Hormones and Behavior 58 (2010) 526–532

Contents lists available at ScienceDirect

Hormones and Behavior

journal homepage: www.elsevier.com/locate/yhbeh

# Short-term exposure to testosterone propionate leads to rapid bill color and dominance changes in zebra finches

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ARTICLE INFO

*Article history:*
Received 4 November 2009
Revised 7 April 2010
Accepted 8 April 2010
Available online 18 April 2010

*Keywords:*
Aggression
Bill color
Dominance
Signaling
Testosterone

ABSTRACT

Testosterone (T) can influence both male–male competition and mate choice displays. In zebra finches, female mate choice is based in part on bill color, and bill color has been shown to be enhanced by long-term testosterone supplementation. However, it is not clear whether bill color plays a role in male–male interactions and how bill color responds to shorter-term changes in T. We tested whether a single injection of testosterone propionate (TP) would influence male–male dominance interactions and lead to rapid (over a three-day period) changes in bill color. In addition, we tested whether bill color predicted aggression and dominance. We allowed birds in triads to establish hierarchies and then injected either dominant or subordinate individuals with TP, in addition to establishing sham control triads. We found that red chroma, but not hue, predicted aggressiveness of males. Exposure to TP led both dominant and subordinate birds to increase dominance scores over three days, longer than the <24 h period in which injected TP stays active. In addition, exposure to TP increased red chroma and hue in three days showing the dynamic nature of allocation of pigments to the bill. Our results suggest that zebra finches can modulate T and bill color levels over short time periods and these changes may occur through positive feedback between T-levels and dominance.



## Introduction

The evolution of colorful ornaments has long been a question of scientific interest (Darwin, 1859, 1871). It is generally accepted that colorful displays in vertebrates, especially those underlain by carotenoid pigments, have evolved in large part through sexual selection. In birds, female mate choice appears to provide the strongest selection pressure on colorful male ornaments (Andersson, 1994; Kokko et al., 2006). However, colorful ornaments can also evolve through male–male competition, particularly when color displays can reveal fighting ability or dominance status (Hunt et al., 2009; Senar, 2006). For example, aggressiveness is linked with carotenoid-based displays in red-collared widowbirds (Pryke and Andersson, 2003a; Pryke et al., 2001), Northern cardinals (Wolfenbarger, 1999), scarlet-tufted malachite sunbirds (Evans and Hatchwell, 1992), red-winged blackbirds (Hansen and Rohwer, 1986; Peek, 1972) and the dusky moorhen (Crowley and Magrath, 2004).

The zebra finch, *Taeniopygia guttata*, is a species with a colorful orange–red bill, underlain by carotenoid pigments (McGraw and Ardia, 2003; McGraw et al., 2003). However, the evolutionary forces driving bill color evolution in zebra finches are not resolved. Some studies have found a female preference for males with redder bills (Blount et al., 2003; Burley and Coopersmith, 1987; Collins and Ten Cate, 1996), while others have found no female preference (Forstmeier and Birkhead, 2004) or an indirect link through the correlation of color with male song (Balzer and Williams, 1998; Collins et al., 1994). Examination of the role of zebra finch bill color in male–male competition has been equally mixed. In indirect assessments, males showed no preference for bill color of other males, inferred to suggest that males of different manipulated bill colors were not viewed differently as rivals (Burley and Coopersmith, 1987; Etman et al., 2001). Cuthill et al. (1997) compared the influence of band color on dominance and found that red-banded birds were dominant over food sources, but did not differ in other aspects of dominance. In the only study to directly examine male–male interactions as a function of natural bill color, bill color did not explain variation in dominance interactions over 5-min trials (Bolund et al., 2006).

However, bill color may have evolved to signal dominance in a social context rather than only as a short-term assessment of a rival's fighting ability, as the role of an ornamental trait may be context-dependent (Chaine and Lyon, 2008). Thus, assessing the link between bill color and dominance over multiple interactions may provide additional insight into whether bill color is a reliable signal of aggression among males. In addition, while it is often assumed that male zebra finches do not form consistent dominance hierarchies

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doi:10.1016/j.yhbeh.2010.04.004

(Evans, 1970), this conclusion is based on only a single flock. Our own observations of male-only groups suggested the opposite, that males housed together appear to form stable hierarchies over a week-long time period. We thus first sought to test whether dominance interactions among males are consistent over this time period. Second, we tested for what factors predicted dominance and aggressiveness in males. In addition to bill color, we also examined the role of body size, as body size tends to reflect resource holding potential and is thus correlated with dominance in many species (Archer, 1988; Enquist and Leimar, 1987). Additionally, in short-term contests larger zebra finch males tended to be more aggressive over 5-min trials, suggesting a role for body size in social hierarchies (Bolund et al., 2006).

