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Experimental evidence of a testosterone-induced shift from paternal to mating behaviour in a facultatively polygynous songbird

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Abstract Previous studies have suggested that testosterone (T) profiles of male birds reflect a trade-off between mate attraction behaviours (requiring high T levels) and parental care activities (requiring low T levels). In this study, we experimentally elevated T levels of monogamous males in the facultatively polygynous European starling (*Sturnus vulgaris*), and compared mate attraction and paternal behaviour of T-treated males with those of controls (C-males). T-males significantly reduced their participation in incubation and fed nestlings significantly less often than C-males. Females paired to T-treated males did not compensate for their mate's lower paternal effort. The observed reduction in a male's investment in incubating the eggs was accompanied by an increased investment in typical female-attracting behaviours: T-males spent a significantly higher proportion of their time singing to attract additional females. They also occupied more additional nestboxes than C-males, although the differences just failed to be significant, and carried significantly more green nesting materials into an additional nestbox (a behaviour previously shown to serve a courtship function). T-males also behaved significantly more aggressively than C-males. During the nestling period, the frequency of mate-attracting behaviours by T-treated and control males no longer differed significantly. Despite the reduced paternal effort by T-males and the lack of compensation behaviour by females, hatching and breeding success did not differ significantly between T- and C-pairs.

Keywords · Testosterone · Sexual selection · Parental investment · Mate attraction · *Sturnus vulgaris*

Introduction

Testosterone (T) has been shown to influence a wide array of social and sexual behaviours in male birds (Wingfield et al. 1990; Ketterson et al. 1992, 1996; Beletsky et al. 1995). Particularly interesting is the pivotal role apparently played by T in regulating the balance between mating and parental effort. A male bird may increase his reproductive success by either advertising for additional mates (investment in a future brood) or participating in parental care activities (investment in the current brood). However, these two kinds of effort are both time and energy consuming and incompatible with each other (Smith 1995). Circulating levels of T appear to reflect this trade-off in males of socially monogamous species: T levels are high during territory establishment, pair formation, nest building and copulation, and decline to baseline levels when eggs or young are present. In contrast, males in polygynous species with no paternal care exhibit sustained secretion of T at peak levels for most of the breeding season, presumably because their periods of male-male aggressiveness and sexual behaviour are more prolonged. Finally, in polygynous species with biparental care, T levels in males gradually decline during the parental phase (Wingfield and Moore 1987; Wingfield et al. 1990; Beletsky et al. 1995; Ketterson et al. 1996).

Studies in which plasma levels of T have been experimentally elevated during the parental phase have shown that T-implanted males reduce their investment in dependent offspring and invest more in other forms of reproductive effort such as mate acquisition, mate-guarding and male-male aggression (Hegner and Wingfield 1987; Ketterson and Nolan 1992, 1999). Some experimental studies have also shown that treatment with T could induce polygyny in socially monogamous males (Watson and Parr 1981; Wingfield 1984). In some studies, females were found to compensate for the reduced paternal care of their mates by increasing their own parental effort, implying that the reproductive success of T-implanted males was not negatively affected (Ketterson et al. 1992; Saino and Møller 1995; Hunt et al. 1999). In

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other studies, however, no evidence for compensation by females was observed, and T-males were found to produce fewer fledglings (Silverin 1980; Hegner and Wingfield 1987; Schoech et al. 1998).

The generalization that males given T implants reduce their parental activity is mainly based on studies that report suppressive effects of elevated T on feeding efforts and nest defence (e.g. Saino and Møller 1995; Cawthorn et al. 1998). However, the effects of high T levels on male incubation behaviour have rarely been investigated. We are aware of only one experimental study that investigated the consequences of elevated T levels on male incubation behaviour. In the sex-role-reversed spotted sandpiper (*Actitis macularia*), in which incubation of eggs and care of young is performed mainly by the male, T-implanted males deserted clutches or showed strongly reduced incubation behaviour relative to control males (Oring et al. 1989). As far as we know, possible effects of experimentally elevated T levels on male incubation behaviour have not yet been examined in passerine birds.

