

Sexually attractive phrases increase yolk androgens deposition in Canaries (*Serinus canaria*)

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Abstract

The androgen concentration in birds' eggs varies with laying order, breeding conditions, and mate attractiveness. In passerine birds, mate attractiveness depends upon song quality. The aim of our study was to evaluate the effect of one criterion used by females to assess male song quality that is to say the presence of sexually attractive phrases on yolk androgen deposition. Twenty-five female Canaries were assigned to three experimental groups; in the first group, the females were allowed to hear songs made up with attractive phrases; in the second group, they were allowed to hear songs made up with non-attractive phrases; and in the control group, the females could not hear any song. Our results show that females allowed to hear songs with attractive phrases deposit significantly higher amounts of androgens (mostly testosterone) in their eggs than females without acoustical stimulation. The females exposed to songs with non-attractive phrases had androgen amounts halfway between the two other groups. This suggests that when females are paired with mates able to sing attractive phrases they can allocate more androgens in their eggs during the pre-laying period.
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1. Introduction

Reproduction involves transferring genetic and non-genetic resources from parents to offspring. Parental investment in terms of breeding behavior and transmission of non-genetic resources is generally costly (Burley, 1988). As a consequence, depending on breeding conditions, females may modulate their maternal investment. In the differential allocation hypothesis, Burley (1988) postulated that females paired with attractive mates invest more in their young than females paired with non-attractive ones. Parental favoritism is another reason for differential investment. This favoritism can be different according to the sex of the parent (see Lessells, 2002 for

a review). In birds, hatching asynchrony is the most widespread way of parental favoritism and may lead to brood reduction. Parents initiate incubating before the last egg of the clutch is laid and therefore favor the earliest hatched chicks compared to the latest hatched ones, as their growth begins first (Evans, 1996). Another way to favor some chicks is to allocate differently non-genetic resources such as eggs' components.

Recent studies have pointed out that variations in the allocation of non-genetic resources influence the development and survival of the young. For instance, in birds, egg yolk contains androgens like testosterone (T), and Schwabl (1993) stated that yolk T is exclusively provided by the female. Moreover, T is present in all eggs, fertilized or not (Eising et al., 2003; Müller et al., 2002; Petrie et al., 2001; Schwabl, 1993). There is also growing evidence that T contained in eggs have different effects on the young. Domestic Canary chicks, *Serinus canaria*,

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born out of eggs containing high concentrations of T beg more often (Schwabl, 1996a); they are also more aggressive and more dominant later in their lives (Schwabl, 1993). Furthermore, T enhances postnatal growth in domesticated Canaries and Black-headed gulls, *Larus ridibundus* (Eising et al., 2001; Schwabl, 1996a), and more specifically enhances the hatching muscle growth, a muscle that is essential for hatching and begging (Lipar and Ketterson, 2000). Nevertheless, Sockman and Schwabl (2000) have shown that androgen injections (T and androstenedione) in American kestrel eggs, *Falco sparverius*, can delay hatching and reduce nestling growth as well as chick survival. However, the study of Eising et al. (2001) on the Black-headed gull seems to contradict those results. T may also have a negative effect on hatching probability as shown in a study on House sparrows, *Passer domesticus* (Mazuc et al., 2003).

Recent studies indicate that, depending on the species and on breeding conditions, females do not deposit the same amount of androgens in all their eggs, therefore causing intra-clutch and inter-clutch variations. Yolk T concentrations increase with laying order in different species, such as in domesticated Canaries (Schwabl, 1993, 1996b), Red-winged blackbirds, *Agelaius phoeniceus* (Lipar et al., 1999a), American kestrels (Sockman and Schwabl, 2000), Lesser black-backed gulls, *Larus fuscus* (Royle et al., 2001) or Black-headed gulls (Eising et al., 2001; Groothuis and Schwabl, 2002). In contrast, T concentrations decrease with laying order in species such as Cattle egrets, *Bubulcus ibis* (Schwabl et al., 1997) and Zebra finches, *Taeniopygia guttata* (Gil et al., 1999), and do not vary in Tree swallows, *Tachycineta bicolor* (Whittingham and Schwabl, 2002). Social conditions can also interfere with yolk androgens allocation. For example, nest site competition (Schwabl, 1997) and female aggressive interactions related to intrusion rate into the territory (Whittingham and Schwabl, 2002) lead to eggs with higher amounts of T. Furthermore, older females eventually lay eggs with more T than 1-year-old females (Pilz et al., 2003). Gil et al. (1999, 2004) have shown that maternal differential deposition of T in Zebra finch and domestic Canary eggs depends on mate attractiveness; they suggest that T deposition represents a way of differential allocation of maternal resources. However, a study testing the differential maternal allocation in House sparrows found no evidence that females mated with males showing larger badges, and supposed to be more attractive, allocated more T in their eggs (Mazuc et al., 2003).

