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Testosterone and helping behavior in the azure-winged magpie (*Cyanopica cyanus*): natural covariation and an experimental test

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Abstract In this study, we investigated patterns of natural covariation between testosterone and reproductive status in a cooperatively breeding bird species, the azure-winged magpie (*Cyanopica cyanus*). To assess the relationship between testosterone and breeding behavior, we also manipulated testosterone (T) levels early in the season (before breeding started) using testosterone-filled or empty implants. Our results do not support the hypothesis that circulating testosterone levels affect the occurrence of helping behavior in the azure-winged magpie. Helping males had similar T levels to breeding males. Furthermore, experimentally augmented plasma T did not affect the likelihood of becoming either a helper or a breeder. Overall, these results are consistent with previous findings in other bird species and only give some support to the behavioral suppression hypothesis, suggesting that helping in the azure-winged magpie is a flexible behavioral option moderated in the short-term by social and ecological factors. Experimentally elevated testosterone levels, however, reduced the reproductive

success of male breeders because of markedly lower levels of paternal care, had similar effects on their mates, but had the contrary effect on helpers, which raised levels of parental effort. We suggest increases in the share of paternity and in social prestige as possible explanations for these results.

Keywords Cooperative breeding · Helpers at the nest · Hormones · Parental care · Testosterone

Introduction

In cooperative breeding systems one or more individuals, in addition to the parents, participate in parental care (Brown 1987; Cockburn 1998). Several ecological and evolutionary factors have been suggested as promoting the delayed breeding and helping behavior in birds (see reviews in Brown 1987, and Cockburn 1998). Relatively few studies, however, have dealt with the proximate mechanisms involved in the expression of helping behavior (Hatchwell and Komdeur 2000).

Several studies have analyzed the relationship between hormonal blood levels and helping behavior in birds (Wingfield et al. 1991; Mays et al. 1991; Vleck et al. 1991; Poiani and Fletcher 1994; Schoech et al. 1991, 1996a, 1996b; Brown and Vleck 1998; Vleck and Brown 1999; Khan et al. 2001; Peters et al. 2001, 2002). It is well documented that elevated levels of testosterone during the mating and laying phases benefit males by favoring the development of secondary sex characteristics and the expression of mating, territorial and mate defense behavior, and by increasing the likelihood of extra-pair copulation (Wingfield et al. 1990; Smith 1995; Vleck and Brown 1999; De Ridder et al. 2000; McDonald et al. 2001). A prolonged high plasma testosterone level can carry a high collateral cost: it can induce immunosuppression (Folstad and Karter 1992; Braude et al. 1999; Peters 2000), increase energy turnover (Beletsky et al. 1995; Smith 1995; Buchanan et al. 2001), disrupt social relationships with the concomitant increased risk of injury

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and predation (Beletsky et al. 1995; Vleck and Brown 1999), and reduce the male's expression of parental care (Duffy 1989; Ketterson and Val Nolan 1992; Smith 1995; De Ridder et al. 2000; McDonald et al. 2001; Stoehr and Hill 2000; Peters et al. 2002; but see Van Duyse et al. 2000, 2002, and Lynn et al. 2002 for different results). Given this trade-off between the benefits of high testosterone levels in terms of mating preference and the costs in terms of reduced parental care, testosterone levels should decrease during incubation (McDonald et al. 2001) and during care of nestlings and fledglings in species where males contribute to parental care (Wingfield et al. 1990; De Ridder et al. 2000; Trainor and Marler 2001).

In cooperative breeding species, one may have to consider an additional trade-off. On the one hand, elevated plasma testosterone in breeding males may reduce their investment in parental care. On the other hand, a breeding male might incur an additional cost from reduced paternity due to extra-pair copulations sneaked by helpers (Green et al. 1995), while sustained high levels of testosterone might help the breeding male to reduce this risk of loss of paternity (Schoech et al. 1996a; De Ridder et al. 2000). Similarly, two options are available for non-breeding males: (1) to maintain relatively high testosterone levels if this improves the chances of filling a vacancy if the breeder dies; and (2) to depress testosterone production in order to avoid aggression from breeding males and increase the likelihood of sneaking extra-pair copulations (Reyer et al. 1986; Schoech et al. 1991).

Several hypotheses have been put forward to explain the sexual inactivity of helpers: (1) delayed maturation (Brown 1987); (2) physiological suppression (Schoech et al. 1996a; Wingfield et al. 1991); (3) lack of appropriate stimuli (Poiani and Fletcher 1994; Schoech et al. 1996b); (4) behavioral suppression (Mumme et al. 1983; Emlen and Wrege 1988; Mays et al. 1991; Schoech et al. 1996b); and (5) inbreeding avoidance (Reyer et al. 1986; Poiani and Fletcher 1994). The implications of these hypotheses on testosterone levels are different: the first three hypotheses imply that male breeders should have higher levels of testosterone than helpers, the fourth that the levels would be similar, and the fifth that helpers should have lower or higher levels depending on whether they help related or non-related females, respectively.

Most studies dealing with the relationship between blood hormone levels and helping behavior in birds have focused on territorial birds. For these species, low levels of testosterone in helpers may have an adaptive value because they obtain major benefits from staying in the parental territory (Woollfenden and Fitzpatrick 1984), when philopatry is imposed by environmental or demographic constraints (Schoech et al. 1991). This, however, requires delayed maturation to avoid aggression from the dominant male (Grant 1990; Schoech et al. 1991).