In the present study, we investigated whether T plays a role in regulating the balance between mating and parental effort in male European starlings (*Sturnus vulgaris*). The starling is an appropriate species for such a study because males contribute substantially to both incubating eggs and feeding the nestlings (Pinxten et al. 1993; Pinxten and Eens 1994). Moreover, starlings are facultatively polygynous and about 40% of males try to attract additional females during the incubation period, and sometimes even during the early nestling period (Pinxten et al. 1989; Pinxten and Eens 1990). We implanted monogamous males with either T-filled or empty silastic tubes and then compared T-implanted males with control males with respect to (1) investment in paternal care (proportion of time spent incubating and number of feeding trips) and (2) investment in mate attraction behaviours during the parental phase. We also evaluated female parental care and examined the effects of experimentally elevated T levels on breeding success.

Methods

Study areas and general methods

Our study was carried out in three nestbox colonies (Kalmthout, Zoersel and Wilrijk) around Antwerp, Belgium. The colonies in Kalmthout (57 nestboxes) and Zoersel (36 nestboxes) were studied during 1998–1999, and the colony in Wilrijk (35 nestboxes) only in 1999. Nestboxes were attached to trees or wooden posts and the distance between neighbouring nestboxes was about 3–5 m. All nestboxes were identical and a 30-cm wooden perch, attached to the bottom of each nestbox, allowed the starlings to sit and display in front of it. All studied populations are usually single-brooded and first clutches are always laid highly synchronously (Pinxten et al. 1990). Most birds were caught for the first time soon after their arrival (February–March) while they were roosting in the nestboxes at night, or with automatic traps while they were inspecting the nestboxes. In 1998, however, two males were captured for the first time during the incubation period. Females that had not yet been captured about a week before clutch initiation were not caught until the nestling period, when the risk of desertion because of capture is minute. Captured birds were aged (second-year versus older

birds; see Svensson 1984) and marked with individually numbered metal and colour rings to allow easy identification in the field. We also measured tarsus length, wing length and body mass.

Hormone manipulations

Ten males (T-males) were implanted subcutaneously with a 15-mm silastic tube (Degania Silicone, inner diameter 1.47 mm, outer diameter 1.96 mm), filled with crystalline T (Sigma) and sealed with silastic medical adhesive (Dow Corning). Due to the adhesive, the total length of the implant actually containing T was about 13 mm. Implants were inserted in the neck region while the birds were under local anaesthesia (10% Xylocaine spray) and the incision was sealed with tissue adhesive (Histoacryl; Braun). The whole treatment took about 10 min and all males were released within 15 min. The implant procedure did not seem to affect the males' behaviour since they always flew away immediately after their release and we frequently observed males singing and displaying vigorously within 20 min after being implanted. Previous experiments with starlings and other species have shown that T implants are effective in increasing T levels for several months (Ketterson et al. 1991; Gwinner and Gwinner 1994; Moreno et al. 1999; E. De Ridder, R. Pinxten, M. Eens, unpublished data). Eleven control males (C-males) were treated in an identical way, but they received empty tubes. Males were arbitrarily assigned to one of the two treatment groups: the first male we captured was given a T implant, the second one was given an empty implant and subsequent males were alternately implanted with filled and empty tubes. All males (with the exception of one C-male and one T-male) were adults. Tarsus length, wing length and body mass at the time of implantation did not differ significantly between T- and C-males (Mann-Whitney *U*-tests: $P > 0.25$ in all cases). In 1998–1999, we implanted ten 'perfectly' monogamous males on the 4th or 5th day of the incubation period (eggs are incubated for about 11 days). Males were referred to as 'perfectly' monogamous if they were paired to a single female, occupied only one nestbox and had never shown attempts to attract an additional female. Five of these 10 males received a T implant (1998: $n=4$; 1999: $n=1$), while the other 5 (1998: $n=4$; 1999: $n=1$) received an empty implant. All males were captured on the nest while they were incubating the eggs. In 1999, another 11 males (5 T-males, 6 C-males) occupying only one nestbox were implanted early in the breeding season, enabling us to study the effects of experimentally elevated T levels on copulation and mate-guarding behaviour, and song activity during the fertile period (E. De Ridder, R. Pinxten, M. Eens, unpublished data). These 11 males were captured on average 14.7 ± 5.9 days (and at the latest 6 days) before clutch initiation of their female.