After testing for the role of bill color in driving dominance interactions, we sought to experimentally investigate one possible mechanism that may underlie hierarchies and bill color signals: testosterone (T). Testosterone is a steroid hormone that plays a role in both aggression and secondary sexual traits. In zebra finches, T is related to bill color (McGraw and Ardia, 2007; McGraw et al., 2006) and aggression (Adkins-Regan, 1999; Arnold, 1975). Because we were interested in the stability of interactions over a week-long time period, we chose to investigate the effect of short-term exposure to rather than use longer-term implants. We injected birds with levels of testosterone propionate (TP) to increase T-levels in subordinate birds to levels found in pilot data collected from dominant individuals. We used TP because its longer esters allow it to be active in the body longer than T alone (Deanesly and Parkes, 1937). We thus allowed triads of birds to establish hierarchies and then gave a single TP injection to either the dominant (rank 1) or subordinate (rank 3) bird in a triad. We predicted that a single injection of TP would lead to increased aggression. We also predicted that when rank 3 birds were injected with TP, established dominance interactions would be disrupted leading to changes in rank.

Lastly, we tested whether our manipulation would lead to short-term dynamic changes in bill color and T-levels. Studies examining changes in bill color in response to a manipulation, such as through immune system activation, have found marked rapid changes in bill color over three to four-week periods (Alonso-Alvarez et al., 2004; Faivre et al., 2003). However, recent studies in American goldfinches and blue-footed boobies have found that non-feather integument signals can change over even shorter time periods (Rosen and Tarvin, 2006; Velando et al., 2006). Thus, we tested whether TP injection induced bill color changes over a 3-day period and whether these changes were associated with change in T-levels and aggression. We predicted that TP-injected birds would produce redder bills. Lastly, we examined how our manipulation would affect T-levels in the blood. Even though injected TP is cleared in less than 6 h (Adkins-Regan and Ottinger, 1988), feedback between short-term increases in T and aggression may lead to an increase in T over longer time periods than the experimental injection is active. Thus, we tested for changes in T-levels three days after injection as a function of change in aggression and bill color. In summary, we tested for (1) the role of bill color in dominance interactions and (2) whether bill color and dominance were influenced by testosterone and (3) the role of T in driving short-term changes in signal strength.

## Methods

This work was conducted with the approval of the IACUC of Franklin and Marshall College. Male zebra finches were obtained from five commercial breeders and allowed to acclimate to a male-only colony in pairs in cages $45 \times 61 \times 61$ cm. The birds received millet seed mix, cuttlefish, grit, and water ad libitum. Birds were maintained at a constant temperature of 24.0 °C ± 1.0 and a relative humidity of 45–60% and a constant photoperiod of 12:12 light:dark.

Three subjects not previously housed together were randomly selected to be combined in a large cage measuring $55 \times 91 \times 51$ cm, which contained two food and two water dishes. We ran a total of 30 triads using 90 individuals. In six triads we assessed only the consistency of aggressive interactions; the next 24 triads were subjected to the injection treatment outlined below. The day of injection was considered day 0 and a one-hour observation (Observation 1) was conducted each day on days −2, −1, 0, 1, 2, and 3. Food was provided ad libitum on days −2, −1, 1, and 2 to a single food dish that was small enough to allow one bird to easily defend them against other individuals. On days 0 and 3, food dishes were removed approximately 4 h prior to observation to attempt to standardize hunger levels to reduce value asymmetry among subjects. We used the behavioral interactions from days 2 to 4 to assign birds to determine ranks. On day 0 following observation, we assigned each triad randomly to one of three treatment groups: (1) sham control, (2) rank 1 injected once with TP, and (3) rank 3 injected once with TP, each with a sample size of 8, for a total of 24 experimental triads. TP-injected males were injected intraperitoneally with 200 μg of testosterone propionate (Sigma Chemical, St. Louis, MO) dissolved in 100 μl of sesame oil. We chose 200 μg as the dose after pilot research revealed higher levels of aggression compared to 100 μg doses with no ill effects (Ardia unpublished data) and that 200 μg raised T-levels to those found in dominant birds (see Results). In the rank 1 treatment, the rank 1 bird was given an injection of TP, rank 2 and 3 birds received 100 μl of sesame oil only. In the rank 3 treatment, the rank 3 bird was given an injection of TP, rank 1 and 2 birds received 100 μl of sesame oil. In the sham control, all three birds received an injection of 100 μl of sesame oil.

During behavioral observations, we noted three categories of interactions (Zann, 1996): (1) bill fence — jabbing or pecking of the bill at the head region of an opponent. A series of fences was considered a single event, (2) displacement — when an individual is rapidly driven off his perch by another, and (3) chase — when an individual displaces and then follows the displaced individual. A displacement followed by a chase was considered a single event. For each hour-long observation, we noted the identities of aggressor and aggressed individuals. To avoid influencing interactions (Cuthill et al., 1997; Hunt et al., 1997), birds were banded with combinations of white, yellow, green, and black plastic color bands as there was no difference in aggression between color sequences (unpublished data). One observer (DMB) conducted 82% of observations; an additional observer was trained by DMB after conducting six observations together; values between the two observers were highly repeatable ($R > 0.95$).

