

Social competition and plasma testosterone profile in domesticated canaries: An experimental test of the challenge hypothesis

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Received 15 July 2004; revised 8 November 2004; accepted 25 February 2005

Available online 5 May 2005

Abstract

The challenge hypothesis predicts that plasma testosterone (T) concentration is high when male–male competitions are high and decreases when males are engaged in paternal care. In monogamous species, T concentration increases at the beginning of the breeding period and decreases after egg laying. According to the challenge hypothesis, increasing competition should also lead to T increase. The aim of our study was to test this hypothesis. In a first experiment, we measured the T profile of domesticated canaries housed with their mate in separated cages without competition. In a second one, we created a competition by housing male and female domestic canaries together (in an aviary) and emphasized this competition by limiting food access. We also studied social status effect. Our results showed no effect of social status in both sexes and no differences in female's T concentration. Concerning males, we obtained a clear monogamous T profile from the ones housed in a low competition situation and a polygamous profile from the others housed in high competition situation. Thus, our results support the hypothesis of the plasticity of the mechanisms controlling T concentration according to environmental conditions.

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Keywords: Domesticated canaries; *Serinus canaria*; Testosterone; Competition; Reproduction; Social status; Challenge hypothesis

Introduction

Sexual hormones regulate behavioral transitions during the reproductive cycle (Silver, 1990) and tend to coordinate behaviors with internal and external events. In male birds, plasma testosterone (T) is involved in sexual behavior (Logan and Wingfield, 1995), in song development (Nottebohm et al., 1987), and in male–male competition (Johnsen, 1998). The challenge hypothesis (Wingfield et al., 1990) predicts that T concentration will fluctuate in regards to competition and parental care. It also predicts differences in T profile according to the reproductive system (Wingfield et al., 1990). These predictions seem to be supported in many species (Beletsky et al., 1992; Vleck and Brown, 1999) but

contradictory results are presented by (Moore et al., 2004). In monogamous species, T levels increase at the beginning of the breeding season and decrease to a breeding baseline concentration after egg laying and hatching (Logan and Wingfield, 1995; Vleck and Brown, 1999). Low breeding concentrations may be adaptive because it has been suggested that T may have negative effects on parental care in monogamous species (Ketterson et al., 2001; Silverin, 1990). In contrast, in polygamous species T levels also increase at the beginning of breeding season but do not decrease during female incubation. Polygamous males try to attract a second mate during the same reproductive cycle and have to compete between each other for a new territory and according to the challenge hypothesis, this competition may be responsible for the T stability.

Males with implanted T are more aggressive than control ones (northern mockingbirds *Minus polyglottos*; Logan,

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1992) and tend to become dominant (white-throated sparrow *Zonotrichia albicollis*; Archawaranon et al., 1991). Thus, T can be related to male aggressiveness (for a review, see Wingfield and Ramenofsky, 1985). Johnsen and Zuk (1995) also suggest that in the red jungle fowl *Gallus gallus*, T increases when winning and decreases when losing an interaction. In dunnocks *Prunella modularis*, Langmore et al., 2002 show that such relationship between T and aggressiveness also exists in females. Besides, dominant males and females often win the majority of interactions (Piper, 1997) and a relation between T levels and social status is expected. Indeed, Peters et al. (2001) show that high status superb fairy-wrens *Malurus cyaneus* have greater levels of T than subordinates. However, contradictory results also exist (Wingfield and Ramenofsky, 1985).

Canary and related species are monogamous, although in the serin (*Serinus serinus*) extrapair attempts are frequent (Gama Mota and Hoi-Leitner, 2003). However, no extrapair fertilization (EPF) is revealed neither in the serin nor in the canary (Gama Mota and Hoi-Leitner, 2003; Voigt et al., 2003). To date, in these species, literature reports T effects on the central nervous system (Appeltants et al., 2003; Sartor et al., 2005) but only few studies deal with the effect of environment on T concentrations (Leitner et al., 2003).

