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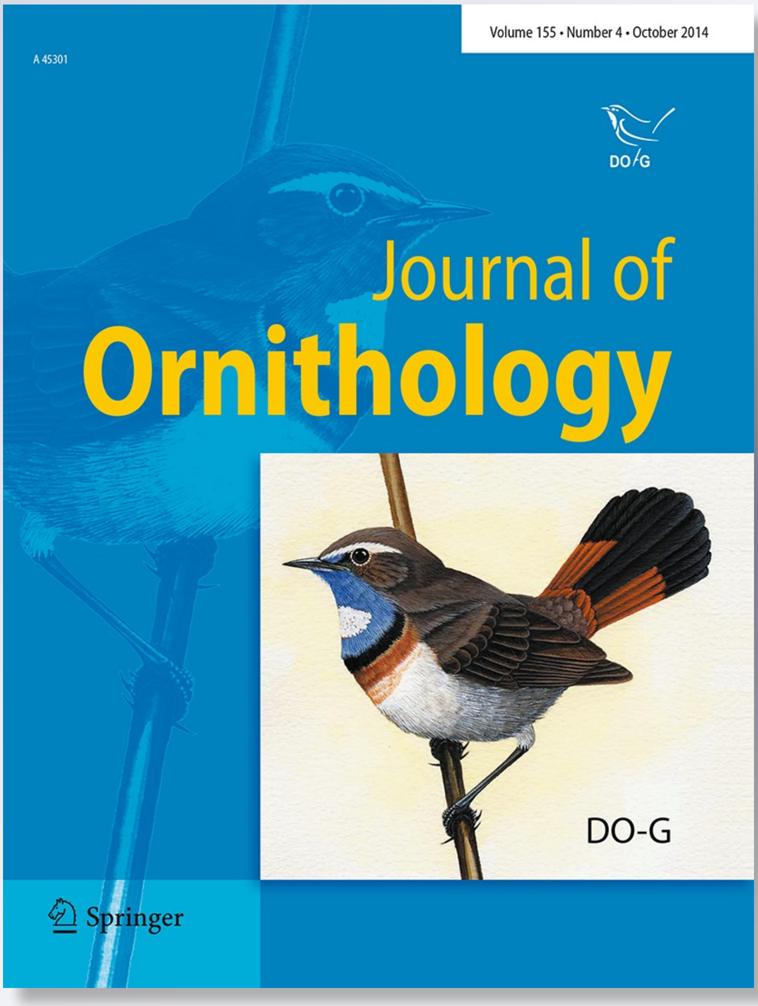
Journal of Ornithology

ISSN 2193-7192
Volume 155
Number 4

J Ornithol (2014) 155:1017-1025
DOI 10.1007/s10336-014-1088-y



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ORIGINAL ARTICLE

Aggression, body condition, and seasonal changes in sex-steroids in four hummingbird species

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Received: 24 September 2013 / Revised: 27 April 2014 / Accepted: 22 May 2014 / Published online: 11 June 2014

Abstract Hummingbirds present a unique combination between extremely high life costs and a number of efficient adaptations to fuel these demands. In addition to cognitive abilities, territorial hummingbirds display aggressive behaviors that allow for access to better food resources. In year-round territorial species, male–male territorial aggression is similar between breeding and non-breeding seasons; however, the endocrine mechanisms underlying control of territoriality during these distinct seasonal periods may differ. In many species, testosterone (T) triggers increased aggression during the breeding season whereas territoriality in the non-breeding season can be regulated by circulating the biologically inert sex steroid precursor dehydroepiandrosterone (DHEA) and converting it to T in target tissues. The seasonal hormonal regulation of hummingbird territorial behavior has heretofore been unknown. Our goal was to assess seasonal changes in sex steroids, territorial aggression levels, and body condition during reproductive and non-reproductive seasons in hummingbirds. To validate the use of cloacal fluid (CF) for the study of sex steroids, steroid levels in plasma and CF were correlated in *Sephanoides sephaniodes*. During the reproductive season, *Calypte. anna, Archilochus alexandri, and Selasphorus rufus* males showed high levels of T that were positively correlated with aggression, but the relationship between T and body condition was not consistent across species. As expected, T levels in females were significantly lower than in males in all seasons, however still detectable. During the non-reproductive season, CF DHEA of *Calypte anna* was high and positively correlated with aggressive behaviors and body condition. Our results suggest that hummingbirds display aggressive behaviors that could be linked to different hormones during the breeding and non-breeding seasons.

Keywords Testosterone · Dehydroepiandrosterone · Hummingbirds · Territoriality · Body condition · Seasonality · Aggression

Communicated by L. Fusani.