### *Measurement of bill color*

We measured bill color and body mass (±0.01 g) of birds on day −4, day 0 (following observation) and day 3 (following observation). Bill color was measured as spectral reflectance using a USB2000+ spectrometer and PX-2 pulsed xenon light (Ocean Optics, Dunedin, FL) using a bi-furcated encased fiber optic probe. The probe was mounted and shielded from external light at a fixed distance of 5 mm perpendicular to the bill. Measurements were taken in duplicate on each side of the bill giving four total measures per individual; the average value was used. All measurements were the proportion of reflectance relative to white standard (WS-1, Ocean Optics). We used the programs CLR (1.05) and RCLR (0.09.28) (Montgomerie, 2008a,b) to analyze colors. Because of the range of possible variables that can be derived from spectral reflectance data (Montgomerie, 2006), we chose *a priori* to measure hue as the wavelength of peak reflectance >500 nm; UV hue as the wavelength of peak reflectance <500 nm; brightness as mean reflectance averaged along the wavelengths of 260 to 720 nm; and red chroma as percentage of total reflectance in the range of 650–720 nm (Montgomerie, 2006). Repeatabilities for all color measures were high

(all $P<0.001$; hue $R=0.533$, UV hue $R=0.612$, brightness $R=0.598$, red chroma $R=0.603$). We used values on day 0 to predict initial dominance status, and the difference between day 3 and day 0 as the change in reflectance as a function of the injection treatment.

### *Measurement of plasma T*

Blood was collected from birds on day 0 and 3 after observations. We collected 50–60 µl of blood with a heparinized micro-hematocrit capillary tube following brachial venopuncture. Blood was immediately stored at 4 °C and within 45 min plasma was removed following centrifuging for 10 min and stored at −80 °C for testosterone analyses. Plasma testosterone concentration levels were determined using an enzyme immunoassay (EIA) test kit (Cat. no. 07BC-1115; MP Biomedicals Diagnostics Division, Orangeburg, NY). Plasma testosterone levels for each bird were run in duplicates and the average was used. Cross reactivities are 0.89 and 0.86% for dihydrotestosterone and androstenedione, respectively (as reported by the manufacturer), and 1.65% for TP (Ardia, unpublished data). To calculate timing of clearance of TP in unmanipulated birds we used two sets of 12 additional male zebra finches that were housed alone in cages to minimize disturbance. For hourly levels of change in T, each bird was injected with 200 µg TP at time 0; six birds were bled at 0 and 6 h after injection, six birds at 3 and 12, all twelve birds were bled at 24 after injection. For shorter-term levels of change in T, each bird was injected at time 0, six birds were bled at 0, 15 and 60 min, while six birds were bled at 0, 30, and 90 min. A blood sample of 30–40 µl was taken at each time point.

### *Data analysis*

A bird was considered dominant to another when it initiated an a priori determined >60% of encounters; no pair-wise-interaction fell between 50 and 60%. We tested dominance statistically using the sign test by examining consistency in the direction of dominance relationships among individuals across the five-day period (Desrochers and Hannon, 1989; Sandell and Smith, 1991). Lastly to verify rankings, we compared the number of aggressive interactions initiated across the three dominance ranks using a general linear model.

We tested variables for normality, all variables except aggressiveness and bill brightness were normally distributed (Shapiro–Wilk's $W\geq0.98$, $P\geq0.23$). We log-transformed aggressiveness and brightness variables to obtain a normal distribution ($W\geq0.97$, $P\geq0.15$) for analyses, but present untransformed values in figures. We tested for factors predicting dominance rank or number of aggressive interactions initiated using a general linear model as a function of body mass, bill brightness, hue, and chroma, while also including triad identity as a random factor using a random slopes term. We examined changes in aggressive interactions, T-levels, or bill color, over a six-day period for aggression and a three-day period for T-levels or bill color, using a repeated measures GLM with the following fixed effects: time, injection treatment (5 levels: sham, rank 1-TP injection, rank 1-non-TP-injected, rank 3-TP injection, rank 3-non-TP-injected), and body mass, while also including triad identity as a random factor using a random slopes term. We tested for correlations among bill color measures to detect colinearity (Graham, 2003); variables were weakly correlated (hue–brightness $R=0.45$, hue–chroma $R=-0.49$, and chroma–brightness $-0.41$), so all three were kept in models. We used a Fisher's exact test to test for changes in dominance ranks following injection using an expected value of no change based on data from control triads. Change in T-levels in singleton birds was tested using a GLM. All means are presented as least square means controlling for other covariates.

