for male redstarts arriving to breed at Hubbard Brook Experimental Forest, North Woodstock, NH, USA in 2008 and 2009.

a) arrival day						
factor	β	SE	LCI	UCI	T	p
intercept	75.06	21.23	31.76	118.36	3.54	0.001
$\delta^{13}\text{C}$	2.83	0.92	0.97	4.70	3.10	0.004
yr	-7.20	30.50	-69.40	55.01	-0.24	0.815
$\delta^{13}\text{C} \times \text{yr}$	0.04	1.38	-2.77	2.86	0.03	0.975
b) androgen						
factor	β	SE	LCI	UCI	T	p
intercept	-103.41	71.70	-249.65	42.82	-1.44	0.159
$\delta^{13}\text{C}$	-6.86	3.08	-13.15	-0.57	-2.22	0.034
year	75.67	98.67	-125.57	276.91	0.77	0.449
$\delta^{13}\text{C} \times \text{yr}$	3.04	4.44	-6.01	12.10	0.69	0.498
c) CP						
factor	β	SE	LCI	UCI	T	p
intercept	12.83	12.05	-12.31	37.97	1.07	0.300
$\delta^{13}\text{C}$	0.42	0.51	-0.65	1.49	0.82	0.421
year	-7.51	12.34	-33.26	18.25	-0.61	0.550
$\delta^{13}\text{C} \times \text{yr}$	-0.30	0.53	-1.40	0.79	-0.58	0.571
d) size-corrected mass						
factor	β	SE	LCI	UCI	T	p
intercept	-1.34	5.63	-12.85	10.16	-0.24	0.813
$\delta^{13}\text{C}$	-0.07	0.24	-0.56	0.43	-0.29	0.778
year	-4.46	8.15	-21.12	12.19	-0.55	0.588
$\delta^{13}\text{C} \times \text{yr}$	-0.19	0.37	-0.95	0.56	-0.52	0.606
e) haematocrit						
factor	β	SE	LCI	UCI	T	p
intercept	0.12	0.45	-0.81	1.06	0.27	0.786
$\delta^{13}\text{C}$	-0.02	0.02	-0.06	0.02	-0.93	0.360
year	0.24	0.49	-0.78	1.26	0.49	0.628
$\delta^{13}\text{C} \times \text{yr}$	0.01	0.02	-0.03	0.05	0.48	0.635

while en route during migration because sperm storage is not yet needed (but see Quay 1985, Moore and McDonald 1993). Since female redstarts arrive later than males (Sherry

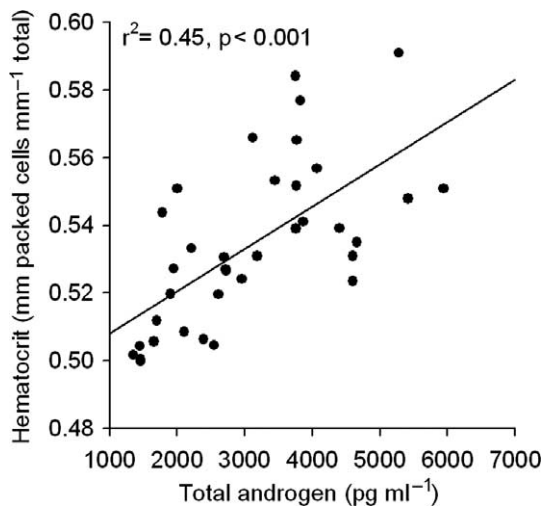


Figure 2. Linear relationship between circulating androgen and haematocrit ratio for male redstarts upon arrival ($n = 36$) to Hubbard Brook Experimental Forest, North Woodstock, NH, USA in 2008 and 2009.

and Holmes 1997), early arriving males have few, if any, opportunities to copulate but are faced with an increasing number of male competitors for breeding territories. Therefore, the primary role of high androgen in early arriving males may be to support territorial behaviour, whereas late arriving males must invest in CP development since many females are present by the time they arrive, potentially representing two different arrival strategies. We did not find a relationship between androgen and latency of response to STI. It is also possible that male redstarts rely on plumage signaling (Reudink et al. 2009b, c), rather than physical aggression, to resolve territorial interactions, resulting in a disassociation between circulating androgen and aggressive behaviour.