Behavioural observations

Observations were made with a telescope from transportable or permanent hides. During the incubation period, all males that were implanted early in the breeding season were observed four times (every second day, starting the 2nd or the 3rd day after clutch completion). Males implanted on the 4th or 5th day of the incubation period were observed twice before implantation (to check that they were perfectly monogamous) and twice after implantation. For these males, we only included the observations made after implantation, which started at the earliest 2 days after implantation to ensure that the implants were 'effective'. Each observation period started when the male or the female entered the nestbox and ended after 1 h. For each observation period, we measured for each sex the total proportion of time spent in the nestbox (nest attendance rate). Average nest attendance rates were used in all analyses. Incubation behaviour was observed at 21 nests (10 T-males and 11 C-males).

During the nestling period, we recorded male and female feeding rates continuously for 1 h when nestlings were 2–4 days old (10 T-males, 10 C-males) and 12–14 days old (8 T-males, 6 C-males). The nestling period was divided into two different age stages because in starlings, delivery of food changes with nestling age

(Tinbergen 1981). Due to predation of some nests, the number of observed males and females differed between the two age stages.

During all observation periods we also determined (1) the number of times males were seen carrying green nesting materials into a nestbox, a behaviour which functions in mate attraction (Eens et al. 1990, 1993, 1994), (2) the number of times males behaved aggressively towards another male and (3) the proportion of time a male spent singing. For the incubation period, we also calculated the proportion of time spent singing/time present in the colony for both male categories, but this provided the same statistical results. For each minute we recorded whether or not a male was singing and in all cases we also noted where he was singing. Therefore, we could determine for each male the proportion of time spent singing (1) towards his own female (i.e. song produced in front of the nestbox in which his female had initiated a clutch or in the tree to which this nestbox was attached) and (2) to attract additional females (i.e. in front of another nestbox, in the tree to which this other nestbox was attached or in a tree very close to another nestbox).

Two T-males were not seen in the colony during the incubation period, but apparently were not dead because they were observed again during the nestling period. These males were excluded from the analyses when comparing the frequency of mate attraction behaviours between C- and T-males, since we cannot exclude the possibility that during the incubation period these males were trying to attract additional females outside the colony. However, when comparing time spent incubating between the two male categories, these males were included since it was evident that they did not assist in incubating the eggs. Two T-males succeeded in becoming polygynous (see Results). For these two males we used only the observations made at the nest of the primary female.

All observations were made between 0700 and 1500 hours in all weather except steady rain. Time-of-day biases were avoided by rotating the order in which T-implanted and C-males were observed. Nestboxes were visited daily to record the start of egg-laying, clutch size, hatching success (the proportion of all eggs hatched), nestling mortality and breeding success (the proportion of hatchlings that fledged). Nestlings were weighed at 5, 10 and 15 days of age with a Pesola spring balance (± 1 g).

Statistical analysis

Data were analysed using the statistical software programs SPSS/PC (SPSS 1986), StatXact-Turbo (Mehta and Patel 1995) and SAS/STAT (SAS 1988). All statistical analyses followed procedures outlined in Siegel (1956) and Sokal and Rohlf (1981). For normally distributed data, parametric tests were used. In some cases, proportions were arcsine square-root transformed to meet assumptions of parametric analyses. When our data did not meet the criteria for the use of parametric statistics, we used non-parametric tests. The probability level for significance was set at $\alpha=0.05$. However, when carrying out multiple comparisons, α was adjusted using a sequential Bonferroni correction (α_a ; Rice 1989) to correct for the increased probability of type I errors. As sample sizes were small for some analyses, we performed power tests. We determined the effect size that would be detected with a power of 80% using simulation techniques (further denoted as effect-P80). We repeatedly (1000 times) generated datasets with increasing difference in mean values and with the observed variances and sample sizes, and calculated power as the proportion of tests that were significant at the 5% level (S. Van Dongen, personal communication). All tests are two-tailed. Values given are mean \pm SE.