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Zusammenfassung

Aggressionsverhalten, Körperkondition und jahreszeitliche Schwankungen der Sexualsteroidspiegel bei vier Kolibriarten

Kolibris verbinden in einzigartiger Weise eine extrem kostspielige Lebensweise mit einer Reihe von Anpassungsleistungen, um ihren Energiebedarf effizient zu decken. Zusätzlich zu ihren kognitiven Fähigkeiten verfügen territoriale Kolibris über aggressive Verhaltensweisen, die ihnen den Zugang zu besseren Nahrungsquellen sichern. Bei Arten, die ganzjährig Reviere verteidigen, ist die territoriale Aggression zwischen Männchen zur Brutzeit ähnlich wie außerhalb; allerdings können sich die der Steuerung der Territorialität zugrunde liegenden endokrinen Mechanismen während dieser jahreszeitlich klar getrennten Zeiträume unterscheiden. Bei vielen Arten löst Testosteron (T) eine Zunahme des Aggressionsverhaltens während der Brutsaison aus, wohingegen die Territorialität außerhalb der Brutzeit durch den Einsatz des biologisch inerten Sexualsteroid-Vorläufers Dehydroepiandrosteron (DHEA) reguliert werden kann, welcher dann im Zielgewebe in T umgewandelt wird. Die jahreszeitliche hormonelle Steuerung des Territorialverhaltens bei Kolibris war bislang unbekannt. Unser Ziel war es, jahreszeitliche Schwankungen des Spiegels von Sexualsteroiden, das Ausmaß territorialer Aggression sowie die Körperkondition der Kolibris jeweils während und außerhalb der Fortpflanzungszeit zu ermitteln. Um die Eignung der Kloakenflüssigkeit (cloacal fluid, CF) für die Untersuchung von Sexualsteroiden zu testen, wurden bei *Sephanoides sephaniodes* die jeweiligen Steroidspiegel in Plasma und CF miteinander in Bezug gesetzt. Während der Fortpflanzungsperiode zeigten Männchen von *C. anna, Archilochus alexandri* und *Selasphorus rufus* jeweils hohe T-Spiegel, welche positiv mit dem Aggressionsverhalten korrelierten; das Verhältnis zwischen T und der Körperkondition stimmte jedoch nicht bei allen Arten überein. Erwartungsgemäß lagen die T-Spiegel bei den Weibchen zu allen Jahreszeiten signifikant niedriger als bei den Männchen, waren aber trotzdem nachweislich vorhanden. Außerhalb der Fortpflanzungszeit lag der CF-Wert für DHEA bei *Calypte anna* hoch und korrelierte positiv mit dem Aggressionsverhalten und der Körperkondition. Unsere Ergebnisse legen nahe, dass die von Kolibris gezeigten aggressiven Verhaltensweisen zur Brutzeit beziehungsweise außerhalb dieser an verschiedene Hormone gekoppelt sein könnten.

Introduction

Energy balance in hummingbirds is the result of a complex interplay between foraging behavior, physiological constraints, cognitive performance, and environmental conditions (Hainsworth 1978; Suarez and Gass 2002). During hovering, hummingbirds achieve some of the highest mass-specific rates of aerobic metabolism known among vertebrates (Suarez 1992). Moreover, their small body size imposes high thermoregulatory costs (González-Gómez et al. 2011a). In order to fuel these high metabolic rates, hummingbirds are able to remember the location and availability of the best resources (González-Gómez et al. 2011b). In fact, recent studies suggest individuals with better cognitive abilities exhibit higher energy intake (González-Gomez et al. 2011c).

In some hummingbirds species such as the Green-backed Firecrown hummingbird (*Sephanoides sephaniodes*) and the Annas hummingbird (*Calypte anna*), males display two strategies, territorial individuals, which actively defend feeding resources by displaying aggressive behaviors, such as performing acrobatic aerial displays, vocalizing, and chasing intruders and non-territorial individuals, which are less aggressive and mostly opportunistic (González-Gómez et al. 2011a; González-Gómez, non-published data). Previous studies conducted by González-Gómez et al. (2011a) showed that, when the cost of aggression is higher than the benefit derived from the defended nectar sources, territorial behaviors cease. Therefore, it is hypothesized that territorial males display a positive correlation between aggression and body condition. Females, in contrast, are in general non-territorial and either perform rapid intrusions into male territories to raid high-quality resources (González-Gómez and Estades 2009) or are forced to forage in nectar-poor, scattered resources (Kodric-Brown and Brown 1978; Temeles et al. 2005).