In the last 30 years, male attractiveness has been extensively studied in domesticated Canaries. Several studies on this species have pointed out the fact that females choose their mates according to their singing capacity. Data from Kroodsma (1976) suggest that females do prefer songs with high variability, in opposition to monotonous songs. Several studies have shown that females also prefer males able to sing certain types of sexually attractive phrases,

named “A” phrases, which elicit high levels of copulation solicitation displays when played to females (Vallet and Kreutzer, 1995; Vallet et al., 1998). Those two criteria, song variability and presence of “A” phrases within the song can potentially influence female reproduction. Kroodsma (1976) has shown that songs with high variability enhance nest building and egg laying; in contrast, songs with “A” phrases do not (Leboucher et al., 1998).

In a previous study in our laboratory, Gil et al. (2004) aimed to test if female Canaries allocated different amounts of T in their eggs depending on song attractiveness; in this study, attractive songs were (i) longer (ii) more variable than non-attractive ones, and lastly, (iii) they contained “A” phrases whereas non-attractive ones did not. The authors showed that female Canaries deposit more T in their eggs when hearing attractive songs (Gil et al., 2004). However, this study did not address the question of which one of the three criteria tested provokes the rise in T. The present experiment is designed to study the effect of the presence of “A” phrases within the songs on yolk androgen deposition in common domesticated female Canaries. Three groups of females were placed in three different acoustic contexts: playback of songs with “A” phrases, songs without “A” phrases, and no song. With regard to duration and variability, all the songs were equivalent; we hypothesized that females would allocate more androgen in their eggs when allowed to hear songs containing “A” phrases than females allowed to hear songs without “A” phrases. Moreover, we presumed that females exposed to songs without “A” phrases would allocate more androgen in their eggs than acoustically isolated females, as song stimulation itself, with or without attractive phrases, stimulates reproduction (Bentley et al., 2000; Leboucher et al., 1998). We also analyzed female plasmatic androgen concentrations because some data suggested that it could be related to yolk androgen variation (Schwabl, 1996b).

2. Methods

2.1. Subjects and breeding conditions

The subjects of this study were 25 2-year-old common domesticated female Canaries, hatched and bred at our laboratory, which weighed 22.9 ± 0.8 g. Before the experiment, they were housed in single-sexed aviaries in a short daylight photoperiod (8L:16D). Four days before the beginning of the experiment, they were housed in individual cages ($38 \times 33 \times 26$ cm), and were randomly assigned to one of the three experimental groups. The first day of the experiment, cages were provided with nest bowl and females were supplied with 8 g of cotton string placed in a dispenser.

Birds were provided daily with seeds, fresh vegetables, and water.

The photoperiod was progressively modified from a short day (8L:16D) to 12L:12D, this photoperiod allows reproductive behavior (Schwabl, 1996b).

2.2. Experimental groups

According to their experimental group, females were housed in three similar experimental rooms acoustically isolated from each other. The females of the attractive song group (A group, $n=8$) could only hear attractive songs. The females of the non-attractive song group (NA group, $n=8$) could only hear non-attractive songs. Playbacks of the different songs types (A or NA) lasted 5 h per day, the females being acoustically isolated the rest of the time. The females of the control group (C group, $n=9$) did not hear any song; they were acoustically isolated from male songs all day long.

2.3. Experimental songs

All the songs used during this experiment were computer-edited songs. Twelve male Canaries were recorded from 1998 to 2000. Using these recordings with Avisoft-SASLab Pro software, we created from each male repertoire one attractive song and one non-attractive song, according to the syllable and phonology organization of the Canary (Güttinger et al., 1978). So, we obtained 12 attractive songs and 12 non-attractive songs.