Cooperative breeding is less widespread in colonial species and consequently few studies have investigated the physiological correlates of helping in such species (Poiani and Fletcher 1994). Territorial and colonial species differ with respect to social interactions: male-

male interactions and risks of paternity loss due to extra-pair copulations are higher in colonial species (Poiani and Fletcher 1994; Vleck and Brown 1999). This has led to the prediction that colonial species should maintain elevated testosterone levels compared to phylogenetically related territorial species, since in colonial species the male-male interactions can be more frequent (Vleck and Brown 1999).

The aims of the present study were: (1) to investigate the temporal variation of blood testosterone (T) levels during the breeding season in the azure-winged magpie (*Cyanopica cyanus*); (2) to determine whether this hormone is involved in the expression of helping behavior; and (3) to assess the effects of elevated T levels on the breeders' and helpers' parental care. Two methods were used for objectives (2) and (3): first, we assessed whether natural T levels differed between breeders and helpers, and second, to ascertain the causal relationship between the hormone and helping behavior, we experimentally increased the T levels of males at the beginning of the breeding season and determined whether this affected: (1) the likelihood of subsequently becoming a breeder or a helper; (2) the likelihood of accepting helpers at nest; and (3) parental investment.

Methods

Study species and site

The azure-winged magpie (*Cyanopica cyanus*) is a small colonial corvid (body mass 70 g), with a cooperative socially monogamous breeding system (Komeda et al. 1987; Cramp and Perrins 1994; Valencia et al. 2003). In our study area, the colony consists of 30–40 pairs, with a low nest density (4–5 nests per ha; Cruz 1988). More than 50% of the nests in the colony have between one and four helpers. Helpers at nests are usually young or adult males with no parent-offspring relationship with the breeders. Sometimes, the helpers feed the female during the incubation period, but normally they appear in the nest only when the nestlings are 4 or 5 days old (Gonzalez 1996; Valencia 2002). In the winter, the birds of the colony form a flock, with the young males remaining in the colony and the young females migrating to other colonies. Aggressive interactions are very rare in this flock, and flocks in general persist for several years (C. de la Cruz, personal observation). The breeding season lasts from April until the first half of July (Cruz et al. 1991). Typically, there is only one clutch per year (5–7 eggs), but replacement clutches are laid if the first fails. Rarely, when the weather is exceptionally good, a pair can raise two successful broods (Valencia et al. 2000).

We studied a colony of azure-winged magpies during three breeding seasons (1999–2001) at Valdesequera, in the province of Badajoz, Spain (39°03'N, 6°48'W). The predominant habitat is dehesa (open holm oak *Quercus rotundifolia* woodland). The climate is typically Mediterranean, with dry hot summers and mild wet winters.

Data collection

Since 1992, azure-winged magpies have been captured and tagged with a metal ring and a unique combination of colored plastic rings for individual recognition. At the beginning of the reproductive period, we measured tarsus length and body mass at each individual to estimate the bird's body condition. The gender, age, and

breeding status of most birds were known from intensive behavioral observations that have been carried out every spring.

In each of the three breeding seasons (1999–2001) we searched extensively for nests. Each tree in the entire study area was inspected at least twice a week. Trees with nests were marked and located with the help of an aerial photograph. Every nest found was monitored (at least on an alternate day basis) and intensively observed until fledging. All nests were observed using a telescope from a blind for at least 1 hour every 2 days. Nests were included in the analyses only when the members of the breeding pair were identified. The gender of the breeders was assessed according to incubation and brooding behavior; only females incubate the clutch and brood the young (Hosono 1966; Komeda et al. 1987). Two age-classes were distinguished on the basis of plumage (Cruz et al. 1992): juveniles (up to 1 year) and adults (more than 1 year old). Nests that produced at least one fledgling were considered successful. As suggested by Brown (1987), we used the term “helper” (except if otherwise stated) for those individuals who assisted the breeding pair in feeding the young and removing the faecal sacs.

We distinguished two types of helpers: (1) first-option (FO) helpers who helped from the beginning of the breeding season instead of attempting to breed by themselves; and (2) second-option (SO) helpers who shifted to helping behavior after attempting their own breeding (regardless of whether or not it failed) (Valencia 2002; Valencia et al. 2003). In analyzing males’ status differences, the males were considered as breeders, although later operated as helpers (SO helpers), when the blood samples were taken before the change of status occurred, but not if samples were taken during the period when they were already operating as SO helpers.

We divided the breeding period in five stages: pre-laying (up to 20 days before the beginning of the clutch), laying (approximately 7 days), incubation (about 14 days), nestling rearing (about 20 days), and fledging.

Blood collection

During the 1999 and 2000 breeding seasons, we conducted extensive capture sessions using specially designed traps. To obtain reliable T levels, we collected a blood sample from each captured bird immediately following capture, collecting it from the brachial vein directly into heparinized haematocrit capillary tubes. When a bird had been captured more than once, only one randomly chosen capture was considered. The blood was immediately centrifuged at 10,000 rpm for 8 min to separate the plasma from the blood cells. The plasma was stored at -20°C until assay.

Testosterone implants

In 2000 and 2001, males captured between February and the beginning of nest building were alternately assigned to one of two treatments. One group ($n=25$) received a testosterone implant (T), the other ($n=24$) received an empty implant (C). The implants consisted of a 20-mm long Silastic tube (Dow Corning, inner diameter 1.47 mm, outer diameter 1.96 mm) packed with crystalline testosterone (Sigma) or left empty, sealed at both ends with Silastic medical adhesive. Before implantation, an area of skin on the back was disinfected with 70% ethanol and a small incision was made. The implant was inserted between the skin and the muscle, and the opening was closed with a stitch. Of these, we were able to observe 19 (76%) T-males and 16 (67%) C-males during the breeding period when their reproductive status was assessed as either breeder or FO helper.