Even though the behavioral patterns of male–male territorial aggression are similar between breeding and non-breeding seasons in year-round territorial avian species, there is extensive evidence showing that endocrine regulation mechanisms are different both seasons in most of bird species (Hau et al. 2000; Wingfield et al. 2001; Wingfield and Soma 2002; Hau 2007; Goymann et al. 2007). In the breeding season, it is well-known that testosterone (T) triggers an increase in male–male competition for territories and mates (Wingfield 1984; Ogawa et al. 1997). Possibly because high levels of testosterone could induce inappropriate behaviors outside of the breeding season such as sexual behavior (Hau 2007), and could have detrimental effects such as decreasing body mass and fat stores (Wingfield and Soma 2002), alternatives to T have been proposed outside of the breeding season. One possibility could be dehydroepiandrosterone (DHEA) a biologically inert androgen precursor that can be converted to biologically active sex steroids in the brain, thus, avoiding peripheral androgenization in a non-reproductive context (Soma and Wingfield 2001; Soma et al. 2000, 2002). For example, simulated territorial intrusions performed with spotted antbirds during the non-reproductive season (i.e., birds had regressed gonads and low levels of androgen and estrogen) showed that plasma DHEA was directly correlated to the intensity of aggressive displays in both males and females (Hau et al. 2000). Interestingly, hormonal regulation of hummingbird territorial behavior has heretofore been unstudied. In this study, we aimed to assess seasonal changes in sex steroids, territorial aggression levels, and body condition during the reproductive and non-reproductive seasons in four hummingbird species that defend food resources such as feeders and flowering trees and shrubs. In this context, we expected that (a) males would have higher levels of testosterone than females during the reproductive season; (b) higher levels of aggression in territorial males would be correlated with higher levels of T during the reproductive season and higher levels of DHEA during the non-reproductive season; and (c) body condition would be positively related to DHEA or T levels in males.

Methods

To assess DHEA and T levels in reproductive and non-reproductive seasons and to determine the relationship between hormone levels in plasma and CF samples, we performed a laboratory study in Chile. Field studies were conducted in California, USA to evaluate the relationship between hormones in CF, aggressive behaviors, and body condition. All protocols were conducted according to Chilean laws, legal permits and the ethical committees of the Universities (IACUC Protocol No. 16095, Federal Bird Banding Permit No. 21535).

Laboratory study

The study was carried out during April (non-reproductive season, $n = 5$) and July (beginning of the reproductive season, $n = 4$) of 2009 in the Andean foothills at the Mediterranean field station of the Universidad Católica de Chile at San Carlos de Apoquindo, central Chile (33°23′S, 70°31′W, 1,100 m elevation above sea level). Subjects were male Green-backed Firecrown hummingbirds, *Sephanoides sephaniodes* (5.8 ± 0.19 g) that had established and were actively defending feeding territories at the study site. Birds were caught with mist nets and housed for seven days in individual mesh cages (1 × 1 × 1 m) under natural photoperiod and temperature at the field site. Birds were fed with nutritionally complete, artificial nectar (Nektar Plus, Nekton USA, Clearwater, FL). All birds were released after the study.

Field studies

Observations and data collection were carried out from September 2010 to August 2011 during the reproductive and non-reproductive seasons, with the objective of relating aggression, hormone levels, and body condition. The study was carried out in Chalk Hill and Baggins End, Davis, CA. Subjects were free-living Anna's hummingbirds (*Calypte anna*, 4.82 ± 0.1 g), which were sampled from September to early December (non-reproductive season, $n = 9$ males) and from middle January to middle May (reproductive season, $n = 13$ females and 34 males); Rufous hummingbirds (*Selasphorus rufus*, 3.15 ± 0.05 g), which were sampled from March to April (migratory/reproductive season, $n = 9$ females and 6 males; see below); and Black-chinned hummingbirds (*Archilochus alexandri*, 3.3 ± 0.06 g), which were sampled from April to late June (reproductive season, $n = 15$ females and 7 males) and from middle August to early September (non-reproductive season, $n = 8$ females and 10 males).

In order to differentiate properly between territorial and non-territorial hummingbirds in a free-living environment, a training period was conducted. Feeders were introduced into the surrounding environment and observations made every day between 8:00 and 11:30 a.m. for a week. Subjects that actively and consistently defended the feeders as part of their territories for more than a day were classified as "territorial birds". Individuals that were able to use the feeders and occasionally displayed aggressive behaviors, but were not consistently observed defending or in the feeder's surrounding area, were classified as "non-territorial" individuals. The artificial feeder consisted of a commercial 50 ml syringe enveloped with red paper. Each feeder was filled twice a day with 50 ml of 25 % w/w sucrose solution. On the day of hormone sampling, the feeder was set up in a cylindrical trap (30 cm diameter, 50 cm height) and aggressive territorial behaviors were observed for 1 h. All the observations were performed between 7:00 and 11:00 a.m. to avoid behavioral variability throughout the day. To assess the intensity of territorial defense, an aggressiveness index described by González-Gómez et al. (2011a) was utilized. In short, this index is composed of three different territorial behaviors: aggressive calls (vocalizations, *V*), chases (*C*), contacts (aggression, *O*), and their combinations weighted by the energy used in the aggressive action. We obtained that V/C/O has the relations 2:3:5, and therefore, we constructed the following aggressive index (AI):

$$\mathrm{AI} = \mathrm{V} * 2 + \mathrm{C} * 5 + \mathrm{VC} * 7 + \mathrm{CO} * 8 + \mathrm{VCO} * 10.$$