The attractive songs were only composed of phrases known to elicit high levels of copulation solicitation displays (CSDs) when played to female Canaries (Vallet and Kreutzer, 1995). These phrases were constructed with the repetition of high frequency modulated notes at a high repetition rate (16 notes per second or more). Moreover, half of the phrases used consisted of 2-note syllables that elicited the most CSDs (Vallet et al., 1998). The non-attractive songs were made up out of phrases with low repetition rate (less than 12 notes per second) and low frequency modulated notes, these phrases were found to elicit low levels of CSDs (Vallet et al., 1998).

The two types of song consisted of the regular alternation of eight different phrases of the same type (A or NA) lasting 1.5 s each. For each male, one song bout was made from the repetition of the same song seven times spaced with a 6 s long silence. Thus the total duration of the song bout was 126 s. During the playback, we played the song bouts of the 12 males; each song bout was separated by 24 s of silence from another. The total duration of the playback lasted 30 min, and was repeated 10 times a day to make it last 5 h.

2.4. Egg collection

All eggs were collected the day they were laid in order to know the laying order and were substituted with dummy eggs. Those dummy eggs were plastic eggs look-

ing alike natural eggs, they were easily accepted by brooding females and none of them was rejected. They were weighed and immediately stored at -20°C . Before immunoassay, frozen eggs were thawed and the yolk and albumen were separated. The entire yolk was weighed and homogenized in 2 ml of distilled water by vortexing with the aid of glass beads.

2.5. Blood sample

Blood samples were collected from each female three times: (i) just before the beginning of the experiment, while females were still housed together on short day photoperiod (Short day sample), (ii) when the female began to build her nest (when she had put 4 g out of 8 g of nesting materials in her nest bowl: Nest sample), (iii) when she laid her first egg (Laying sample). One hundred microliters of blood sample was collected by wing venipuncture using a heparinized syringe. It is worth noting that this method and the volume collected did not induce long-term effect on body weight of small birds (Stangel, 1986), and did not affect the female's willingness to continue laying and brooding. Samples were immediately centrifuged and plasma was stored at -20°C until assayed for testosterone.

2.6. Testosterone assays

Yolk concentrations of testosterone were determined by radioimmunoassay at the CEBC (CNRS). T extraction consisted of adding 3 ml of diethyl ether to the entire yolk sample, vortexing for 1 min, and centrifuging for 5 min (4°C , $2000\times$ RPM). The ether phase was decanted after snap-freezing the tube in an alcohol bath at -30°C and evaporated. The dried extract was redissolved in 300 μl of phosphate buffer. Tritiated testosterone (1000 CPM) (Amersham-Pharmacia Biotech Europe, 91898-Orsay, France) was added to the samples for the calculation of extraction recovery. This extraction technique is the standard technique used for plasma samples (Mauget et al., 1994) and is commonly used for yolk samples (Gil et al., 2004; Mazuc et al., 2003); the recovery values for all the yolk samples were $>90\%$. The rest of the method follows standard RIA techniques. T was determined in duplicates. Duplicate aliquots of the extracts redissolved in 0.01 M phosphate-buffered saline (pH 7.4) containing 0.1% bovine albumin (PBS-BSA) were incubated overnight at 4°C with ca. 6000 cpm of [^3H]testosterone and a specific antibody. Specific T antibody was kindly provided by Dr. G. Picaper (Médecine Nucléaire, CHU, 45900—La Source, France). Bound and free fractions were separated by adsorption with dextran-coated charcoal and centrifuged and aliquots of the bound fractions were counted with a Packard 1600 liquid scintillator counter. Female plasma T levels were determined using the same RIA technique.

The minimal levels of T detected were 50 pg/ml and 15 pg/mg for plasma and yolk, respectively. Yolk T as well as plasma T were determined in several assays. For the yolk samples, the mean intraassay coefficient of variation was 8.0% and the mean interassay coefficient of variation was 15.1%. For the plasma samples, the mean intraassay coefficient of variation was 7.3% and the mean interassay coefficient of variation was 9.5%.