## Results

### *Do male zebra finches show consistency in aggression?*

Differences in dominance stayed consistent over time (sign test $P<0.05$). Individuals classified as rank 1 individuals initiated 96.2% of encounters with rank 2 and 3 individuals (±4.1 SD; range 86.2–100%), while rank 2 individuals initiated 76.7% of encounters with rank 3 individuals (±9.8 SD; range 67.3–100%). There was individual consistency in aggressiveness, as initial aggressive scores on day −3 were correlated with scores on day 0 ($\beta=0.91$, $R^2=0.72$, $P<0.001$; $N=90$).

### *What predicts initial dominance position?*

Initial dominance rank prior to injection with TP was best predicted by red chroma ($F_{1,65}=4.4$, $P=0.04$), and weakly predicted by brightness ($F_{1,65}=2.9$, $P=0.09$), but not hue ($F_{1,65}=1.1$, $P=0.30$) or UV hue ($F_{1,65}=0.9$, $P=0.33$), or body mass ($F_{1,65}=1.8$, $P=0.19$). Consistent with this finding, birds with greater red chroma initiated a higher number of aggressive interactions ($\beta=0.45$, $F_{1,65}=5.2$, $P=0.02$; Fig. 1A). There was a weak negative effect of bill brightness ($\beta=-0.11$, $F_{1,65}=3.0$, $P=0.09$) and no effect of hue ($F_{1,65}=0.6$, $P=0.44$) or UV hue ($F_{1,65}=1.2$, $P=0.27$). Heavier birds had a trend towards being more aggressive ($\beta=0.12$, $F_{1,65}=3.1$, $P=0.08$). Initial T-levels were correlated with the number of interactions initiated ($\beta=0.39$; $F_{1,65}=7.8$, $P=0.007$; Fig. 1B), as well as weakly with body mass ($\beta=0.24$; $F_{1,65}=3.2$, $P=0.08$). In addition, initial T-levels were correlated with red chroma ($\beta=0.32$; $F_{1,65}=6.2$, $P=0.01$), but not hue, UV hue, or brightness ($Fs_{1,65}<1.4$, $Ps>0.24$).

### *How do birds respond to a single exposure to TP?*

There was no effect of time on aggressive interactions in control birds over a six-day period ($time_{5,11}=0.56$, $P=0.72$). Individuals injected with TP showed changes in dominance interactions over time, while uninjected birds did not (time $F_{5,61}=7.1$, $P=0.009$; treatment $F_{4,61}=11.3$, $P<0.001$; time*treatment $F_{4,61}=10.1$,

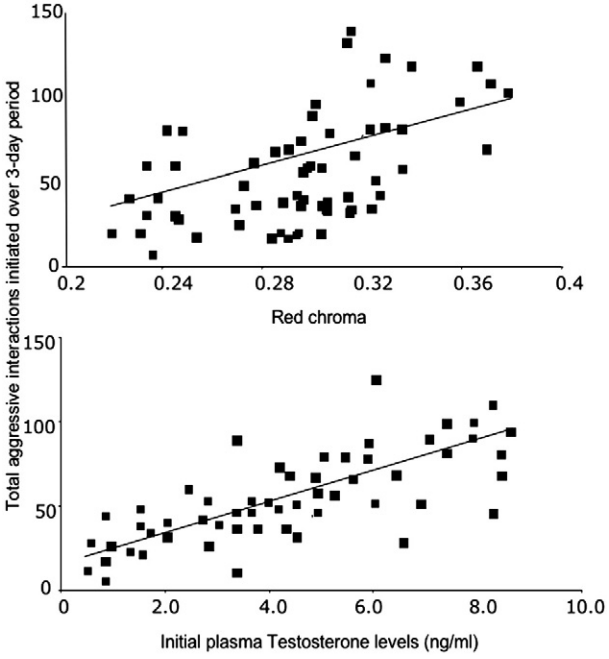


**Fig. 1.** Relationship between aggressive interactions over a 3-day period and red chroma (saturation between 650 and 700 nm) and initial plasma testosterone levels. See text for more detail on aggression scoring.

$P=0.001$; Fig. 2). There was no effect of body mass on changes in aggressive interactions over the 7-day period ($F_{1,61}=0.52$, $P=0.47$). These changes in aggressive interactions led rank 1 birds to maintain their dominance status, while rank 3 birds injected with TP increased to rank 2 in 7 of 8 triads (Fisher's exact test $P=0.01$).