Twenty-five implanted males were recaptured several times during the breeding season and a blood sample was collected to assess the efficacy of the T-implants. The T levels of the T-implanted males (mean=1.18 $\mu\text{g/ml}$, SE=0.09, range=0.14–2.86) were at the high end of the physiological range in non-experimental birds (mean=0.5 $\mu\text{g/ml}$, SE=0.032, range=0.07–2.45), while those of the C-males (mean=0.66 $\mu\text{g/ml}$, SE=0.084, range=0.11–2.55) did

not differ from the levels of free-living non-experimental birds (ANOVA $F_{2,86}=6.71$, $P=0.002$; posthoc comparisons Scheffé multiple-comparison procedure: T-males vs non experimental males, $P<0.05$; T-males vs C-males, $P<0.05$; and C-males vs non experimental males, $P>0.05$), showing therefore that the manipulation had been effective. When possible, the implant was withdrawn at the end of the breeding period.

Testosterone assay

Testosterone titers were determined by radioimmunoassay at the Centre d’Etudes Biologiques de Chizé, using testosterone specific antibodies (Lormée et al. 2000). Testosterone antisera were kindly provided by Dr. Gérard Picaper (Medecine Nucléaire, Centre Hospitalier Universitaire la Source, Orléans, France). Testosterone was extracted in duplicate from 50 μl plasma samples in diethyl-ether and redissolved in 0.01 M phosphate-buffered saline (Ph 7.4) containing 0.1% bovine albumin (PBS-BSA). Aliquots were incubated overnight at 4°C with ca. 6000 cpm of $^3\text{H-T}$ and antiserum. Only one assay was performed and the intra-assay coefficient of variation was 11% ($n=3$ duplicates).

Statistical analyses

We used general linear models (ANOVAs and ANCOVAs) unless the assumptions of these tests were violated, in which case we used non-parametric statistics. Sample size differences between analyses reflect missing values.

Results

Seasonal changes in T levels and determinants of helping behavior

The temporal pattern of T levels depended on the year (the levels were higher in 2000) and the category of the individual breeding males, breeding females, and helper males (Fig. 1, Table 1). Post-hoc comparisons showed that the T levels of breeding males were significantly higher in the laying period (i.e., the period of female fecundity) than in the chick rearing period ($F_{1,28}=17.7$, $P=0.0002$; Fig. 1). Breeding males had higher T levels than females throughout the breeding cycle ($F_{1,118}=21.6$, $P<0.0001$; Fig. 1). Helpers and breeding males had similar T levels ($F_{1,88}=2.2$, $P=0.14$), although few data were available for the laying and incubation period (Fig. 1).

There was no difference between in T levels of males helping at their mother’s or at an unrelated female’s nest. (Mann-Whitney test: $U=25.0$; $P=0.21$; $n=21$).

The absence of correlation between T levels and the propensity to breed or to help was confirmed by the experimental manipulation of testosterone early in the breeding season. Twenty-six per cent (5 out of 19) of T-males were first-option (FO) helpers versus 19% (3 out of 16) for C-males (Fisher exact test: $P=0.70$). Similarly, the proportion of males who participated in reproduction either as breeders or as FO helpers did not differ between the two hormone treatments (T-males: 76% reproduction, 19/25; C-males: 70% reproduction, 16/23; Fisher exact test: $P=0.75$; in this analysis, males which did not

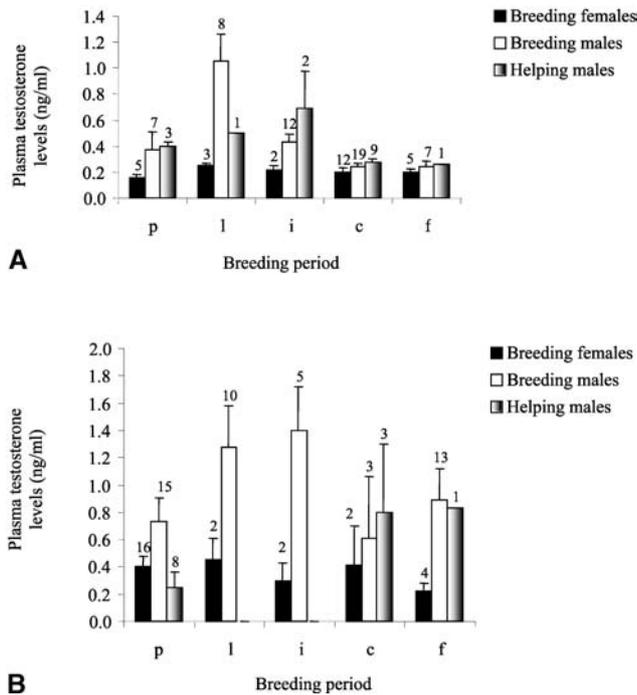


Fig. 1 Seasonal pattern of testosterone levels in breeding females, breeding males and helping males in **A** 1999, **B** 2000. Breeding periods are *p* pre-laying, *l* laying, *i* incubation, *c* chick rearing, *f* fledging. Bars refer to SE; numbers refer to sample sizes

Table 1 ANOVA on log-transformed T levels of breeding males ($n=70$), breeding females ($n=46$) and male helpers ($n=16$). Breeding periods are: pre-laying, laying, incubation, chick rearing and fledging. Year is 1999 or 2000. None of the interactions (two-way and three-way) was significant and they were dropped from the final model

Source of variation	Type III SS	df	F	P
Breeding status	4.51	2	10.54	0.0010
Breeding period	2.98	4	3.48	0.0100
Year	3.60	1	16.81	0.0001
Error	26.55	124		

participate in reproduction included floating birds and individuals that disappeared from the population).