Results

Parental care

Incubation behaviour

All 11 C-males assisted their female in incubation, compared with 6 out of 10 T-males (Fisher's exact test:

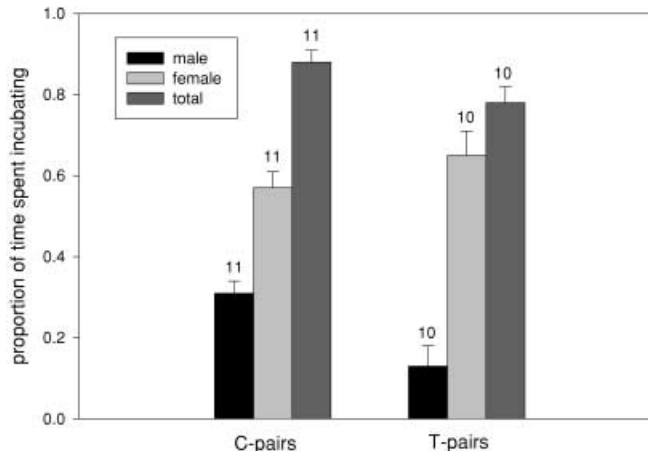


Fig. 1 Male, female and total nest attentiveness (proportion of time spent incubating) in pairs in which the male received an empty (*C-pairs*) or testosterone-filled (*T-pairs*) implant. Data shown are the mean \pm SE

$P=0.035$). On average, C-males spent 31% of the observation time incubating, which is significantly more than T-males which spent only 13% of the time incubating (t -test: $t=2.99$, $df=19$, $P=0.007$; Fig. 1). Females mated to T-males (T-females) spent more time incubating the eggs than females mated to C-males, but the difference was not significant (Mann-Whitney U -test: $U=35.5$, $P=0.17$, effect-P80=0.21; Fig. 1). Total nest attendance rates (proportion of time spent incubating by male and female combined) of C-pairs were significantly higher than those of T-pairs (t -test: $t=2.07$, $df=19$, $P=0.05$; Fig. 1).

Feeding behaviour

All 10 C-males were observed feeding 2- to 4-day-old chicks compared with 7 out of 10 T-males. When nestlings were 12–14 days old, again all 6 observed C-males and 5 out of 8 T-males were observed feeding. In both periods, this difference was not significant (Fisher's exact tests: $P=0.21$). Nevertheless, the intensity of male investment in feeding the nestlings was clearly affected by the experimental treatment. Mann-Whitney U -tests with a sequential Bonferroni correction revealed that T-males made significantly fewer feeding trips per hour than C-males (days 2–4: $U=15$, $P=0.007$; days 12–14: $U=3.5$, $P=0.005$, $\alpha_a=0.025$; Fig. 2). This was not due to differences in brood size between the two categories of nest (Mann-Whitney U -tests: $P>0.8$ in both cases). Feeding rates of C- and T-females however did not differ significantly (Mann-Whitney U -test – days 2–4: $U=45.5$, $P=0.739$, effect-P80=3.06; days 12–14: $U=24$, $P=1$, effect-P80=3.4; Fig. 2). Total feeding rates (combining rates of both parents) were lower in nests of T-pairs, but the differences were not significant (Mann-Whitney U -tests – days 2–4: $U=28$, $P=0.1$, effect-P80=5; days 12–14: $U=9.5$, $P=0.06$, effect-P80=5.6, $\alpha_a=0.025$; Fig. 2).

Table 1 Song activity (proportion of time spent singing) of control (C-) and testosterone-implanted (T-)males. Data are the mean±SE (number of pairs in *parentheses*). Differences between

Song activity	C-males	T-males	<i>U</i> -test	α_n
(1) Total time spent singing	0.03±0.01 (11)	0.26±0.03 (8)	<i>U</i> =0, <i>P</i> <0.0001	0.017
(2) Directed towards their mate	0.02±0.01 (11)	0.07±0.02 (8)	<i>U</i> =21, <i>P</i> =0.06	0.05
(3) Directed towards a prospecting female	0.01±0.009 (11)	0.18±0.03 (8)	<i>U</i> =3, <i>P</i> =0.0002	0.025

the two male categories were tested using Mann-Whitney *U*-tests. α was adjusted using a sequential Bonferroni readjustment (see Methods)