Exposure to TP led to dynamic changes in bill color. TP-injected birds increased red chroma in both the dominant and subordinate treatments, while control birds maintained red chroma (Fig. 3A; effect of treatment $F_{4,60}=6.3$, $P=0.003$; body mass $F_{1,60}=0.7$, $P=0.40$; initial bill chroma $F_{1,60}=0.1$, $P=0.75$). Hue increased more in the rank 3 birds (Fig. 3B; treatment $F_{4,60}=7{,}2$, $P=0.001$; body mass $F_{1,60}=0.9$, $P=0.34$; initial bill hue $F_{1,60}=4.4$, $P=0.04$). In addition, TP-injected birds decreased brightness similarly, while uninjected birds did not (Fig. 3C; effect of treatment $F_{4,60}=9.3$, $P=0.001$; body mass $F_{1,60}=1.1$, $P=0.29$; initial brightness $F_{1,60}=1.5$, $P=0.22$). There was no change in UV hue ($F$s<1.2, $P$>0.27). Changes in red chroma were negatively correlated with changes in brightness; as birds increased red chroma they decreased in brightness ($\beta=-0.43$, $F_{1,65}=6.9$, $P=0.01$) and increased in hue ($\beta=0.18$, $F_{1,65}=4.6$, $P=0.04$). We found similar results when comparing absolute levels of bill color on day 3, as TP-injected birds had bills with greater red chroma than control-injected birds (red chroma ± SE, TP-injected 0.299 ± 0.01, $N=16$; control 0.291 ± 0.005, $N=48$; $F_{1,50}=13.2$, $P<0.01$), regardless of initial dominance rank ($F_{2,50}=1.2$, $P=0.30$). In addition, we found similar results when comparing change from day −4 to day 3 (change in red chroma ± SE, TP-injected 0.009 ± 0.0004, $N=16$; control −0.001 ± 0.0001, $N=48$; $F_{1,50}=11.0$, $P<0.01$).

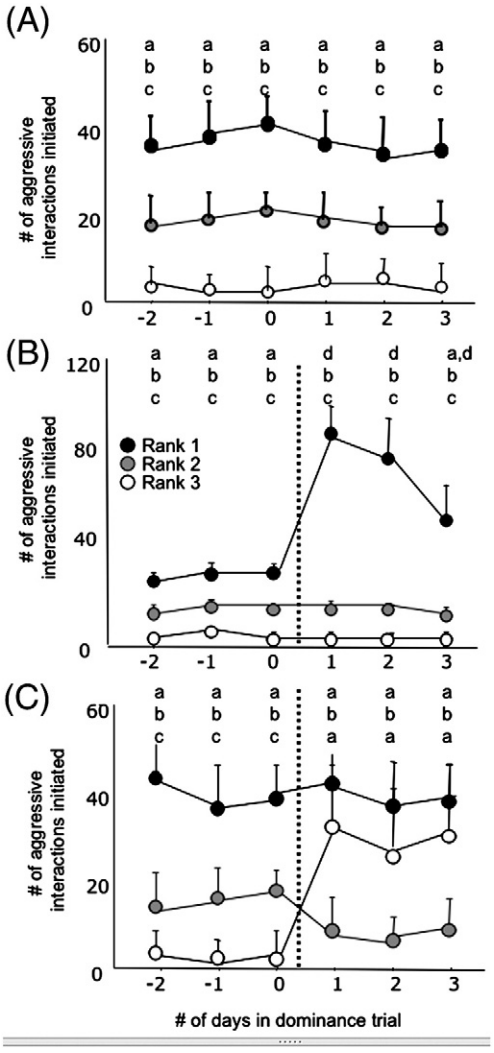


**Fig. 2.** Change (±SD) in the number of aggressive interactions initiated over a six-day period of zebra finches in (A) sham control, (B) rank 1 individuals injected with testosterone propionate and (C) rank 3 individuals injected with testosterone propionate. All non-testosterone individuals were injected with vehicle only. Injections were given after observations on day 0. See text for more detail on aggression scoring and sample sizes. Letters refer to significant differences among mean in rank order in column for each day.