We also analyzed whether the decision of failed breeders to attempt replacement or to become a helper was affected by the hormone treatment. All T-male breeders ($n=9$) who failed in their first breeding attempt made a replacement clutch; of the 11 C-male breeders that failed in their first breeding attempt, 9 laid a replacement clutch (82%) whereas 2 (8%) became SO helpers (Fisher exact test: $P=0.48$).

Laying date of females paired with T-males did not differ from the laying date of females paired with C-males (mean: 12 April for both groups; Mann-Whitney test: $U=53.5$; $P=0.91$; $n=21$).

Body condition, measured as the residuals of the linear regression between tarsus length and body mass, did not differ between male breeders and helpers (one-way

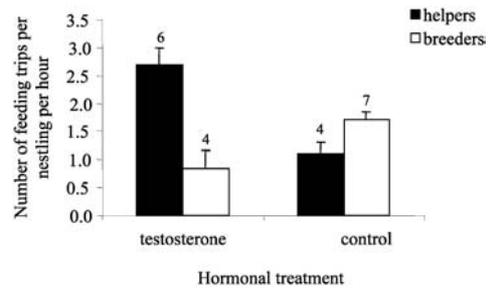


Fig. 2 Effect of testosterone implants on feeding effort (number of feeding trips per nestling per hour) of T-implants breeder and helper males. Bars refer to SE; numbers refer to sample sizes

Table 2 ANCOVA with number of feeding trips as dependent variable, nest, hormonal treatment and breeding status as factors, number and age of nestlings as covariates. Hormonal treatment by breeding status was the only significant interaction, and therefore the other interaction terms were dropped from the final model

Source of variation	Type III SS	df	F	P
Nest	30.95	15	2.36	0.009
Hormonal treatment (HT)	0.04	1	0.05	0.83
Breeding status (BS)	3.16	1	3.61	0.06
Number of nestlings	9.69	1	11.08	0.001
Age of nestlings	9.26	1	10.59	0.002
HT×BS	8.13	1	9.29	0.003
Error	58.61	67		

ANOVA: $F_{1,106}=0.01$, $P=0.94$). Similarly, there was no significant correlation between body condition and plasma testosterone concentration ($r=0.03$, $P=0.85$, $n=35$).

T levels of breeders and their likelihood of accepting helpers

To determine whether the circulating T levels of breeders affected their likelihood of accepting helpers at the nest, we examined the mean testosterone levels for males during the chick and fledgling rearing period. There was no statistically significant correlation between the number of helpers at nest and the testosterone levels of the breeding male (two-way ANOVA: number of helpers, $F_{1,19}=1.73$, $P=0.21$; year, $F_{1,19}=5.20$, $P=0.03$; number of helpers × year, $F_{1,19}=0.53$, $P=0.47$). We also tested whether testosterone implants affected the likelihood of accepting helpers. Among the T-males, one out of four benefited from at least one helper, whereas four out of seven C-males had helpers at nest (Fisher exact test: $P=0.54$).

Feeding rate

The natural levels of testosterone in breeding males were not significantly correlated with the rate of nestling feeding (partial correlation coefficient controlling for nestling age: $r=-0.10$, $P=0.69$, $n=19$). However, for the

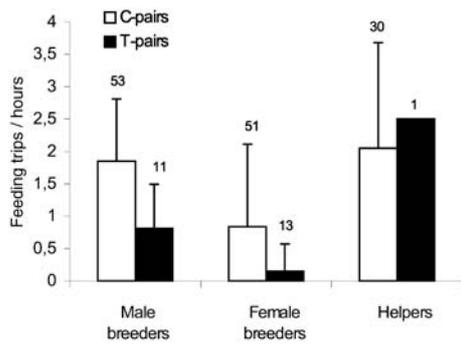


Fig. 3 Male, female and helper's nestling provisioning rates (feeding trips per hour) in nests in which the male received an empty (C-males) and a testosterone implants (T-males). Bars refer to SD; numbers refer to sample sizes

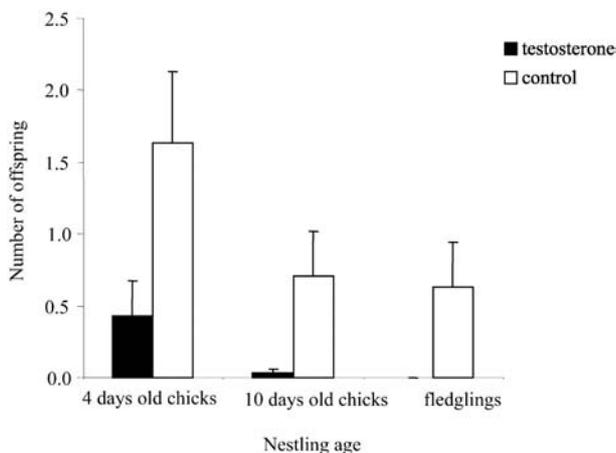


Fig. 4 Effect of testosterone implants on brood size and on reproductive success (for statistics, see main text). Bars refer to SE. Sample size: T-nests =29, and C-nests =25

experimental groups, we found that T affected the feeding rates of the breeding and helper males in opposite ways (Fig. 2). The T-implant breeders made less frequent feeding visits than C-breeders, whereas T-helpers contributed more to parental care than C-helpers (Fig. 2). This was reflected in a significant statistical interaction between breeding status and hormonal treatment after controlling for nest, number, and age of nestlings (Table 2).