Birds were captured in the trap immediately after the period of observation and CF was collected. All CF samples were collected in the first 3 min after capture. In addition, body weight and tarsus length were recorded for each bird. Birds were weighed with a Pesola Micro-Line Spring Scale (capacity 10 g) and measured with a Fowler (Brantford, Ontario) UltraCal Mark III Electronic Caliper. Body condition index was calculated as the residuals of the regression between tarsus length and body mass ($R^2 = 0.17$, $p = 0.03$). Because of the volume constraint (i.e., each sample was roughly 30–50 μL), CF samples were analyzed for either T (reproductive season, Anna's, Black-chinned and Rufus hummingbirds) or DHEA (non-reproductive season, Anna's hummingbirds) using a radioimmunoassay (RIA) as described below.

Blood samples

A 30 μL blood sample (roughly 5 % of body mass) was taken from the tarsus vein immediately after capture with a heparinized capillary tube. Tubes were sealed using sealing clay. This procedure was repeated 72 h later in order to obtain the necessary volume to perform the hormone analysis (i.e., 25 μL of plasma). All birds maintained or increased body mass during captivity and were released after two days of the last blood collection when we were confidant they had recovered. Samples were immediately centrifuged at 2,000 rpm for 5 min and plasma was moved to a −20 °C freezer.

Cloacal fluid samples

Samples were collected non-invasively by holding an 80 μL microcapillary tube directly outside the cloaca. During aspiration, care was taken to avoid fecal masses. If a sample had visible fecal masses, it was discarded. Samples were frozen immediately after collection in a −20 °C freezer.

Controlling for urine dilution

In order to control for the animal's hydration state and its possible effect on hormone levels, CF creatinine was used as a reference for *S. sephaniodes* (Hiebert et al. 2000). Creatinine concentration was determined with reagents from Cayman Chemical (Cat No. 500701), following the protocol described by Hiebert et al. (2000).

The levels of creatinine were undetectable in CF from the other species in this study. Instead, specific gravity (hereafter SG) was used, which refers to the density (mass per volume) of a sample in comparison to pure water (Armstrong 2005). SG was determined using a clinical temperature compensated refractometer (Westover Scientific, RHC200ATC). Unfortunately, due to the limited amount of CF obtained from free-living hummingbirds, most of the samples were completely used in the unsuccessful creatinine assay and hormonal assay. Therefore, to assess the variation in urine dilution among free-living birds, an additional study was performed. We recorded SG in the samples from free-living birds of the three studied species for which we still had at least 10 μL ($N = 43$ out of a total of 94 samples). We compared the SG of these samples with that of CF from newly captured *C. anna* that were maintained in captivity for 30 min and then released ($n = 4$). This study was performed during the reproductive season at the same dates as the general urine sampling. Birds were individually maintained in collapsible tulle cages (60 × 60 × 60 cm); a CF sample was taken when the birds were caught and then at 15 and 30 min, respectively. All birds were then released. Ad libitum sucrose solution (25 % w/w) was offered in a syringe nectar feeder (see below), but none of the birds fed; therefore, we were able to measure the effect of dehydration on SG by comparing the SG of the three CF samples collected during this procedure.

Radioimmunoassay (RIA)

Plasma

After centrifugation, plasma samples (30 μL) were brought to 200 μL by adding distilled water. Samples were incubated overnight with $[^3H]$ steroid (i.e., testosterone or DHEA, approximately 10,000 cpm, New England Nuclear) for recovery determination. Steroid extraction was performed with redistilled dichloromethane. Levels of T and DHEA in plasma were assessed using one direct RIA per each hormone (e.g., Wingfield et al. 1992). Intra-assay variation was 8.3 % and 7.9 %, respectively. A detection limit of 0.19 and 0.20 ng/ml was used for DHEA and T, respectively.

Cloacal fluid

Prior to analysis of DHEA in cloacal fluid, a sulfatase enzyme was used to hydrolyze DHEA-S into DHEA (Soma and Wingfield 2001). For this, 0.75 ml of 0.2 M sodium acetate buffer (pH 5.0) with sulfatase (Sigma, partially purified from *Helix pomata*) was added to 30 μL of each sample; samples were incubated overnight at 37 °C. The following day 20 μL of $[^3H]$ DHEA was added to each sample and samples were again incubated overnight. DHEA extraction was performed with redistilled dichloromethane. RIA was performed as described previously (Wingfield et al. 1992).

Levels of T in CF were determined using direct RIA with no extraction (Hiebert et al. 2000). Specifically, the sample was brought to 200 μL with distilled water; then 100 μL of $[^3H]$ T and 100 μL of T antiserum were added (Hiebert et al. 2000). Inter-assay variation was T 11.74 %. DHEA samples were run in one assay.