The failure to find a direct relationship between androgen levels upon arrival and breeding success may be due to the fact that we did not measure paternity, only overall fledging success. Reudink et al. (2009a) found that high levels of extra-pair paternity and greater incidence of polygyny in redstarts were associated with early arrival. Although rates of polygyny were very low in our study population (one male in each year of the study), it is possible that fitness benefits from high androgen upon arrival may be manifest in the number of genetic offspring. Further, it is important to emphasize that we measured

androgen only during the arrival period and studies examining intra-seasonal change in androgens, such as testosterone, have primarily found that peak breeding levels are not reached until the nest building and egg laying phases of the nesting cycle (Wingfield et al. 1990, Wingfield and Farner 1993, Schwabl et al. 2005). Our data do, however, support the idea that plasma androgen levels during the pre-arrival period could influence fitness through a proximate effect on migration.

Androgen prior to and upon arrival may be acting to influence arrival date, and ultimately breeding success, through its anabolic effects on structural tissues such as muscle, adipose, and bone. But, androgen's role in regulating tissues is complex and studies in which the androgen testosterone was experimentally elevated in birds have shown varying effects on physical condition, often depending on life-history stage. Elevated levels of exogenous testosterone during breeding have ranged from no effect (Lynn et al. 2000, Alonso-Alvarez et al. 2002) to catabolic effects on energy stores (Ketterson et al. 1991). Artificially elevated testosterone has had anabolic effects on overall body composition during development (Navara et al. 2006, Muller et al. 2007), can advanced the phenology of migratory preparation (Tonra et al. 2011), and testosterone has been shown to increase activity independent of daily energy expenditure (Lynn et al. 2000) and promote migratory hyperphagia (Deviche 1995). Therefore, although we did not find any relationship between arrival androgen and concurrent condition (as measured by size-corrected mass), elevated androgen may have enabled males reach condition thresholds more rapidly, through its support of increased foraging activity. In addition, males who elevate androgen may achieve more advanced physiological migratory condition, independent of mass. In support of this, we found that males arriving with high androgen have high haematocrit. Further, in agreement with previous studies (Ninni et al. 2004, Hatch et al. 2010), we found that haematocrit was positively associated with early arrival. Together, these findings suggest that males who elevate androgen benefit from associated erythropoietic effects, increasing oxygen capacity. It is unlikely that the elevated haematocrit in early arriving males was due to differential dehydration levels compared with later arrival birds, as birds can quickly restore water lost metabolically during migration within several hours of arrival (Morton 1994). Further, metabolic water loss during migratory flight cannot explain why, for example, bar-tailed godwits *Limosa lapponica* increase haematocrit prior to departure from staging areas (Piersma et al. 1996), or why red knots *Calidris canutus* actually decreased haematocrit after being exercised in a wind tunnel (Jenni et al. 2006). Jenni et al. did show that the slight decrease in hematocrit was associated with migratory flight corresponds to reduction in muscle scores, indicating that birds with high hematocrit are in superior physical condition, however that was not reflected in our analyses of size-corrected mass.

Migratory birds face a conflicting set of energetic demands when simultaneously migrating and transitioning from a non-breeding state to a breeding one. Physiological mechanisms that minimize conflicts, perhaps through diversifying hormone function, would, if achieved, be advantageous to both survival and breeding success. The

potential direct effects of androgen on migratory performance, and through it, breeding success, could indicate that androgen is playing a dual role in the transition between life history phases; breeding and migration. More experimental work manipulating androgen activity during migratory preparation on the wintering grounds and at stopover sites is necessary to examine this model further.

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