The females paired with T-implanted males, which had reduced their parental effort, did not compensate for this loss. Instead, we observed an 80% decline in their parental effort with respect to the females paired with C-control males (Fig. 3), although the difference was not statistically significant ($F_{1,60}=0.19$, $P=0.66$).

Breeding success

Breeding pairs with helpers had significantly higher reproductive success (number of fledged young) than

pairs without helpers (two-way ANOVA with year as factor: year, $F_{1,40}=2.12$, $P=0.153$; number of helpers at nest, $F_{1,40}=4.42$, $P=0.042$).

There was no correlation between the natural T levels of breeding males during the chick raising period and reproductive success (Spearman correlation coefficient, $R_s=-0.268$, $n=16$, $P=0.316$). However, the pairs with a T-implanted breeding male had higher chick mortality and lower reproductive success (number of fledglings) than the C-male broods (Fig. 4; number of 4- and 10-days-old nestlings: ANOVA: $F_{1,52}=5.21$, $P=0.027$ and $F_{1,52}=5.86$, $P=0.019$ respectively; number of fledglings: ANOVA: $F_{1,52}=5.25$, $P=0.026$).

Discussion

We found that testosterone plays little if any role in determining helping behavior in the cooperative breeding azure-winged magpie. Both the correlative and the experimental data suggest the absence of any effect of elevated T levels on reproductive status, but it did have a negative impact on the breeding males' reproductive success by reducing their parental behavior.

Testosterone and breeding status

In cooperative breeding birds, the pattern of help at the nest varies greatly in response to multiple ecological and genetic factors (Cockburn 1998). For instance, some studies have shown the expression of helping behavior to be related to blood testosterone levels (Reyer et al. 1986; Mays et al. 1991; Schoech et al. 1991, 1996a, 1996b; Vleck et al. 1991; Vleck and Brown 1999; Poiani and Fletcher 1994; Khan et al. 2001). Nonetheless, intra- or inter-species variation in testosterone levels might not always lead to predictable effects in determining breeding status. Florida scrub-jays (*Aphelocoma coerulencens*) (Schoech et al. 1991, 1996a, 1996b), white-browed sparrow weavers (*Plocepasser mahali*) (Wingfield et al. 1991), and bell miners (*Manorina melanophrys*) (Poiani and Fletcher 1994) are examples where sexually active male breeders have testosterone levels that are consistently higher than those of reproductively inactive male helpers, as is consistent with the hypotheses of delayed maturation (Brown 1987), absence of suitable stimuli (Poiani and Fletcher, 1994; Schoech et al. 1996b), and physiological suppression (Schoech et al. 1996b; Wingfield et al. 1991; Peters et al. 2001). Conversely, in the Australian magpie (*Gymnorhina tibicen*) (Schmidt et al. 1991) and the Mexican jay (*Aphelocoma ultramarina*) (Vleck and Brown 1999) helpers and breeders have similar testosterone levels, findings that have been interpreted as supporting the behavioral suppression hypothesis (Mumme et al. 1983; Emlen and Wrege 1988; Mays et al. 1991; Schoech et al. 1996b). In the superb fairy-wren (*Malurus cyaneus*), a species whose mating system presents particularly high levels of extra-

pair copulation, helpers are reproductively competent and sexually active as shown by their high frequency of involvement in the extra-pair copulation, and have testosterone levels that are lower than those of the dominant breeding males in groups, but similar to those of paired males (Peters et al. 2001). This suggests that helpers are reproductively suppressed by aggressive interactions with dominant males (Peters et al. 2001, 2002) and/or because females are not receptive to them as subordinate males (Peters et al. 2001).

In the azure-winged magpie, breeders and helpers had statistically indistinguishable levels of circulating testosterone throughout the breeding season. However, few helpers were sampled during the laying and incubation periods (indeed none in 2000, mainly because of the bad weather conditions), when testosterone is at its highest level in breeding males. Notwithstanding this low statistical power, we are confident that testosterone is not involved in the expression of helping behavior in this species, because the experimentally induced increase in testosterone early in the season did not affect the likelihood that a bird would become a breeder or a helper. Our results therefore do not support the hypotheses of delayed maturation (Brown 1987), absence of appropriate stimuli (Poiani and Fletcher 1994; Schoech et al. 1996b), or physiological suppression (Schoech et al. 1996b; Wingfield et al. 1991), which all predict lower levels of testosterone in helpers than in breeders. Moreover, the first of these hypotheses predicts that helpers are incapable of breeding due to their delayed development, but 1-year-old azure-winged magpie males frequently breed. Furthermore, several individuals have bred in one year but helped the next, so that the decision to be a helper appears to be a highly flexible option available to any member of the colony of any age or reproductive experience (Valencia 2002; Valencia et al. 2003).

The physiological suppression hypothesis predicts that helpers should tend to have a worse body condition than breeders (Wingfield et al. 1991; Khan et al. 2001). However, in the azure-winged magpie we found no relationship between body condition and reproductive status or plasma testosterone concentration. The inbreeding avoidance hypothesis (Reyer et al. 1986; Poiani and Fletcher 1994) is also unsupported by our results. Although there are mechanisms to prevent inbreeding in this population (29% of male helpers born in the colony, seven individuals in the three study years, were observed as helpers at their mother's nest, but they never formed a pair-bond) it does not seem that inbreeding avoidance is testosterone driven because helpers related or unrelated to the recipient female had similar T levels.