Statistical analysis

To assess the effect of season (reproductive, non-reproductive) on sex steroid levels, we performed a multivariate analysis of variance (MANOVA). Levels of hormones in plasma and CF were correlated with a simple regression.

Comparisons between territorial and non-territorial individuals were assessed utilizing the Mann–Whitney test since one of the distributions did not meet the normality criterion. To assess the effect of DHEA on aggressive behaviors and body condition, simple regressions were performed. To assess the effect of species on hormone levels during the reproductive season, an ANOVA and a posteriori comparison among species through Tukey HSD for unbalanced sample size was performed. Differences in T levels between reproductive and non-reproductive seasons within a species were compared using the Mann–Whitney test when they did not meet conditions for normality and the *t* test when they did.

To compare SG in CF between free-living and captive birds at the moment of capture, a Mann–Whitney test was performed; in addition, a repeated measures Friedman ANOVA was used to compare SG over time (0, 15 and 30 min) in captive individuals. Analyses were performed with Statistica 6.0. Data are expressed as mean ± SE.

Results

Laboratory study

Plasma DHEA levels were significantly higher during the non-reproductive season compared to the early reproductive season in *S. sephanoides* (Fig. 1a). In contrast, plasma T was not significantly different between seasons (Fig. 1a). Steroids in CF and plasma were positively correlated (Fig. 1b).

Urine dilution

Creatinine in *S. sephaniodes* was not significantly different between non-reproductive and reproductive seasons (0.30 ± 0.06 and 0.31 ± 0.09 mg/dL, respectively, mean ± SE; CV = 0.37 %, Mann–Whitney $U = 4.5$, $P = 0.85$). We found no significant relation between creatinine and body condition index ($r^2 = 0.08$; $F_{1,4} = 0.35$; $P = 0.58$).

Cloacal fluid SG for free-living hummingbirds showed minimal variation among individuals and species (1.0037 ± 0.00038, mean ± SE; CV = 0.25 %, range = 1.002–1.008). There was no difference between the SG of CF from free-living birds,all three California species, and from *C. anna* that was kept in captivity at the moment of capture (Mann–Whitney $U = 78.5$, $p = 0.72$) or after 15 min of fasting (1.008 ± 0.0019, $p = 0.13$) but significantly increased after 30 min (1.011 ± 0.002, Friedman ANOVA $X^2 = 6.53$, $P < 0.05$). On the basis of these results, we are confident that there is minimal variation in urine dilution in free-living hummingbirds. We, therefore, did not incorporate a correction for urine dilution in the analysis of CF obtained from birds in the field.

Field studies

Males

During the reproductive season, levels of T in CF of *A. alexandri* were 3.5 times higher than in Anna's and 2.6 times higher than in *S. rufus* (ANOVA ($F_{(1,43)} = 3.99$, $P < 0.05$, Fig. 2). Levels of T in CF were significantly greater in *A. alexandri* than in *C. anna* (Tukey HSD $P < 0.05$), but not significantly different between *A*. alexandri and *S. rufus* (Tukey HSD $P = 0.15$) or between *S. rufus* and *C. anna* (Tukey HSD $P = 0.91$). Body condition index was positively correlated with T levels in *C. anna* (Fig. 3a), but tended to be negatively correlated with T

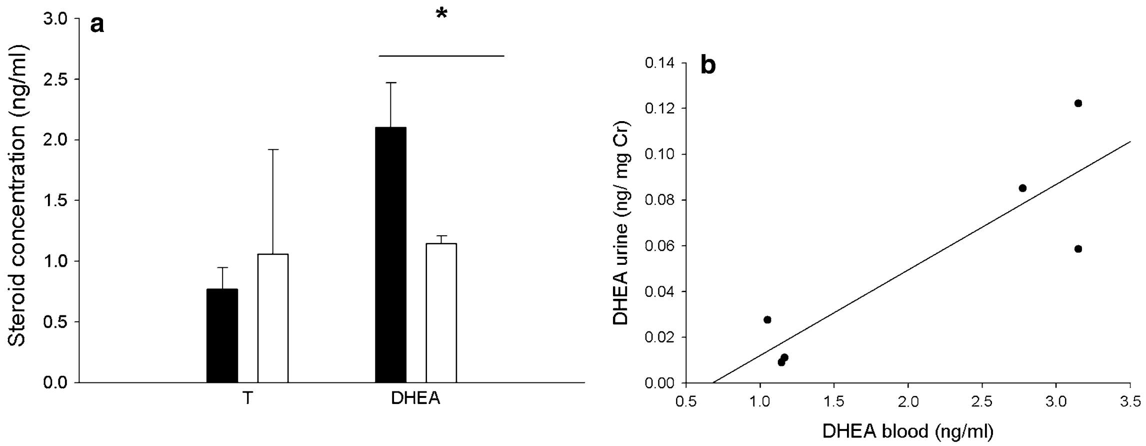

Fig. 1 Levels of plasma DHEA in *S. sephanoides* ($n = 9$) were significantly higher during the non-reproductive season (*black bars*) than during the early reproductive season (Mann–Whitney $U_9 = 2.32$, $P < 0.05$, *white bars*). In contrast, levels of T were not significantly different between seasons ($U_9 = 7.01$, $P = 0.60$, **a**). Levels of plasma and urine creatinine referenced DHEA were positively correlated ($r^2 = 0.69$, $F_{(1,4)} = 8.7$, $P < 0.05$, $n = 5$; **b**)