In two separated experiments, we aimed to test one of the predictions of the challenge hypothesis (Wingfield et al., 1990): T concentrations will increase with increasing competition. In the first experiment, we studied the T profile of domestic male canaries (*Serinus canaria*) caged with a mate, thus monogamous, in a not competitive environment. Then, in a second experiment, we studied T concentration of individuals housed in an aviary within an experimental competition context (daily competition for mate and food access). Since male–male competition may also be related to social status, we tested whether dominant and subordinate birds present different T concentration profiles.

Materials and methods

In experiment 1, we studied the T concentration of males housed in cages with their mate. In experiment 2, we studied the T concentration of males and females housed in an aviary. In this second experiment, we also studied the relationship between dominance status and T levels in both males and females.

Experiment 1: Low competition situation

Subject and housing

We used 18 male and 18 female domestic canaries. These birds, raised in our laboratory, were 2–3 years old. Males

and females had previous reproductive experience but did not mate with one another before this experiment. Males and females stayed at least 3 months in single-sexed aviaries on a short daylight schedule (8:16-h light/dark) before the beginning of this experiment. Females and males were brought into reproductive conditions by lengthening the photoperiod (16:8-h light/dark). Males and females were randomly paired and housed in individual cages (38 × 33 × 26 cm), fitted with perches, a nest bowl and nesting material.

Blood samples and testosterone assays were conducted on males following the method described below (see the Blood samples for experiments 1 and 2 and the Testosterone assays for experiments 1 and 2 sections).

Experiment 2: High competition situation

Subject and housing

We used 27 male and 24 female domestic canaries randomly chosen from our laboratory's aviaries. Birds were color banded for individual recognition and then housed in aviaries (320 × 140 × 300 cm). All along the experiment, perch, water, and baths were provided. Food was given ad libitum aside from the food deprivation period.

Experimental procedure

There were initially 3 groups of 9 males and 3 group of 8 females housed in single-sexed groups during the short day period of 1 month (SD: 8:16-h light/dark). From each group of 9 males, a group of 6 males was generated by removing the 3 middle ranked birds. Groups of 6 females were generated in the same way. A remaining group of 6 males (3 dominants and 3 subordinates) was introduced to a group of 6 females during the short day mixed period (SDMi). After 1 month, birds were switched to long days (16:8-h light/dark). This reproductive period lasted for 3 month and the birds were provided with nest construction materials (cotton string) and nest bowls. Courtship feeding of males, copulation solicitation displays of females, copulations, and helping behavior during reproduction were observed and allowed us to identify pairs. During these periods, dominance tests were conducted according to the method described in the following section.

Dominance tests

Social status was determined using a method of competition for access to a food source (following the definition given by Drews, 1993). These tests were conducted in the aviary. After a food deprivation period of 50 min, birds were tested using “extra-food” (fruits or vegetables). Food deprivation had no adverse effects on the canaries' health or weight (Parisot et al., 2004a). All of the experiments were

done at least 1 h before the lights went out, so that all birds could eat at satiety before dark. Each test lasted 15 min and was repeated three times per day.

We recorded 4 different items: the number of victorious interactions, the rank of access, the time spent feeding, and the number of accesses to food. For each item, birds were classified from rank N to 1 where N represents the total number of birds. For example for the rank of access, if bird A comes to the extra-food in the first position in 6 tests out of 10, bird B in only 2, and bird C in only 1, then A will be given the rank N , B the rank $N-1$, C the rank $N-2$, etc. Then, for each bird, we summed ranks obtained for each item. This sum is called the “dominance score”. For example, if bird A obtained the rank N for each item, its dominance score will be $4N$ which is the highest possible score. Dominant individuals are often described as the ones obtaining more access to food and winning most interactions over food access (Drews, 1993). Following this description, we considered as most dominant the one that obtained the highest dominance score. The mean number of victorious interactions per period was used as an index of male–male competition.