Our findings seem to support the behavioral suppression hypothesis (Mumme et al. 1983; Emlen and Wrege 1988; Mays et al. 1991; Schoech et al. 1996b). Indeed, the azure-winged magpie helper males are physiologically capable of breeding and have similar T levels to those of the breeder males. Also, the decision to breed or to help is found to be reversible from one year to the next or even within a given breeding season (Valencia et al. 2003).

Both social and ecological factors may affect the likelihood of any individual bird breeding in a particular season.

Testosterone and parental care

Since testosterone inhibits patterns of parental behavior (Ketterson and Val Nolan 1992; Smith 1995), in accordance with the trade-off hypothesis, males of bird species living in temperate areas usually exhibit high levels of testosterone during territory establishment, mate attraction, and mate fertility, but low levels during periods of active parental care (Wingfield et al. 1990; Peters et al. 2001; Van Duyse et al. 2002). Male azure-winged magpie breeders presented the typical pattern of monogamous species in which males share parental care (Lynn et al. 2002) and present T levels that are high during laying and incubation and low during the nestling and fledgling stages (Fig. 1). We were only able to capture very few helper males during laying and incubation and could not determine the helpers' T levels during these periods. In the nestling phase, however, the values were similar to those of the breeders. This is coherent with the general pattern since both are involved in parental care.

Experimentally elevated T is known to inhibit male incubation (McDonald et al. 2001) or feeding of offspring to varying degrees (Dittami et al. 1991; Saino and Møller 1995; Ketterson et al. 1996; De Ridder et al. 2000; Stoehr and Hill 2000; Peters et al. 2001, 2002), although recent studies have shown that there are other species whose males are insensitive to the T treatment (Van Duyse et al. 2000, 2002; Lynn et al. 2002). The difference in mating system, future mating opportunities, or the possibility of extra-pair copulation, and the importance of paternal care for successful breeding might explain in part this differences (Stoehr and Hill 2000; Lynn et al. 2002; Van Duyse et al. 2000, 2002). In the azure-winged magpie, the T-males reduced their parental effort, with 55% fewer feeding visits relative to the C-males (Fig. 2). Surprisingly, the T-implanted helpers had a 100% increase in feeding visit rate compared with C-helpers. Two explanations could be put forward. First, benefits of helping behavior might at least partially reside on the possibility of shared paternity. In that case, experimental elevation of T levels of helpers could lead to an increased proportion of fathered offspring in the brood (Schoech et al. 1991). The enhanced paternity insurance could therefore motivate a greater feeding effort. Second, if the function of helping is to signal the individual's quality resulting in greater "social prestige" within the group, and hence forming part of the set of sexual signals on which the females will base their mate choice, helpers could be increasing their possibilities for future pairing by signaling their quality as breeders on the basis of raising the frequency of their feeding visits to the chicks (Zahavi 1995; Zahavi and Zahavi 1997).

According to Winkler (1987) for species with biparental care, in the case of a decline in the parental care

taken on by one of the pair, the optimal strategy for its mate would be to attempt compensate the loss at least partially. Thus Saino and Møller (1995) in barn swallow (*Hirundo rustica*) and Ketterson et al. (1992) in dark-eyed junco (*Junco hyemalis*) found at least partial evidence for these models, because the female increased her parental effort when the participation of the male was experimentally reduced. Other studies, however, have failed to find this relationship and the feeding visit behaviour of the females was insensitive to the reduction in paternal effort of the T-males (Schoech et al. 1996a; De Ridder et al. 2000; Stoehr and Hill 2000). In the superb fairy-wren, a cooperative breeding species, not only did Peters et al. (2002) observe no compensation effect but they even found the opposite tendency, i.e., the females paired with T-males, which had reduced their parental effort and also presented a lower feeding visit rate, although to a lesser degree than their mate. Similarly, in the azure-winged magpie, the females with a T-male mate did not compensate for the loss of the male's parental care, but also presented an up to 80% reduction compared to the females of C-males, although the differences were not significant (Fig. 3). The overall reduction in feeding visit rate for the nests with a T-male was 65%. The female may be stimulated by the activity of the male to increase her own parental effort, and reduce it when there is little or no participation by the male. This would be especially important in the first nestling phases when the male also brings food to the female (unpublished data), when the female would have to increase the time spent on her own feeding to the detriment of her direct parental behaviour towards the chicks.

For the T-treated nests, the reduction in feeding the chicks would provoke an increase in their mortality, and hence a correspondingly reduced breeding success (Fig. 4). While at 4 days after hatching, the control nests had a mean of 1.6 chicks per nest, this value was only 0.45 in the T-male nests. At 10 days of age, only one chick survived in all the T-nests, while the mean was 0.75 chicks in the control nests. No chick of the T-nests reached the stage of flight, as against 0.6 chicks in the C-nests. In the azure-winged magpie the participation of the male has to be of great importance for breeding success: on one hand, this is because the male is the member of the family unit who usually takes on the greater parental care (Valencia 2002), and on the other, because his behaviour seems to influence that of the female, which matches her parental investment to that of her mate. The helpers might attempt to make up for any reduction of parental effort in the T-nests, but their presence does not usually begin until 4 or 5 days after hatching, so that they could arrive too late to compensate for the negative effect of the reduction of parental effort of the breeding pair on the breeding success. This question can only be resolved by further experiments in which the breeding males are removed at different ages of the chicks.