The specificity of the T antibody related to dihydrotestosterone (5 α -DHT) and Δ 4 androstenedione (Δ 4) cross-reaction was evaluated to 35.5 and 1.8%, respectively (30% bound). Specific radioimmunoassay in yolk and plasma for those three androgens showed that T is the major androgen in plasma (DHT/T < 5%, Δ 4/T < 5%) and that both T and DHT are major androgens in yolk (DHT/T = 42.7%, Δ 4/T = 5.2%). Likewise, in a previous experiment, carried out in our laboratory, the same assay was used (Gil et al., 2004); in this experiment, concentrations of T, DHT, and Δ 4 were assayed in a subsample of yolks. The results showed high correlations among the three androgens ($P < 0.001$ in each case). Moreover, the mean concentrations of the three androgens were 80.68, 43.71, and 10.03 pg/mg, respectively, for T, DHT, and Δ 4 (Gil et al., 2004). So, T appears to be the main androgen in the Canary egg yolk. However, as the T antibody cross-reacts with DHT (see above), the present data allow us to describe the T–DHT variation in Canaries yolk and T variation in plasma; consequently our results will be interpreted in terms of major androgen concentrations rather than T concentrations.

2.7. Statistical analysis

Parametrical statistics were used to analyze our data (Sokal and Rohlf, 1995). Analysis was computed using SigmaStat version 2.03 (SPSS, Chicago, IL). One-way ANOVAs were used to analyze the number of eggs laid, the egg mass/female body mass ratio, and the laying latency for the three groups taken independently. To compare egg mass, yolk, and plasma androgen concentrations depending on experimental conditions and laying order, we used two-way ANOVAs for repeated measures. A Pearson test was used to analyze the correlations between plasmatic and yolk androgen concentrations.

3. Results

3.1. Clutch size and laying latency (Table 1)

The statistical analysis revealed no effect of the different treatments on the number of eggs laid (one-way ANOVA, $F_{(2)} = 0.876$, $P = 0.431$). The average clutch size was 4.160 ± 0.149 , and only eight females laid five eggs (three C females, three NA females, and two A females).

Due to this result, only the data of the first four eggs were taken into account for further analyses (Table 1).

The statistical analysis also indicated that the treatments had no significant effect on laying latency, i.e., the time spent between the beginning of the experiment and the laying of the first egg (one-way ANOVA, $F_{(2)} = 0.107$, $P = 0.899$). Moreover, the egg mass/body mass ratio was constant (one-way ANOVA, $F_{(2)} = 1.275$, $P = 0.285$).

The data analysis revealed no significant effect of the treatments on egg mass. In contrast, there was a significant effect of laying order, whereas the interaction effect was not significant (two-way repeated measures ANOVA, group effect, $F_{(2)} = 1.362$, $P = 0.277$; egg laying order, $F_{(3)} = 3.335$, $P = 0.025$; interaction, $F_{(6)} = 1.443$, $P = 0.214$). The post hoc analysis revealed a significant difference between the mass of the first two eggs and the fourth egg, independently of the treatments (Tukey test, 1 vs 4, $P = 0.042$; 2 vs 4, $P = 0.037$), the last egg being heavier than the first two ones.

3.2. Yolk androgens (Fig. 1)

A significant effect of the treatment and a significant effect of laying order on the concentrations of androgens in egg yolk were found, but there was no significant interaction effect (two-way RM ANOVA, group effect, $F_{(2)} = 8.479$, $P = 0.002$; laying order effect, $F_{(3)} = 8.747$, $P < 0.001$; interaction, $F_{(6)} = 0.696$, $P = 0.654$). A post hoc comparison indicated that the eggs of the A group had a higher yolk androgen concentrations than the ones of the C group (Tukey test, $P = 0.002$). Otherwise, there was no significant difference between C females and NA females (Tukey test, $P = 0.098$) and no significant difference between A and NA females (Tukey test, $P = 0.237$). Concerning egg laying order, the post hoc analysis indicated a difference between androgen concentration in the first egg and in the three other eggs (Tukey test, 1 vs 2, $P = 0.027$; 1 vs 3, $P < 0.001$; 1 vs 4, $P < 0.001$), the first egg having a lower level of androgens than the three other ones (Fig. 1).