Dominance tests were conducted during all periods (SD, SDMi, and reproductive period) and after each period a hierarchy was established for each group: each bird was given an individual rank from N (dominant) to 1 (subordinate). Furthermore, during the reproductive period 4 intermediate mixed hierarchies (males and females together) were established every 30 tests.

Blood samples and testosterone assays were conducted following the method described below.

Blood samples for experiments 1 and 2

Blood samples (approximately 0.1 mL) were collected by venipuncture from the wing vein using a heparinized

syringe and immediately centrifuged. As described by Stangel (1986) this method and the volume collected did not affect small bird weight's and their willingness to reproduce. Plasma was stored at -20°C until assayed for T.

During the first experiment, 5 blood samples were collected from males at different periods, and during the second experiment 9 blood samples were collected from all individuals (males and females) at different periods according to the schedule presented in Fig. 1.

Testosterone assays for experiments 1 and 2

Plasma concentrations of T were determined by radioimmunoassays at the CEBC (CNRS). T extraction consisted of adding 3 mL of diethyl ether to 50 μL of the blood sample, then vortexing for 1 min and centrifuging for 5 min (4°C , 2000 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 T (1000 CPM) (Amersham Pharmacia Biotech Europe, 91898-Orsay, France) was added to the samples for the calculation of extraction recovery. This extraction technique is a standard method used for plasma samples at the CEBC (Mauget et al., 1994). The rest of the method follows standard RIA techniques. T was determined by using duplicates in two separate assays (one for each experiment). Duplicate aliquots of the extracts, redissolved in 0.01 phosphate-buffered saline (pH 7.4) containing 0.1% bovine albumin (PBS-PSA), were incubated overnight at 4°C with approximately 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 absorption with Dextran-coated Charcoal and centrifuged. Aliquots of the bound fractions were counted with Packard 1600 liquid scintillator counter. The minimal

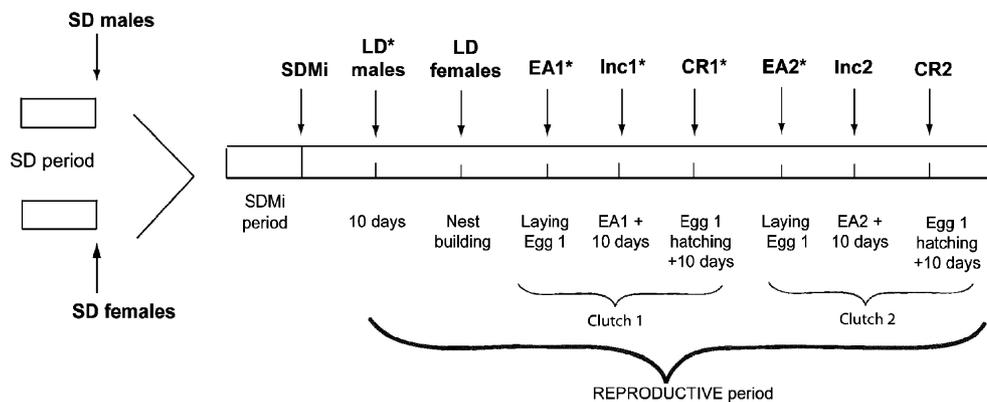


Fig. 1. Chronological representation of blood sampling. Arrowheads indicate the different blood samples (in bold): SD males and SD females: end of the SD period (when males and females were separated), SDMi: end of the SDMi period (males and females were sampled at the same moment), LD male: 10 days after photoperiod modification, LD females: at the beginning of nest-building, EA1: for pairs when the first egg of the first clutch was laid, Inc 1: 10 days after egg 1 was laid, CR1: 10 days after egg 1 hatched, EA2: when the first egg of their second clutch was laid, Inc 2: 10 days after EA2, and CR2: 10 after egg 1 of the second clutch hatched. In experiment 1, 5 blood samples were performed (indicated by *), and 9 blood samples were performed in experiment 2 (in bold).

levels of T detected were 50 pg/mL. The mean intra-assay and inter-assay coefficient of variation were 8% and 9.5%, respectively.