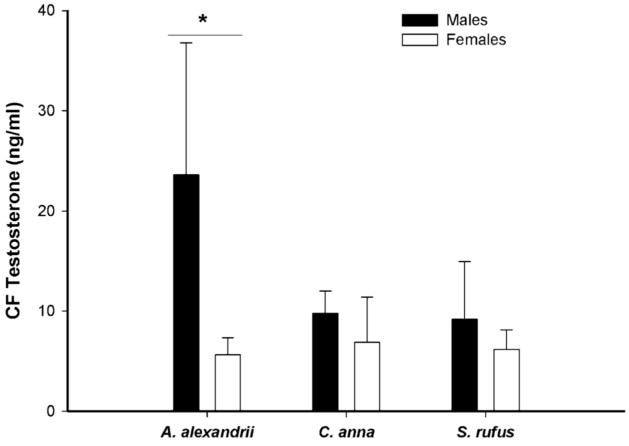

Fig. 2 Testosterone levels in cloacal fluid (CF) during the reproductive season from males and females of three species of free-living hummingbirds that were briefly captured to obtain the sample. *Asterisk* significantly differences, $P < 0.05$

levels in *A. alexandri* (Fig. 3b). In addition, T levels in CF of *A. alexandri* were marginally positively correlated with the aggression index (Fig. 3c).

During mid-August, in the non-reproductive pre-migration period, levels of CF T in *A. alexandri* were 29 times lower than in the reproductive season (0.79 ± 0.15 ng/ml CF mean ± SE, $t = -2.26$, $P < 0.05$, $n = 17$; data not shown); however, the relation between body mass and hormone levels during this period was not significant ($R^2 = 0.22$, $F_{(1,8)} = 2.4$, $P = 0.16$, $n = 10$).

During the non-reproductive season, DHEA in CF of male *C. anna* was significantly higher in territorial than in non-territorial males (Fig. 4a). DHEA was positively correlated with aggression index (Fig. 4b). Body condition was marginally positively related to DHEA levels (Fig. 4c).

Females

Levels of T in CF were not significantly different across species during the reproductive season (ANOVA ($F_{(1,21)} = 2.78$, $P = 0.08$, $n = 24$, Fig. 3). T levels in CF from *A. alexandri* during the reproductive season (5.65 ± 1.71 ng/ml) were nine times higher than during the pre-migration period (0.57 ± 0.19 ng/ml, Mann–Whitney $U = 0.0$, $P < 0.05$, $n = 14$; data not shown). During the reproductive season, we found levels of T in CF significantly higher in males than in females in *C. anna* (Mann–Whitney $U = 100$, $P < 0.01$) and in *A. alexandri* ($U = 63$, $P < 0.05$), but not in *S. rufus* ($U = 26$, $P = 0.90$).

Discussion

In this study, we showed a positive relationship between sex steroids in plasma and their metabolites in CF (Fig. 1b). The small blood volumes available from hummingbirds makes this technique especially useful for studying endocrine mechanisms in these birds. In comparison to other birds (Wasser et al. 1993; Goyman 2005), hummingbirds present advantages to use this methodology because they have relatively stable diets (López-Calleja and Bozinovic 2003; González-Gómez et al. unpublished data) and ingest large amounts of water, which visibly separates urine from feces.

Reproductive season

As it was earlier reported by Stiles (1971), *C. anna* did not defend feeding territories consistently during reproductive season, although they displayed inter-specific and intra-