Conclusion

Our results lend some support to the behavioral suppression hypothesis and are consistent with previous findings (Valencia et al. 2003), indicating that helping is a behaviorally flexible option, probably influenced by social interactions as stated by hypothesis (3), but also by ecological conditions affecting the probability of a successful breeding attempt. Helpers have similar T levels to breeders, suggesting that their reproductive status might be controlled by both inter- and intra-sexual behavioral interactions. Whereas testosterone provides no benefits in terms of access to reproductive status, it can lead to costs in terms of reduced paternal contribution to nestling care and reduced reproductive success. These selective factors should contribute to maintaining T levels at low values during the period of parental care. T-helpers increase their parental effort because they can use their alloparental behavior as a signal of quality to allow them to acquire greater social prestige within the group or an increased possibility of the paternity of some chick of the clutch.

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References

- Beletsky LD, Gori GH, Freeman DF, Wingfield JC (1995) Testosterone and polygyny in birds. In Power DM (ed) Current ornithology, vol 12. Plenum, New York, pp 1–41
- Braude S, Tang-Martínez Z, Taylor, G (1999) Stress, testosterone, and the immuno-redistribution hypothesis. *Behav Ecol* 10:345–350
- Brown JL (1987) Helping and communal breeding in birds. Ecology and evolution. Princeton University Press, Princeton, N.J.
- Brown JL, Vleck CM (1998) Prolactin and helping in birds: has natural selection strengthened helping behavior. *Behav Ecol* 9:541–545
- Buchanan KL, Evans MT, Goldsmith AR, Bryant DM, Rowe LV (2001) Testosterone influences basal metabolic rate in male house sparrows: a new cost of dominance signalling? *Proc R Soc Lond B* 1474:1337–1344
- Cockburn A (1998) Evolution of helping behavior in cooperatively breeding birds. *Annu Rev Ecol Syst* 29:141–177
- Cramp S, Perrins CM (1994) Birds of the Western Palearctic, vol 8. Oxford University Press, Oxford
- Cruz C (1988) Contribución al conocimiento de la biología del rabilargo (*Cyanopica cyanus*). PhD thesis. Universidad de Extremadura, Spain