Statistical analysis

We used non-parametric statistics to analyze data related to hierarchy (Siegel and Castellan, 1988) and parametric statistics to analyze hormonal and other behavioral data (Sokal and Rohlf, 1995). Data were log-transformed when necessary so that data with different variances could be compared parametrically. Dominance stability was tested using Spearman correlations to compare each individual rank within each group during the three different periods (SD, SDMi, and reproductive period) and to compare the 4 intermediate mixed hierarchies obtained during the reproductive period. For experiment 1, variations of T level between the 5 blood samples were analyzed using one-way ANOVA for repeated measures, using Tukey tests for post hoc comparison. For experiment 2, variations between the T levels of the 9 samples were analyzed using two-way ANOVA for repeated measures using status as grouping factor and sample period as repeated factor. Post hoc comparison were analyzed using Tukey tests. All analyses were done using Sigmastat 2.03 for Windows (1992–1997 SPSS Inc). During experiment 2, one subordinate male remained unpaired during the breeding period so its data were removed from hormonal analyses.

Results

Experiment 1

Testosterone concentration

Statistical analyses revealed a significant difference between male LD samples and the other samples (one-way ANOVA for repeated measures, $F_{4,18} = 6.78$, $P < 0.001$, Tukey tests, in all case $P < 0.05$; see Fig. 2).

Experiment 2

Dominance

Hierarchies remained relatively stable during SD, SDMi, and the reproductive period. Statistical analyses revealed a positive correlation between the 3 hierarchies (SD-SDMi, $r = 0.82$, $P < 0.001$; SD-reproductive season, $r = 0.70$, $P < 0.001$; SDMi-reproductive season, $r = 0.71$, $P < 0.001$, $N = 36$). Within the reproductive period, intermediate mixed-hierarchies were correlated (see Table 1).

Male–male competition

The statistical analyses revealed that male–male competition did not remain stable during the experiment (one-

way ANOVA for repeated measures, $F_{8,17} = 3.10$, $P = 0.003$; see Fig. 2. for the profile). Post hoc comparison revealed significant differences between SDMi and Inc 1 period (Tukey test, $P = 0.03$) and between SDMi and EA2 (Tukey test, $P = 0.003$) and between EA2 and CR2 (Tukey test, $P = 0.018$).

Breeding behavior

We considered that a male and a female were paired when the male fed the female, copulated with her, and fed her young. Birds were paired with 0–3 mates (see Table 2 for details). Among 18 established pairs, 12 of them were composed of males and females sharing the same status: 7 were dominant pairs and 5 were subordinate pairs.

Testosterone concentration

Male testosterone concentrations. Statistical analyses revealed no significant difference between high and low status birds but did show a significant effect of period (two-way ANOVA for repeated measures, $F_{8,17} = 5.46$, $P < 0.001$, see Fig. 3a). There was no significant interaction between the two factors. Post hoc comparisons revealed significant differences between SD samples and all reproductive samples (in all cases Tukey test, $P < 0.05$) except for CR2 (Tukey test, $P = 0.38$). SDMi sample was not significantly different from reproductive samples (in all cases Tukey test, $P > 0.05$) except for EA2 (Tukey test, $P = 0.011$) and Inc 2 (Tukey test, $P = 0.003$).

Female testosterone concentrations (see Fig. 3b). Statistical analyses revealed no significant difference between high and low status females, no significant effect of period, and no significant interactions between these two factors.