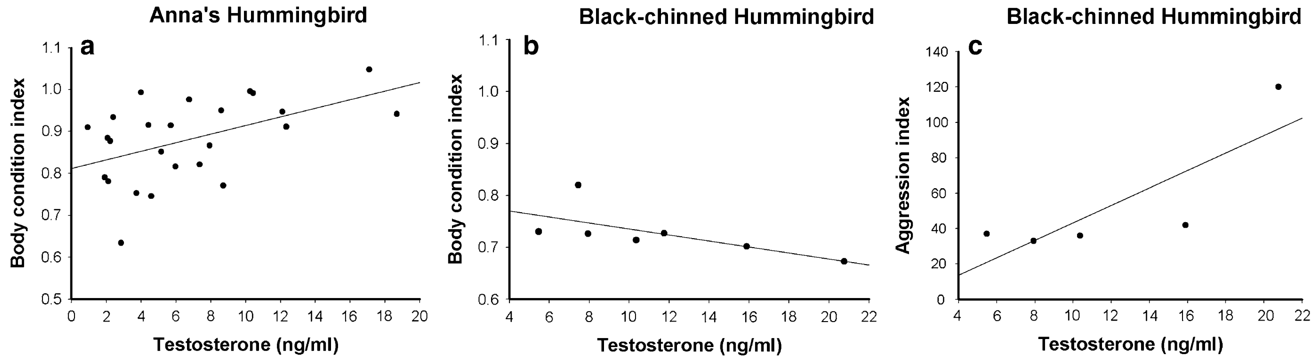

Fig. 3 Body condition index was positively related to testosterone in cloacal fluid (CF) in Anna's hummingbird (*C. anna)* during the reproductive season ($r^2 = 0.211$, $F_{(1,23)} = 7.435$, $P < 0.05$, **a**). Although the relation between T and aggression in Black-chinned (*A. alexandri*) was marginally positive during the reproductive season ($r^2 = 0.75$, $F_{(1,3)} = 8.13$, $P = 0.058$, **c**), we found that body condition tended to be lower in birds with higher T ($r^2 = 0.55$, $F_{(1,5)} = 6.1$, $P = 0.055$, **b**)

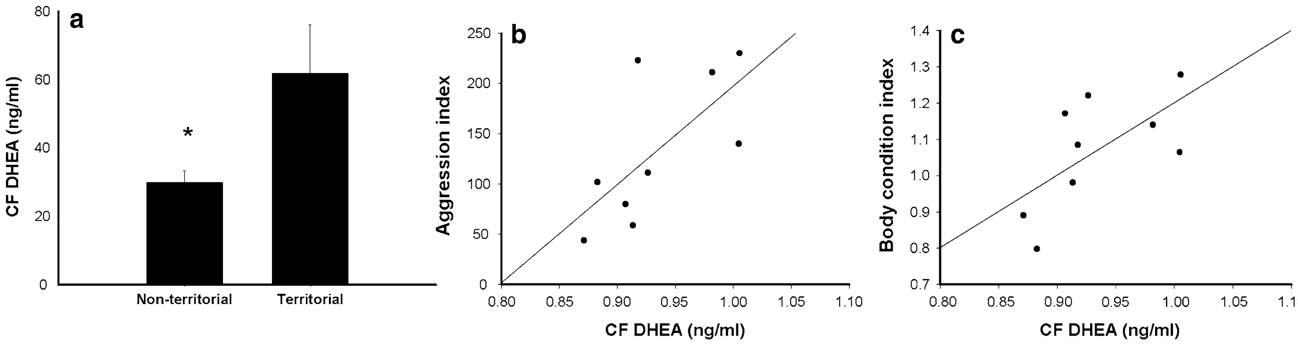

Fig. 4 In cloacal fluid (CF) obtained during the non-reproductive season from free-living *C. anna*, DHEA was significantly higher in territorial males than in non-territorial males (Mann–Whitney test $U = 2.00$, $P < 0.05$, $n = 9$, **a**). During the non-reproductive season, aggression was positively correlated with the DHEA levels in CF ($F_{(1,7)} = 6.29$, $P < 0.05$, $n = 9$, **b**). In addition, the body condition index was marginally positively related with the hormone level in *C. anna* ($F_{(1,7)} = 4.98$, $P = 0.06$, $n = 9$, **c**)

specific aggressiveness. Therefore, we were unable to relate territorial aggression levels with sex steroid levels; we did, however, observe that higher levels of T were positively related with body condition (Fig. 3a). This finding supports the idea that aggression levels may be related to resource acquisition and could provide an honest signal for sexual selection, either directly, in displays of physical attractiveness or motor performance, or indirectly, through the acquisition and defense of high quality food sources (McGlothlin et al. 2007; Byers et al. 2011).

When *A. alexandri* arrived at our field site in early April, they were consistently territorial and actively displaced *C. anna* from feeders. We found aggression levels in this species tended to be positively correlated with T (Fig. 3b). However, we observed a marginally negative relation between T and body condition (Fig. 3c). Possible explanations could be related to the lower capacity of *A. alexandri* for fat storage in comparison to bigger hummingbird species such as *C. anna* (Hainsworth 1978). In addition, *A. alexandri* males face higher energy expenditures than *C. anna* during the reproductive season. They perform a long distance migration to breeding grounds where they defend feeding resources at the same time that they perform expensive courtship displays. In middle August, during molt and before migration, *A. alexandri* showed T levels significantly lower in comparison to the reproductive season. This was expected at the end of the breeding season as it does in many other seasonal species (Wingfield et al. 1990, 2001). In addition, elevated levels of T could inhibit or delay molt (Schleussner et al. 1985).