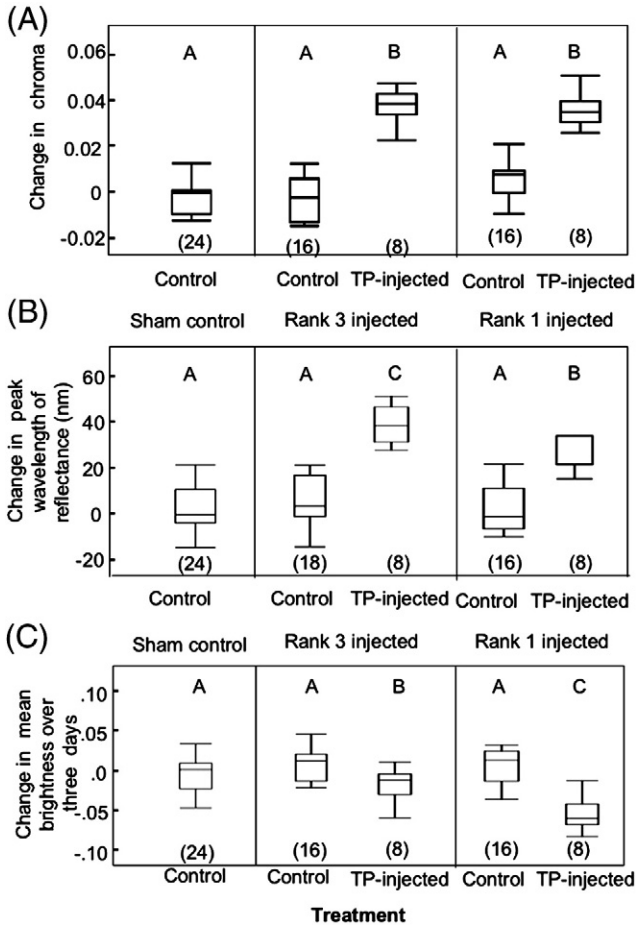


**Fig. 3.** Box plots of change in bill color over a three-day period following injection of testosterone propionate in either rank 1 or rank 3 individuals in triads of zebra finches. (A) Red chroma, (B) hue, and (C) mean brightness. All non-testosterone individuals were injected with vehicle only. See text for more detail on aggression scoring. Letters refer to significant differences among means. Sample size in parentheses.

In order to determine how a single injection of TP affected T-levels, we examined the time course of T in singleton unmanipulated birds not involved in this experiment. A single injection of TP increased blood T-levels within 30 min, with a decline but still elevated levels at 6 h, and no difference relative to initial levels by 12 h (Fig. 4). We compared T-levels in injection treatment birds three days after injection and found that both rank 1 and rank 3 birds injected with TP had increased blood T-levels, with rank 3 birds increasing at greater levels than rank 1 birds; control birds showed no change (Fig. 5; effect of treatment $F_{2,57}=9.8$, $P<0.001$; injection $F_{1,57}=53.1$, $P<0.001$; treatment*injection $F_{1,42}=18.0$, $P<0.001$). T-levels of singleton injected males at 1.5–3 h were similar to those of dominant males in control triads (plasma T-levels ng/ml ± SD: T-injected males 7.3 ± 1.2, $N=12$; dominant males in control triads 6.7 ± 2.1, $N=8$; $t_{18}=0.82$, $P=0.42$). Birds that showed higher levels of increased T were more aggressive (treatment: $F_{1,13}=1.7$, $P=0.20$; change in T: $\beta=0.65$; $F_{1,13}=8.2$, $P=0.02$), and had a greater increase in red chroma (treatment: $F_{1,13}=0.93$, $P=0.35$; change in T: $\beta=0.53$; $F_{1,13}=5.6$, $P=0.04$), but not hue, UV hue, or brightness ($F$s<1.3, $P$s>0.28).

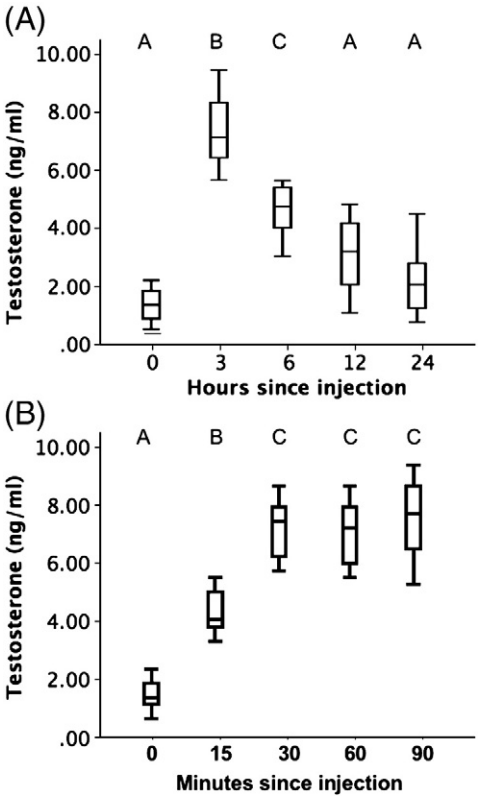


**Fig. 4.** Change in blood testosterone levels in singleton male zebra finches following injection with 200 µg of testosterone propionate. (A) Repeated measures GLM $F_{4,28}=61.4$, $P<0.001$, (B) $F_{4,28}=47.3$, $P<0.002$. ($N=6$ for all time periods except 24 h where $N=12$).