Discussion

The aim of our experiment was to study the T profile of domestic canaries experiencing two different levels of competition (Wingfield et al., 1990). We also tested whether dominants and subordinates show different T concentrations. Our results revealed no difference between dominant and subordinate T levels whatever the period or the sex although hierarchy remained relatively stable throughout the experiment. Dominant birds are generally more aggressive as they win more interactions (Drews, 1993; Green, 1983). Some studies show a positive correlation between aggressiveness and T levels (in the Red-Legged partridge *Alecto rufa* Green, 1983, in the spotted antbird Hau et al., 2000, in the northern mockingbirds Logan, 1992; Wingfield et al., 1987). Nevertheless, contradictory results also exist in males and in females (dark-eyed junco *Junco hyemalis* Cristol et al., 1990, in the males barn swallow *Hirundo rustica* Saino and Møller, 1995; Wingfield and Ramenofsky, 1985). In the white-throated sparrow, Schlinger (1987) found no correlation

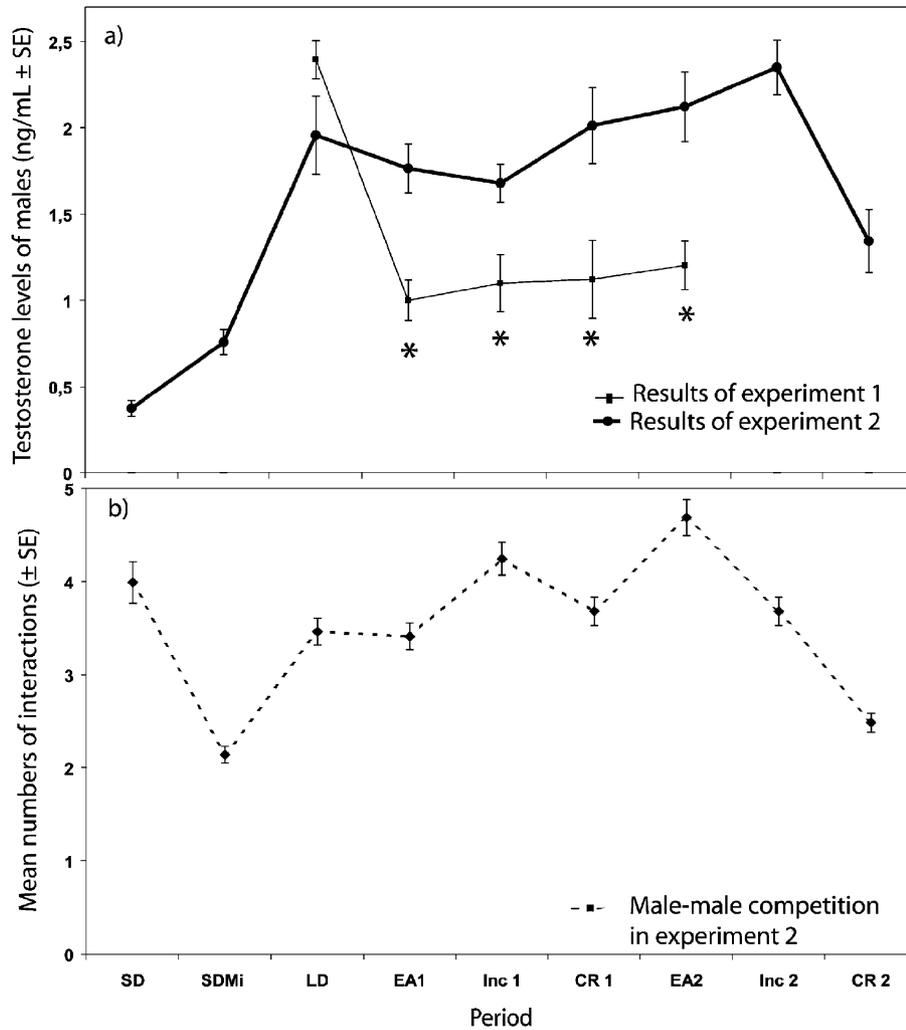


Fig. 2. (a) Plasma T concentration: plasma testosterone concentration of male domesticated canaries housed in cage with a female (experiment 1: thin line, $N = 18$, *indicates a significant difference with LD sample, one-way ANOVA for repeated measures) and plasma T concentration of males housed in an aviary with daily competition (experiment 2 bold line, $N = 18$, detailed data are presented in Fig. 3). (b) Male–male competition during experiment 2, measured as mean number of victorious interactions per period (dashed line, mean number of interactions for each period, \pm SE).