3.3. Female plasma androgen levels (Tables 2 and 3)

There was a significant difference between the three experimental groups and between the three samples but there was no significant interaction effect (two-way RM ANOVA, group effect, $F_{(2)} = 7.267$, $P = 0.003$; sampling order effect, $F_{(2)} = 6.537$, $P = 0.004$; interaction, $F_{(4)} = 1.531$, $P = 0.215$). The females of the A group had lower plasma androgen concentrations than the females of the NA and C groups (Tukey test, A vs C, $P = 0.007$, A vs NA, $P = 0.015$). Independently of the treatment, the plasma androgen concentration increased between the short days and the egg laying period (Tukey test, Short day sample vs Laying sample, $P = 0.004$). A separate analysis showed that the difference between the three experimental groups

Table 1
Female body mass, eggs' characteristics, and egg mass/body mass ratio depending on the experimental groups (means ± SE)

Group	Female body mass (g)	Clutch size	Egg mass (g)				Mean	Egg mass/body mass	Egg laying latency (days)
			First	Second	Third	Fourth			
A (n = 8)	22.163 ± 1.332	4.250 ± 0.250	1.450 ± 0.118	1.462 ± 0.127	1.450 ± 0.108	1.591 ± 0.0287	1.485 ± 0.0515	0.673 ± 0.00250	47.250 ± 7.208
NA (n = 8)	22.675 ± 1.639	3.875 ± 0.295	1.613 ± 0.151	1.635 ± 0.133	1.670 ± 0.136	1.762 ± 0.136	1.662 ± 0.0673	0.0742 ± 0.00414	49.125 ± 6.613
C (n = 9)	23.900 ± 1.576	4.333 ± 0.236	1.675 ± 0.0810	1.592 ± 0.0686	1.685 ± 0.0750	1.626 ± 0.0739	1.644 ± 0.0362	0.702 ± 0.00231	45.222 ± 4.275
ANOVAs' results	P = 0.708	P = 0.431	P = 0.380	P = 0.535	P = 0.245	P = 0.359	P = 0.277	P = 0.285	P = 0.899

Experimental groups: C, control females, non-stimulated; NA, females stimulated with non-attractive songs; and A, females stimulated with attractive songs.

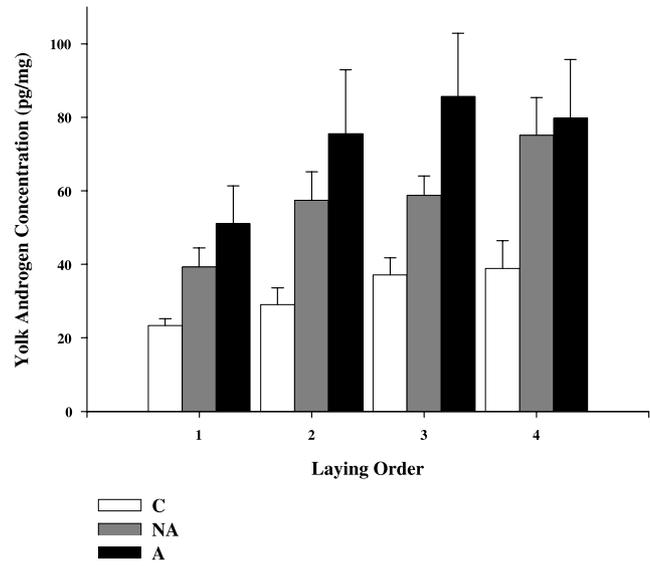


Fig. 1. Yolk androgen concentration (pg/mg) of the first four eggs depending on laying order and on experimental group (means ± SE). Experimental groups: C, control females, non-stimulated; NA, females stimulated with non-attractive songs; and A, females stimulated with attractive songs. For statistical analysis, see Section 3.

occurred even before the beginning of the experiment (Short days sample) while all the females were housed together in the same room and in the same conditions (one-way ANOVA, $F_{(2)} = 10.194$, $P < 0.001$, Tukey test for multiple comparison, A vs C, $P = 0.0032$, A vs NA, $P < 0.001$). The androgen concentrations are presented in Table 2. No significant correlation was found between any of the three plasma androgen concentrations (Short day sample, Nest sample, and Laying sample) and yolk androgen concentrations of the four eggs ($P > 0.05$ in each case). However, we should interpret those negative results cautiously due to the small size of some samples. The results of correlation analysis are presented in Table 3.