## Discussion

Our results support the hypothesis that bill color plays a role in male–male interactions in zebra finches as we found that the most aggressive and dominant birds had higher red chroma (i.e. more saturated bills in red wavelengths). In addition, we found that aggressive interactions and the strength of the bill color signal were modified over short time periods by the hormone testosterone (T). Birds with higher endogenous levels of T initiated more aggressive interactions and had greater red chroma in their bills. Furthermore, an experimental increase of T-levels led to increases in aggression, changes in dominance rank, and rapid changes in bill color. While we cannot demonstrate a direct effect because we did not directly manipulate bill color, our results indicate that bill color may be reflective of aggression and dominance abilities.

We found that triads of zebra finches formed stable hierarchies for week-long periods, in contrast to Evans (1970), whose conclusions are derived from a single group of finches. Thus, our results indicate that male–male interactions in small groups of zebra finches may be more stable than previously thought and therefore the potential for social dominance exists in this species. Without repeating our study with larger group sizes and under a range of environmental conditions, we cannot rule out that stable hierarchies only form under certain conditions. Our results suggest that the potential for dominance hierarchies exists in zebra finches, however the artificial nature of our triads suggests more research is needed to determine the range of social contexts under which male zebra finches maintain stable hierarchies. In addition, by housing males without females, access to food was likely the only defensible resource. Rank 1 males were the first to access the food dish in all trials, suggesting that dominance in this context provided greater food access.

In fact, the patterns reported here suggest that bill color may function only as a signal in multiple interactions among males, in contrast to the results reported in Bolund et al. (2006). In their study, using a large sample size, they found no effect of bill color on aggression in short pair-wise interactions. However, here we found that bill red chroma predicts aggression levels and dominance status in a triad over a week-long period. This difference between our study and Bolund et al. (2006) suggests that if bill color indeed serves to convey fighting ability it has evolved in a social context, rather than through a series of pair-wise interactions with individuals that had not been encountered previously. A signal mediates aggressive interactions by serving as a reliable predictor of rank and thus can minimize encounters that an individual is likely to lose. In some species, a single encounter is sufficient to judge a plumage-based signal, particularly when the *production* costs of an ornament, such as a melanin-based bib, are low (Lemel and Wallin, 1993; Senar and Camerino, 1998). But in other studies, a signal may reflect longer-term social dominance and may thus reflect a time-integrated measure of dominance that occurs through multiple interactions with rivals (Griggio et al., 2007; Pryke et al., 2001; Reudink et al., 2009). Our results suggest that bill color may serve this role in zebra finches. However, the fact that bill color does not predict aggression between novel males suggests that additional signals are involved in mediating dominance interactions in zebra finches. Thus bill color may either play a contributing causal role or be correlated with other status-reflecting traits (Senar, 2006). The clear next step is to manipulate bill color directly under a variety of contexts to examine the direct role of bill color as a social signal among males.

High levels of red chroma are produced by a greater proportion of reflectance in red wavelengths. The negative correlation between increasing chroma and decreased brightness (reflectivity) suggests that changes in blood carotenoid levels may underlay this change, as carotenoids are considered subtractive colorants that reduce reflectivity (Andersson and Prager, 2006). Carotenoid-based displays generally act as honest signals by indicating carotenoid deposition, as carotenoids are acquired only through diet and are subject to tradeoffs between health and display (Lozano, 1994; McGraw and Ardia, 2003). In this study, we found high levels of red chroma and low levels of bill brightness, but not hue or UV hue, were correlated with dominance and aggression. Our results support the growing evidence that carotenoid-based signals can signal dominance (Crowley and Magrath, 2004; Evans and Hatchwell, 1992; Hansen and Rohwer, 1986; Peek, 1972; Pryke and Andersson, 2003a,b; Pryke et al., 2002). Dominance was also correlated with body mass, but larger birds did not have redder bills. This supports the idea that multiple

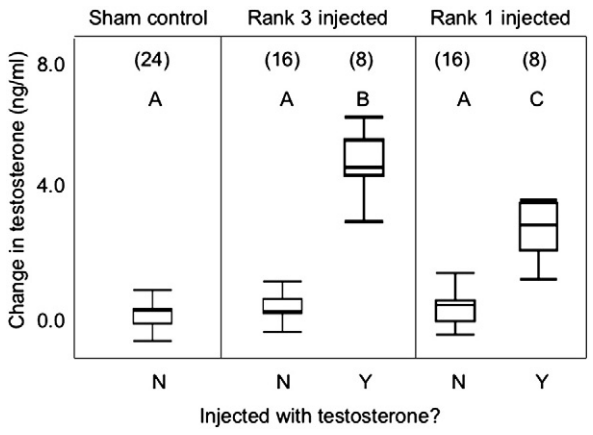


**Fig. 5.** Box plots of change in blood testosterone levels over a three-day period in zebra finches following injection of testosterone propionate. (A) Sham control, (B) rank 1 individual injected, and (C) rank 3 individual injected. Letters refer to significant differences among means. Sample size in parentheses.

traits influence resource holding potential in zebra finches, or that bill color is strongly correlated with other variables that affect dominance.