between social hierarchy and T levels but did find a positive correlation between aggressive displays and T levels. Indeed, aggressiveness is implicated in hierarchy but it is not the only behavioral tendency involved (Chalmers, 1979; Drews, 1993). In house sparrows *Passer domesticus*, social status of males is related to signals that are T dependent (Buchanan et al., 2001; Gonzales et al., 2001). For instance, in badge signaling system, birds with the largest badge are more often

dominant (in the white-crowned sparrows, *Zonotrichia leucophrys* Keys and Rothstein, 1991, and in the siskin, *Carudelis spinus* Senar and Camerino, 1998). Taken together, these results suggest that T is involved in some components of social status (aggressiveness or badge) but that social status does not depend exclusively on T plasmatic levels of birds.

Table 1
Results of Spearman correlations between the 4 intermediate mixed hierarchies established for each group of 12 individuals every 30 tests during the reproductive period ($N = 36$)

	Period 2	Period 3	Period 4
Period 1	$r = 0.62, P < 0.001$	$r = 0.60, P < 0.001$	$r = 0.38, P = 0.02$
Period 2		$r = 0.49, P = 0.002$	$r = 0.63, P < 0.001$
Period 3			$r = 0.46, P = 0.005$

Note. Period 1: tests 1–30; period 2: tests 30–60; period 3: tests 60–90; and period 4: tests 90–120.

Table 2
Pairs observed during the experiment

Females	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R
Males	5	2	1	6	11	7	12	9					13	15	14	16	16	17
		3						8										18
								10										

Note. Letters indicate females and numbers indicate males, bold characters indicate dominants and thin characters indicate subordinates. Three females were paired with 2 or 3 males (C, H, R), 1 male was paired to 2 females (16), 4 females remained unpaired (B, F, K, L), and 1 male remained unpaired (4).