- Cruz C, de Lope F, da Silva E (1991) La muda postnupcial en el rabilargo (*Cyanopica cyanus cooki*). *Ardeola* 38:101–115
- Cruz C, de Lope F, Sánchez JM (1992) Postjuvenile moult in the azure-winged Magpie *Cyanopica cyanus cooki*. *Ring Migr* 13:27–35
- De Ridder E, Pinxten R, Eens M (2000) Experimental evidence of a testosterone-induced shift from paternal to mating behaviour in a facultatively polygynous songbird. *Behav Ecol Sociobiol* 40:32–42
- Dittami JP, Hoi H, Sageder G (1991) Parental investment and territorial/sexual behavior in male and female reed warblers: are they mutually exclusive. *Ethology* 88:249–255
- Dufty AM (1989) Testosterone and survival: a cost of aggressiveness? *Horm Behav* 23:185–193
- Emlen ST, Wrege PH (1988) The role of kinship in helping decision among white-fronted bee-eaters. *Behav Ecol Sociobiol* 23:305–315
- Folstad I, Karter AJ (1992) Parasites, bright males and the immunocompetence handicap. *Am Nat* 139:603–622
- Gonzalez B (1996) Comportamiento reproductivo del rabilargo (*Cyanopica cyanus*). PhD thesis. Universidad de Sevilla, Spain
- Grant R (1990) The significance of subadult plumage in Darwin's finches, *Geospiza fortis*. *Behav Ecol* 1:161–170
- Green DJ, Cockburn A, Hall ML, Osmond HL, Dunn PO (1995) Increased opportunities for cuckoldry may be why dominant male fairy-wrens tolerate helpers. *Proc R Soc Lond Ser B* 262:297–303.
- Hatchwell BJ, Komdeur J (2000) Ecological constraints, life history traits and the evolution of co-operative breeding. *Anim Behav* 59:1079–1086
- Hosono T (1966) A study of the life history of Blue Magpie (1). Breeding biology. *Misc Rep Yamashina Inst Ornithol* 4:481–487
- Ketterson ED, Nolan V (1992) Hormones and life histories: an integrative approach. *Am Nat* 140S: 33–62
- Ketterson ED, Nolan V, Cawthorn MJ, Parker PG, Ziegenfus C (1996) Phenotypic engineering: using hormones to explore the mechanistic and functional bases of phenotypic variation in nature. *Ibis* 138:70–86
- Khan MZ, McNabb FMA, Walters JR, Sharp PJ (2001) Patterns of testosterone and prolactin concentrations and reproductive behavior of helpers and breeders in the cooperatively breeding Red-Cockaded Woodpecker (*Picoides borealis*). *Horm Behav* 40:1–13
- Komeda S, Yamagishi S, Fujioka M (1987) Co-operative breeding in azure-winged magpies, *Cyanopica cyana*, living in a region of heavy snowfall. *Condor* 89:835–841
- Lormée H, Jouventin P, Lacroix A, Lallemand J, Chastel O (2000) Reproductive endocrinology of tropical seabirds: sex-specific patterns in LH, steroids, and prolactin secretion in relation to parental care. *Gen Comp Endocrinol* 117:413–426
- Lynn SE, Hayward LS, Benowitz-Fredericks ZM, Wingfield JC (2002) Behavioural insensitivity to supplementary testosterone during the parental phase in the Chestnut-Collared Longspur, *Calcarius ornatus*. *Anim Behav* 63:795–803
- Mays NA, Vleck CM, Dawson J (1991) Plasma luteinizing, steroid hormones, behavioral role and nest stage in co-operatively breeding Harris' Hawks (*Parabuteus unicinctus*). *Auk* 108:619–638
- McDonald PG, Buttemer, WA, Astheimer LB (2001) The influence of testosterone on territorial defence and parental behavior in male free-living Rufous Whistlers, *Pachycephala rufiventris*. *Horm Behav* 39:185–194
- Mumme RL, Koenig, WD, Pitelka FA (1983) Mate guarding in the acorn woodpecker: within group reproductive competition in a co-operative breeder. *Anim Behav* 31:1094–1106
- Peters A (2000) Testosterone treatment is immunosuppressive in superb fairy-wrens, yet free-living males with high testosterone are more immunocompetent. *Proc R Soc Lond B* 267:883–889
- Peters A, Astheimer LB, Cockburn A (2001) The annual testosterone profile in cooperatively breeding superb fairy-wrens, *Malurus cyaneus*, reflects their extreme infidelity. *Behav Ecol Sociobiol* 50:519–527
- Peters A, Cockburn A, Cunningham, R (2002) Testosterone treatment suppress paternal care in superb fairy-wrens, *Malurus cyaneus*, despite their concurrent investment in courtship. *Behav Ecol Sociobiol* 51:538–547
- Poiani A, Fletcher T (1994) Plasma levels of androgens and gonadal development of breeders and helpers in the bell miner (*Manorina melanophrys*). *Behav Ecol Sociobiol* 34:31–41
- Reyer HU, Dittami JP, Hall MR (1986) Avian helpers at nest: are they physiologically castrated? *Ethology* 71:216–228
- Saino N, Møller AP (1995) Testosterone-induced depression of male parental behavior in the bar swallow: female compensation and effects on seasonal fitness. *Behav Ecol Sociobiol* 36:151–157
- Schmidt LG, Bradshaw SD, Follet BK (1991) Plasma levels of luteinizing hormones and androgens in relation to age and breeding status among co-operatively breeding Australian magpie (*Gymnorhina tibicen* Latham). *Gen Comp Endocrinol* 83:48–55
- Schoech SJ, Mumme RL, Moore MC (1991) Reproductive endocrinology and mechanisms of breeding inhibition in cooperatively breeding Florida scrub jays (*Aphelocoma c. coerulescens*). *Condor* 93:354–364
- Schoech SJ, Mumme RL, Wingfield, JC (1996a) Prolactin and helping behaviour in the cooperatively breeding Florida scrub-jay, *Aphelocoma c. coerulescens*. *Anim Behav* 52:445–456
- Schoech SJ, Mumme RL, Wingfield, JC (1996b) Delayed breeding in the co-operatively breeding Florida scrub-jay (*Aphelocoma coerulescens*): inhibition or the absence of stimulation? *Behav Ecol Sociobiol* 39:77–90
- Smith HG (1995) Experimental demonstration of a trade-off between mate attraction and parental care. *Proc R Soc London B* 260:45–51
- Stoehr AM, Hill GE (2000) Testosterone and the allocation of reproductive effort in male house finches (*Carpodacus mexicanus*). *Behav Ecol Sociobiol* 48:407–411
- Trainor BC, Marler A (2001) Testosterone, paternal behavior, and aggression in the monogamous California Mouse (*Peromyscus californicus*). *Horm Behav* 40:32–42
- Valencia J (2002) Factores ambientales y comportamiento reproductor en el rabilargo (*Cyanopica cyanus*). PhD thesis. Universidad de Extremadura, Spain
- Valencia J, Cruz C, Carranza J (2000) Second broods in a Mediterranean cooperatively-breeding corvid: the azure-winged magpie. *Etologia* 8:25–28
- Valencia J, Cruz C, González B (2003) Flexible helping behaviour in the azure-winged magpie. *Ethology* 109:545–558
- Van Duyse E, Pinxten, R, Eens M (2000) Does testosterone affect the trade-off between investment in sexual/territorial behaviour and parental care in male Great Tits? *Behaviour* 137:1503–1515
- Van Duyse E, Pinxten R, Eens M (2002) Effects of testosterone on song, aggression and nestling feeding behavior in male Great Tits *Parus major*. *Horm Behav* 41:178–186
- Vleck CM, Brown JL (1999) Testosterone and social and reproductive behaviour in *Aphelocoma* jays. *Anim Behav* 58:943–951
- Vleck CM, Mays MA, Dawson JW, Goldsmith AR (1991) Hormonal correlates of parental and helping behavior in cooperatively breeding Harris' Hawks (*Parabuteo unicinctus*). *Auk* 108:638–648
- Winkler DW (1987) A general model for parental care. *Am Nat* 130:526–543.
- Wingfield JC, Hegner RE, Dufty AM Jr, Ball GF (1990) The "Challenge hypothesis": theoretical implications for patterns of testosterone secretion, mating system, and breeding strategies. *Am Nat* 136:829–846
- Wingfield JC, Hegner RE, Lewis DM (1991) Circulating levels of luteinizing hormone and steroid hormone in relation to social

- status in the cooperatively breeding white-browed sparrow weaver, *Plocepasser mahali*. *J Zool* 225:43–58
- Woolfenden GE, Fitzpatrick JW (1984) The Florida scrub jay: demography of a cooperatively breeding bird. Princeton University Press, Princeton, N.J.
- Zahavi A (1995) Altruism as a handicap – the limitations of kin selection and reciprocity. *J Avian Biol* 26:1-3
- Zahavi A, Zahavi A (1997) The handicap principle. A missing piece of Darwin's puzzle. Oxford University Press, Oxford