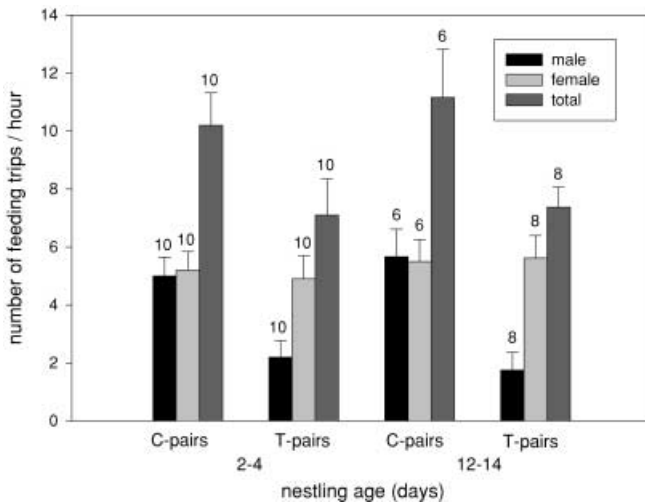


Fig. 2 Absolute male, female and total feeding rate (number of feeding trips/h) on days 2–4 and 12–14 of the nestling period in pairs of which the male received an empty (*C*-pairs) or testosterone-filled (*T*-pairs) implant. Data shown are the mean±SE

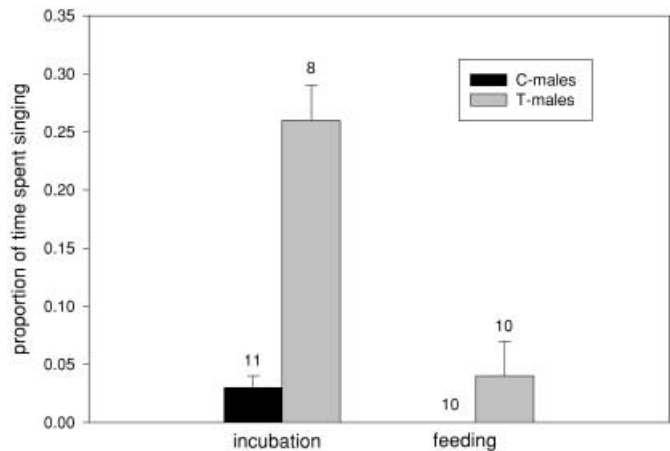


Fig. 3 Song activity (proportion of time spent singing) of control (*C*) and testosterone-implanted (*T*) males during the incubation and feeding period. Data shown are the mean±SE

Mate attraction behaviour

Five out of 10 T-males occupied at least one additional nestbox for several days (range: one to five additional nestboxes) compared to only 1 out of 11 C-males. This difference was not significant (Fisher's exact test: *P*=0.06).

T implantations clearly affected male singing behaviour during the incubation period. All 8 T-males (excluding the 2 T-males not present in the colony during the incubation period; see Methods) were observed singing during this period compared to only 5 out of the 11 C-males (Fisher's exact test: *P*=0.02). The proportion of total time spent singing was significantly higher in T-males than in C-males (Table 1, Fig. 3). However, only song activity to attract additional females differed significantly between the two male groups, while song activity directed at their own female was not significantly higher in T-males (Table 1). All 8 T-males directed song activity at a female other than their mate, compared with only 2 out of 11 C-males (Fisher's exact test: *P*=0.0007). During the incubation period, the proportion of T-males (3 out of 8) observed flying into an additional nestbox with green nesting materials was higher than the proportion of C-males (0 out of 11), but the difference was just not significant (Fisher's exact test: *P*=0.06). However, it became significant when including also males that were

known to have carried green nesting materials in an additional nestbox based on the daily nestbox checks, but were not observed doing so (5 out of 8 versus 0 out of 11, Fisher's exact test: *P*=0.005). Four out of 8 T-males were seen at least once behaving aggressively towards another male. Such aggressive interactions were never recorded during observations of the 11 C-males (Fisher's exact test: *P*=0.02).