4. Discussion

In our experiment, the playback of attractive songs had no effect on the number of eggs laid and on the egg laying date. Those results are consistent with Leboucher and collaborators previous study on domesticated Canaries (Leboucher et al., 1998). However, attractive songs had surprisingly no effect on egg mass; we would have expected females hearing attractive songs to invest more in their eggs in terms of mass as it had already been shown in the Mallards, *Anas platyrinchos* (Cunningham and Russel, 2000). In this study, female Mallards paired with attractive males, i.e., the dominant males in this species, laid heavier eggs. Overall, those results agree with Mazuc et al.'s (2003) previous findings in House sparrows; in this study females paired with attractive males, i.e., the males with the larger badges, did not lay more eggs or eggs with heavier yolk.

Table 2

Androgen concentrations (ng/mL) of the three plasma samples (Short days sample, Nest sample, and Laying sample) depending on the experimental groups (means \pm SE)

Group	Plasma androgen concentrations (ng/mL)		
	Short day	Nest	Laying
A ($n = 8$)	0.271 \pm 0.0615	0.304 \pm 0.129	0.576 \pm 0.133
NA ($n = 8$)	0.705 \pm 0.0892	0.722 \pm 0.109	0.723 \pm 0.0702
C ($n = 9$)	0.528 \pm 0.0477	0.788 \pm 0.101	0.912 \pm 0.119

Experimental groups: C, control females, non-stimulated; NA, females stimulated with non-attractive songs; and A, females stimulated with attractive songs. For statistical analysis, see Section 3.

Table 3

Correlation analysis (Pearson correlation) between plasma androgen concentrations and egg yolk androgen concentrations depending on the three experimental groups

	Yolk samples Plasma samples	Egg 1			Egg 2			Egg 3			Egg 4		
		<i>R</i>	<i>P</i>	(<i>n</i>)									
C group	Short day	-0.463	0.209	(9)	-0.543	0.131	(9)	-0.234	0.544	(9)	-0.132	0.755	(8)
	Nest	-0.0810	0.879	(6)	-0.769	0.0737	(6)	-0.479	0.336	(6)	-0.530	0.358	(5)
	Laying	0.371	0.325	(9)	0.141	0.718	(9)	0.382	0.310	(9)	0.673	0.0674	(8)
NA group	Short day	0.0470	0.912	(8)	-0.255	0.543	(8)	-0.357	0.385	(8)	0.192	0.757	(5)
	Nest	-0.0169	0.979	(5)	-0.467	0.428	(5)	-0.513	0.377	(5)	0.996	0.0593	(3)
	Laying	-0.0496	0.907	(8)	0.253	0.545	(8)	0.102	0.810	(8)	0.459	0.437	(5)
A group	Short day	-0.0557	0.896	(8)	-0.228	0.587	(8)	-0.317	0.444	(8)	0.453	0.308	(7)
	Nest	0.545	0.342	(5)	0.739	0.154	(5)	0.730	0.161	(5)	0.216	0.784	(4)
	Laying	-0.649	0.115	(7)	-0.468	0.289	(7)	-0.443	0.320	(7)	-0.241	0.602	(7)

R, Coefficient of correlation, *P*, statistical probability; and (*n*), number of samples.

About androgen concentrations, i.e., chiefly T, in egg yolks, our results are consistent with previous studies on Canaries as the last egg laid contained more androgens than the first one (Gil et al., 2004; Schwabl, 1993). This pattern of T distribution within the eggs of a clutch is commonly observed in different species, as in Red-winged blackbirds (Lipar et al., 1999a), American kestrels (Sockman and Schwabl, 2000), Lesser black-backed gulls (Royle et al., 2001) or Black-headed gulls (Eising et al., 2001; Groothuis and Schwabl, 2002). It has been suggested that it could be a mechanism to mitigate the effect of hatching asynchrony (Eising et al., 2001; Schwabl, 1996b). The offspring with a greater amount of T (the latest hatched in that case) would have competitive advantages over the other as they would beg more food and they would grow faster (Schwabl, 1993, 1996a). Moreover, in our study, the last egg was significantly heavier than the two first ones; this could also be a way to mitigate the effect of hatching asynchrony. This mitigation should be an adaptive mechanism when reproductive conditions are good, allowing parents to breed a great number of chicks. In such favorable circumstances, brood reduction may appear to be unnecessary.