Conversely, we found high variation in T levels in *S. rufus,* which could be at least partially explained by the location of our field site. Specifically, our field site is in the migration route of this species. Therefore, it is possible some of the birds could have been summer residents while others were likely to be migrants heading north. Further research is needed to assess the link between survival, androgen levels, reproductive success, and energy expenditure in long distance migrant hummingbirds.

Non-reproductive season

In *S. sephanoides,* plasma levels of DHEA were higher than T during the non-reproductive season (Fig. 1). However, during the (early) reproductive season, we found plasma levels of DHEA to be lower and levels of T highly variable. We found a consistent pattern in *C. anna* where levels of DHEA were significantly higher in territorial than in non-territorial individuals and positively correlated with aggression levels. In this species, body condition tended to be higher in males with higher DHEA (Fig. 4c), suggesting that more aggressive subjects were able to secure higher quality resources (Copenhaver and Ewald 1980; Camfield 2006). Our results could suggest that androgen precursors such as DHEA are linked to aggression levels during the non-reproductive season in year-round territorial hummingbird species; however new studies and manipulative experiments are needed to confirm this hypothesis.

In our field site, the aggression displayed by *C. anna* (measured with the aggression index) was five times lower than that previously reported for *S. sephanoides* during the same time of the year (González-Gómez et al. 2011a). These behaviors were displayed to maintain feeding territories during the non-reproductive season (Stiles 1971) and included chases, contacts, and vocalizations. Despite climatic conditions and habitat structure similarities in the areas inhabited by *C. anna* in central California and *S. sephanoides* in central Chile, *C. anna* maintained territories that were roughly five times bigger than typical *S. sephanoides* territories (Stiles 1971; González-Gómez and Vasquez 2006). This may offer an explanation at the ecological level for the observed differences in aggression levels during the non-reproductive season. Previous studies have shown that under similar resource conditions, the intensity of territory defense tends to be higher in smaller territories and where open vegetation structure easily allows territory owners to detect intruders (Norton et al. 1982).

Female aggression

Although we did not quantify aggression in females, we did observe intra-specific and inter-specific aggressive encounters among female *C. anna*, *A. alexandri*, and *S. rufus*. Even when females did not display territorial behaviors consistently, levels of T in CF of female *S. rufus* were similar to those in males of this species. Previous studies demonstrate that levels of T in females of sexually polygynous species are significantly lower than levels of T in conspecific males and lower than levels of T in females of monogamous species (Ketterson et al. 2005). Because hummingbirds are sexually polygynous, we expected T in males and females to show a similar pattern; the fact that they did not could be an effect of small sample size and/or large variation in T levels of males.

The fact that females show detectable levels of T during the reproductive season may be related to the role of T in ovulation and egg production. For example, during ovulation the thecal layer of the developing follicle produces T, which is converted to an estrogen by adjacent granulosa cells (Gomez et al. 2001). T also regulates the production of albumin by the oviduct (Yu and Marquardt 1973; Müller et al. 2002). In addition, T that is not converted to estradiol in the ovary can circulate and affect behavior (Brenowitz and Arnold 1993). Considering that female hummingbird species solely display parental care, it would be very difficult for females to hold a feeding territory during the reproductive season. However, aggressive behaviors against other hummingbird males or females could be advantageous in terms of improving food access (González-Gómez et al. 2011a). That could be especially significant in species such as *A. alexandri* and *S. rufus*, in which males are highly territorial and aggressive during this period, often forcing the females to poor and scattered resources (Kodric-Brown and Brown 1978). On the other hand, it has been argued that aggression in females could be related to circulating levels of estradiol (Pärn et al. 2008, but see Elekonich and Wingfield 2000). Our preliminary data show that at the end of the reproductive season, estradiol in females was 25 times higher than levels of T, but further sampling would be necessary to address the relationship between estradiol and aggressive behaviors in female hummingbirds.

Conclusion

High levels of steroids supporting territorial behaviors are likely to be adaptive in territorial animals. Our results suggest that the regulation of aggressive behavior in hummingbirds and, therefore, the ability to secure scarce resources, could be mediated by different hormones during the reproductive season and non-reproductive seasons in year-round territorial species such as *S. sephanoides* and *C. anna*. Manipulations of DHEA and T are needed to confirm the functional relationship between hormone levels, aggressive behaviors and body condition. Further research is needed to describe these relationships through the annual life cycle, especially in migratory species.

Acknowledgments This paper is dedicated to the memory of Loreto Godoy. We thank Baggins End Community, and Lee James of Tierra Verde Vegetables for allowing us to work in their properties. We thank Kara Sweeney and Holly Ernest for contacts and sampling help. This research was supported by CONICYT, Postdoc Becas Chile to PGG and the Endowed Professorship in Physiology to JCW, College of Biological Sciences, University of California, Davis and Grant Number IOS-0750540 from the National Science. Foundation to JCW.

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