The proportion of T-males (2 out of 10) and C-males (0 out of 10) showing song activity during the nestling period did not differ significantly (Fisher's exact test: *P*=0.5). Likewise, the proportion of time spent singing did not differ significantly between C- and T-males (Mann-Whitney *U*-test: *U*=40, *P*=0.48, effect-*P*₈₀=0.1; Fig. 3). Carrying of green nest materials into the nest and aggressive behaviour towards other males were never observed during the nestling period.

Two out of 10 T-males became polygynous compared to none of the 11 C-males (Fisher's exact test: *P*=0.21).

Breeding success

The experimental treatment had no significant effect on hatching success (C-pairs: 0.812±0.06; T-pairs: 0.762±0.07; Mann-Whitney *U*-test: *n*₁=11, *n*₂=10, *U*=51, *P*=0.81, effect-*P*₈₀=0.28). Due to predation during the nestling stage, four of the ten broods of C-males in which feeding rates were recorded, and three of the ten

broods of T-males failed completely. Considering the other nests, fledging success did not differ significantly between C-nests (0.97 ± 0.03) and T-nests (0.9 ± 0.05 ; Mann-Whitney U -test: $n_1=6$, $n_2=7$, $U=14.5$, $P=0.36$, effect- $P_{80}=0.19$). Average body mass of 5-, 10- and 15-day-old nestlings also did not differ between the two categories of nests (Mann-Whitney U -tests: $P > 0.5$ in all cases, $5.2 < \text{effect-}P_{80} < 8.4$).

Discussion

Effects on male behaviour

The results of our study indicate that in male European starlings, experimentally elevated T levels result in a reduction, and in some cases even in the absence, of male participation in incubation. To the best of our knowledge, our study is the first to report that experimentally elevated T levels decrease or inhibit incubation behaviour in a male songbird. Previously, experimentally elevated T levels were shown to disrupt the expression of normal incubation behaviour in male spotted sandpipers (Oring et al. 1989). However, the spotted sandpiper is a sex-role-reversed species (representing only about 1% of all bird species; Eens and Pinxten, in press) in which males provide most or all parental care.

Our results revealed that experimentally elevated T levels also interfered with the full expression of male feeding behaviour, as has been found in several other bird species (e.g. Saino and Møller 1995; Schoech et al. 1998; Hunt et al. 1999; Moreno et al. 1999).

The strong decrease in incubation behaviour by T-males was accompanied by an increased investment in song and other mate attraction behaviours, strongly suggesting that elevated T levels cause a shift from parental to sexual investment. The T-induced increase in the frequency of mate attraction behaviours during the incubation period confirms the results of previous experiments showing that T implants elicit mate attraction behaviours in non-breeding male starlings (unpublished data). It also confirms the results of other studies reporting an increase in song activity in socially monogamous male passerine birds treated with exogenous T (Nowicki and Ball 1989; Hunt et al. 1997). In the sex-role-reversed spotted sandpiper, T implants also appeared to heighten sexual receptivity and hence reduce incubation constancy (Oring et al. 1989). In our study, T-implanted males also behaved significantly more aggressively during the incubation period than C-males, which is not surprising since the regulation of aggression by T during the breeding season is well-established (Balthazart 1983). By contrast, male spotted sandpipers implanted with T during the incubation period did not engage in more chases or fights. This may be explained by the sex-role-reversed breeding system of this species – a system in which females tend to be more aggressive than males (Oring et al. 1989). Our results during the incubation period seem to be consistent with the idea that depressed T levels

during the time of male parental care are seen as a compromise between the conflicting demands of parental care and male-male competition/mate attraction behaviour (Hegner and Wingfield 1987).