The main result of our study is that females deposit higher quantities of androgens when hearing songs with "A" phrases. Indeed, the amount of androgens deposited in eggs by females allowed to hear songs with "A" phrases (A group) is significantly higher than the amount of androgens deposited in the eggs laid by

females acoustically isolated (C group). Females exposed to songs without "A" phrases (NA group) had androgen amounts halfway between the two other groups. Moreover, androgen concentration in eggs tended to be higher in the A group than in the NA group, and higher in the NA group than in the C group, but those differences did not reach a significant level.

The study of Gil et al. (1999) pointed out that Zebra finch females paired with attractive males deposited greater amount of T in their eggs than did females paired with non-attractive males. In a further study, Gil et al. (2004) showed that female domesticated Canaries exposed to attractive songs deposited more T in their eggs than females either exposed to non-attractive songs or non-exposed to songs. In this previous study, attractive songs were longer and more variable than non-attractive ones, and they contained "A" phrases whereas non-attractive ones did not. Our present results are consistent with this previous study. Moreover, they go further in emphasizing the key-role of the "A" phrase within the attractive songs.

Petrie et al. (2001) have recently found that the amount of yolk maternal steroids differ according to the sex of the future offspring; male eggs having more androgens (androstenedione and T) than female ones in the Peafowl, *Pavo cristatus*. They also suggest that this differential investment of steroids can affect sex ratio by influencing sex-chromosome segregation at the first meiotic division. Recently, those results have been reconsid-

ered; Eising et al. (2003) and Gil et al. (2004) have pointed out that the steroid concentration measurement was done after 10 days of incubation, suggesting that the steroid concentration observed was not reflecting only the maternal allocation but also the own embryo steroid production. Moreover, in the same study on the White Leghorn chicken, *Gallus domesticus*, Eising et al. (2003) found no differences of maternal androgen allocation (androstenedione and T) relating to the sex of the embryo. Considering all these previous results and taking into account that our Canary eggs were not fertilized, our results in terms of androgen concentration only reflect the maternal investment and the influence of the embryonic development on androgen concentration can be discarded.

Regarding female plasma androgen concentrations, there are three major points. (i) The plasma androgen concentration increased between the short day period and the egg laying, this result is consistent with Schwabl's (1996b) previous results on fecal T; this increase was independent of the treatment. (ii) There was a significant difference in plasma androgen concentrations between the A group females and the females of the two other groups; females of the A group having the lowest androgenic plasma concentrations. Unexpectedly, this difference occurred even before the beginning of the experiment while all the females were housed together under a short photoperiod schedule. As a consequence, it seems that any effects of the treatments on this difference can be discarded. (iii) No correlation was found between androgen concentrations of any of the plasma samples and yolk samples. Our data suggest that plasmatic androgen concentration do not influence directly the androgens present within the yolk. As noted by Lipar et al. (1999b), results of previous studies indicated that steroid plasma concentration does not closely reflect follicular steroid concentration (Doi et al., 1980; Hammond et al., 1980). In the same study, Lipar and others have suggested that the primary influence on T yolk deposition is the follicle. They stated that "the partial separation of follicular and circulating plasma steroid levels could be a mechanism that has evolved to help control the amount of hormones that is delivered to eggs." Such a mechanism would explain the lack of relationship between plasma and yolk androgen concentrations. However, due to the small size of some samples we should interpret those negative results cautiously.

It is assumed that when females are paired with preferred mates, their chicks grow and survive better (Petrie, 1994). Therefore, when a female Canary allocates more androgens in its eggs when hearing high quality songs, it is expected that its whole clutch would undergo positive effects of T such as enhancing growth, aggressiveness, and begging (Eising et al., 2001; Lipar and Ketterson, 2000; Schwabl, 1993, 1996a). However, when injected into eggs, androgens (T and androstenedione) can

induce negative effects on the chick development while they delay hatching and reduce nestling growth as well as survival in American kestrels (Sockman and Schwabl, 2000); in contrast, androgens enhance chicks' growth in Black-headed gulls (Eising et al., 2001).

To conclude, when female Canaries hear songs with attractive phrases, they allocate more androgens in their eggs. In respect to the various effects of androgens on chicks, it can be considered as a benefit for the whole clutch, but further studies must be done in order to evaluate the effects of this yolk androgen variation on the clutch development.

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