Dominance interactions in zebra finches were affected by T, which was to be expected, as previous studies have found a direct link in zebra finches (Adkins-Regan, 1999; Arnold, 1975). First, we found that aggressiveness was correlated with blood T-levels. In addition, our single experimental administration of TP led to an increase in aggression. In fact, rank 3 birds, which in control triads were consistently subordinate, increased aggression sufficiently to outrank previously rank 2 individuals in 7 of 8 triads. Increased aggression persisted for three days following injections, even though we found that a single injection of TP is active for less than 12 h. This increased aggression three days following injection could be due to a number of factors. First, cascading effects of changes in T-levels can occur that affect neuronal processes and other cellular level effects (Ball and Balthazart, 2008), leading to persisting effects of a single injection. A non-mutually exclusive alternative is that a positive feedback loop may have occurred between T and aggression (Soma, 2006; Wingfield, 1985; Wingfield et al., 1990; Wingfield and Wada, 1989), whereby injected birds raised their own production of T in response to feedback from their experimentally changed behavior. So, while our results are consistent with a winner effect (Oliveira et al., 2009), we cannot differentiate possible causes in our study.

The fact that rank 3 birds were able to increase T-levels and aggression and modify their position in the hierarchy raises the question of why are they initially subordinate. Testosterone has been proposed as a main driver of signal honesty through immunosuppression (Folstad and Karter, 1992), immunodistribution (Braude et al., 1999), or energetic tradeoffs (Buchanan et al., 2001). Thus, it is likely that rank 3 individuals suffer costs we did not measure and/or they will eventually suffer increased defeats and return to rank 3 status. Thus, a signal of dominance, in this case bill color, needs to be backed by long-term dominant behavior (Jarvi et al., 1987; Rohwer and Rohwer, 1978).

A particularly novel result we report relates to the dynamic changes observed in bill color in response to TP injection. Hue, chroma, and brightness of bills all changed over a 3-day interval. This result supports the handful of other studies that have found similar rapid changes. For example, goldfinch males show fading in bill color when deprived of carotenoids for 24 h (Rosen and Tarvin, 2006). In addition, immune activation causes rapid (7 days or less) bill or foot color changes, a result found in mallards (Peters et al., 2004) and blue-footed boobies (Velando et al., 2006). Interestingly, while TP-injected birds modified all three aspects of color (hue, brightness, and chroma), only changes in red chroma and brightness, the best predictors of aggression and dominance, were directly related to changes in blood T-levels. This suggests that males with rising T-levels may preferentially increase the most relevant social signal. Testosterone has been found to cause changes in blood carotenoid levels and color in zebra finches (McGraw and Ardia, 2007; McGraw et al., 2006) and red-legged partridge (Blas et al., 2005).

The dynamic nature of carotenoid-based non-feather signals, such as bills and legs, suggests they represent a complementary condition-based signal relative to more frequently considered feather color ornaments (Hill, 2006; Hill et al., 2009; Pomiankowski and Møller, 1993). Interactive effects of food and carotenoid supplementation affected yellow chroma of bills but not feather color in house finches (Hill et al., 2009). In ring-necked pheasants, the wattle has male–male interaction functions (Mateos and Carranza, 1997; Papeschi et al., 2003) and is underlain, in part, by carotenoids (Smith et al., 2007). Thus, bill color is both a more honest and a less honest signal than feather displays. More honest from the perspective of reflecting current conditions (Perez-Rodriguez, 2008), in contrast to feather-based displays, which reflect conditions at the time of feather creation. However, given the dynamic nature of bill color, it may be easier to cheat for short time periods by elevating bill color, as we appear to have induced in our rank 3 birds. Perhaps the dynamic nature of bill color makes it a less valuable signal of dominance status in novel interactions and thus helps explain why Bolund et al. (2006) found no predictive effect of bill color over short time periods, while we report a link between bill color and dominance over 7-day periods.

In summary, our results demonstrate that bill color is correlated with dominance and aggression in zebra finches and that changes in bill color are driven in part by T. In addition, we report the dynamic nature of bill color in this species and that these rapid changes are mirrored by changes in T-levels. Thus bill color in zebra finches likely falls within the armament–ornament model (Berglund et al., 1996; Wong and Candolin, 2005), whereby signals evolve through both male–male competition and female choice (Griggio et al., 2007; Hunt et al., 2009; Tarof et al., 2005).

## Acknowledgments

We thank Alyssa Ackerman Crawford, Brent Schneider, and Jacob Gantz for their assistance with data collection, Charles Heyser and Rachel Blaser for their technical assistance, and three anonymous reviewers and Elizabeth Adkins-Regan for their comments on earlier versions of this work. Brent Schneider developed initial testosterone assays. We thank Franklin and Marshall College for vivarium space and the Hackman Scholars fund for support for DB and MG.

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