In contrast to the events during the incubation period, T-males did not show an increase in the frequency of mate-attracting behaviours during the nestling period, although their feeding behaviour was clearly depressed. This lack of an increased investment in mate attraction behaviours during the nestling period makes sense, since a field study carried out in the same nestbox colonies (Pinxten et al. 1989; Pinxten and Eens 1994) revealed that males succeed in attracting additional females mainly during the egg-laying or incubation period. Moreover, the few males still occasionally trying to attract an additional female during the nestling period always stopped their attempts at the latest when the nestlings were 10 days old. Due to the low availability of unmated females during the nestling period, the possible benefits of trying to attract additional females at that time are probably outweighed by the high costs incurred from not feeding the chicks. Our results thus suggest that elevated T during the time of male parental care does not always induce a shift to mate attraction behaviour, but that the likelihood of an implanted male starling showing mate attraction behaviour during the parental phase may also be influenced by the degree to which males are exposed to prospecting females and thus by the opportunities to attract additional mates. Individual male quality may also play a role in regulating the balance between mating and parental effort. An alternative explanation for the lack of T-induced sexual investment during the feeding stage could, however, be that sensitivity to T changes during the breeding season so that the effect of T on mate attraction behaviour was lower during the feeding than during the incubation stage.

We are currently investigating whether natural plasma T levels are higher in male starlings trying to attract an additional female during the incubation (or nestling) stage than in males that never show such behaviour during this period. Given that males trying to attract additional mates sing during a prolonged period (Eens et al. 1994) and that they provide less parental care than monogamous males (Pinxten et al. 1993; Pinxten and Eens 1994), we would expect to find a positive relationship between T and mate attraction behaviour. On the other hand, as already mentioned, variation in the frequency of mate attraction behaviours during the parental phase may not be explained solely by variation in T levels, but may be further influenced by situational variables such as the availability of unmated females.

Previous experimental studies using T implants in socially monogamous birds have resulted in a shift in the mating system towards polygyny (Wingfield 1984), although not always (Ketterson and Nolan 1992; Saino and Møller 1995). Despite the fact that experimentally elevated T levels clearly increased investment in mate attraction behaviours in T-males during the incubation period, there was no significant difference in the propor-

tion of T- and C-males that became polygynous. However, in the population we studied, the proportion of males successful in becoming polygynous can vary strongly between years (from 20 to 70%; Pinxten et al. 1989), depending on several variables such as the availability of unmated females and the behaviour of the primary female, which can vigorously try to prevent her mate from becoming polygynous (Eens and Pinxten 1995, 1996).

Effects on female parental care and breeding success

In our study, T-females did not spend significantly more time incubating than C-females. By contrast, Pinxten et al. (1993) found that female European starlings mated to polygynous males did compensate, although not fully, for the reduced nest attendance rates of their partners. This different response may be explained by the fact that in the latter study, the difference in attendance rates of polygynous and monogamous males was much greater than the difference between control and T-implanted males in this study, putting more pressure on females to compensate. Although total nest attentiveness was reduced in T-pairs, the reduction was apparently not large enough to negatively affect hatching success.

In contrast to studies that report compensation by T-females for their mates' shortfall in feeding effort (e.g. Ketterson et al. 1992, 1996; Saino and Møller 1995), we did not find that T-females increased their feeding frequencies to compensate for the mate's reduced feeding effort. Likewise, Silverin (1980), Dittami et al. (1991) and Schoech et al. (1998) established that female pied flycatchers (*Ficedula hypoleuca*), reed warblers (*Acrocephalus arundinaceus*) and dark-eyed juncos (*Junco hyemalis*), respectively, did not compensate for the decreased feeding rates of their T-implanted mates.

Despite reduced T-male feeding effort and an apparent lack of female compensation, overall breeding success did not differ between C- and T-pairs, as has been found in several other studies (e.g. Dittami et al. 1991; Saino and Møller 1995; Hunt et al. 1999). This result may be explained if T-females, although they did not increase their feeding rates, still compensated for lower male provisioning rates by bringing larger prey at each visit (see Sejberg et al. 2000). It should however also be emphasized that, due to the small sample sizes, the power of our tests were rather small (see Results). Moreover, we examined breeding success only until the time of fledging and did not investigate other potential costs of high T, such as reduced offspring or male survival. Quite clearly, further research is necessary to clarify all fitness costs and benefits of high plasma T levels in European starlings and other facultatively polygynous songbirds.

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