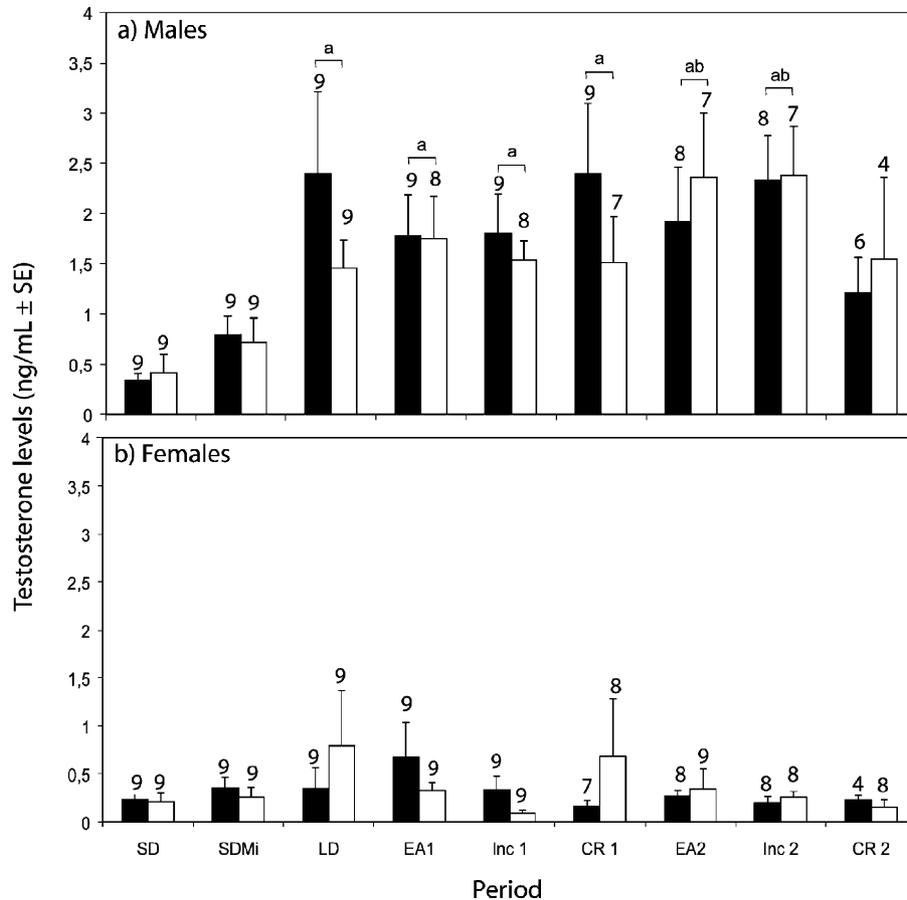


Fig. 3. (a) Males' plasma T concentration: dominants (black bar) and subordinates (white bar) for the 9 different blood samples (ng/mL \pm SE), "a" indicates significant difference with SD sample, "b" indicates significant difference with SDMi sample, two-way ANOVA for repeated measures. (b) Females' plasma T concentration: dominants (black bars) and subordinates (white bars). Two-way ANOVA for repeated measures. Periods are as follows: SD (short day); SDMi (short day in mixed group); LD: long day; for clutch 1: EA1 (Egg A1); Inc 1 (Incubation 1) and CR1 (chick rearing 1); for clutch 2: EA2 (Egg A2); Inc2 (Incubation 2) and CR 2 (chick rearing 2).

The challenge hypothesis predicts that the pattern of T concentration varies in regard to competition and parental care. In monogamous species, T increases at the beginning of the reproductive period, when competition is high and decreases when birds are engaged in parental care. In this study, we tested one prediction of the challenge hypothesis (Wingfield et al., 1990): T increases with increasing competition. It has been shown that this hypothesis can also be applied to females (Langmore et al., 2002). However, in our experiment, we do not observe any differences between T levels of our different samples from females whose T concentration remained constant at an average level which was lower than the males'. These T levels of females may be adaptive to assume their reproduction. T is known to have negative effects on female reproductive activities, for instance, females implanted with T may show non-functional ovaries (Shoemaker, 1939; Staub and De Beer, 1997). In males, data from the first experiment indicate that T profile of males caged with their mate within a low competition environment show a clear monogamous T profile. Their T concentration is high at the

beginning of the reproductive period and decreases when males are engaged in parental care. When males and females are housed together, birds are not strictly monogamous; indeed in an experiment conducted in our laboratory, genetic analyses revealed that males perform EPF during reproduction in aviaries (Parisot et al., 2004b). Furthermore, in our second experiment, we observed that 3 females and 1 male were paired with several mates. In this condition, our results show that males' T profile follows the polygamous profile as described by Wingfield et al. (1990). T increased at the beginning of the reproductive period and remained stable all along this period. As suggested by the challenge hypothesis, daily competition may have an influence on T concentration and may be related to its stability (Wingfield et al., 1990). Males' T profile in experiment 2 generally parallels the male–male competition profile (see Fig. 2). In our study, male–male competition occurred during the whole sampling period and increased during EA2 when females are receptive once again. In canaries, females lay their second clutch while males still feed chicks. Males fed chicks during CR 1 and also during

EA2 and Inc 2. During these periods, males are in competition for obtaining food.

In conclusion, this study shows that a same species may show different T profiles according to the level of competition. Monogamous species are affected by environmental cues as suggested by Wingfield et al. (1990). They state that “Since these males all show parental care, levels of T tend to be lower for much of the breeding season. However, mechanisms exist to increase the T level when necessary (such as a challenge from an intruding male)”. Here, we experimentally demonstrated that such mechanisms might modulate plasma T concentration in male domestic canaries.

Acknowledgments

This research was supported by grants from Université Paris 10 Nanterre, Université Paris 13, Villetaneuse and from CEBC, CNRS Chizé. We thank Stéphanie Ruault and Colette Trouvé for their help during T analysis. We also thank Colette Desaleux who cared for the birds and Marie Monbureau.

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