REPRODUCTIVE ENDOCRINOLOGY AND MECHANISMS OF BREEDING INHIBITION IN COOPERATIVELY BREEDING FLORIDA SCRUB JAYS (*APHELOCOMA C. COERULESCENS*)[1]

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Abstract. Although the ecological and evolutionary bases of cooperative breeding have received close scrutiny, few studies have explored the physiological mechanisms responsible for delayed breeding by helpers. We chose the Florida Scrub Jay, *Aphelocoma c. coerulescens,* to determine whether breeders and nonbreeders have different hormone profiles during the reproductive season. We found that male nonbreeders had significantly lower testosterone levels and higher progesterone levels than did male breeders. Nonbreeder and breeder males had similar plasma levels of corticosterone. Elevated plasma levels of progesterone in nonbreeder males may interfere with the reproductive activity at the behavioral or physiological level. Alternatively, progesterone may enable these nonbreeding males to express parental behavior in the absence of a parent/offspring relationship, but the occurrence of the highest levels of progesterone during nest building and egg laying supports the former interpretation. In females, we found that nonbreeders had significantly lower levels of estradiol and corticosterone than breeders. The low levels of primary sex steroid hormones in male and female nonbreeders may physiologically and behaviorally inhibit reproductive activity and suggest that these individuals are reproductively incompetent. The absence of high levels of corticosterone in nonbreeders suggests that this reproductive inhibition is not due to glucocorticoid-mediated stress imposed by dominant breeders.

Key words: Florida Scrub Jay; Aphelocoma c. coerulescens; *reproductive hormones; breeding inhibition; cooperative breeding.*

INTRODUCTION

Cooperative breeding in birds has received considerable attention because it presents an apparent evolutionary paradox. So-called "helpers-at-the nest" (Skutch 1935) in many cooperatively breeding birds delay their own reproductive efforts and instead help a breeding pair rear offspring. Much progress has been made in explaining this apparently altruistic behavior, but these explanations remain controversial (see reviews by Emlen 1984; Emlen and Vehrencamp 1985; Brown 1987; Jamieson and Craig 1987; Jamieson 1989; Koenig and Mumme 1990). Although there has been considerable interest in the ecological and evolutionary factors promot-

ing delayed breeding and helping behavior, there have been few studies of the proximate (physiological) mechanisms involved (Reyer et al. 1986; Wingfield et al., in press; Mays et al., in press). What are the mechanisms that allow otherwise mature birds to forego breeding and yet still exhibit many breeding behaviors, such as feeding nestlings and nest defense?

Three primary causes of breeding suppression demonstrated in vertebrates may also apply to cooperative breeding. First, adrenal glucocorticoids, such as corticosterone, secreted in response to chronic stress may inhibit reproductive physiology and behavior (Moore and Miller 1984, Moore and Zoeller 1985, Wingfield 1988). Second, reproductively competent individuals may be denied access to breeding opportunities (Mumme et al. 1983, Emlen and Wrege 1986, Reyer et al. 1986). For example, males may aggressively defend their mates or, alternately, females may simply refuse to copulate with nonbreeding helper males. Third, due to sexual

immaturity or lack of appropriate stimuli individuals do not produce adequate levels of reproductive hormones to render them reproductively competent (Reyer et al. 1986).

We studied a population of cooperatively breeding Florida Scrub Jays (*Aphelocoma c. coerulescens*) to discriminate between these hypotheses for inhibition of breeding in helpers of this species. We measured the circulating levels of the primary sex steroids (progesterone, testosterone, and estradiol) and the primary adrenal glucocorticoid (corticosterone) in male and female helpers and breeders throughout the reproductive cycle.

MATERIALS AND METHODS

GENERAL METHODS

We studied a population of Florida Scrub Jays adjacent to the population which has been followed by Woolfenden and co-workers at Archbold Biological Station, Highlands County, Florida (27°10′N, 81°21′W, elevation 38–68 m) since 1969 (Woolfenden 1975, Woolfenden and Fitzpatrick 1984). We conducted fieldwork from March through June 1989.

All individuals in the study population could be identified by unique combinations of color bands and numbered aluminum United States Fish and Wildlife Service bands which they received either at the onset of the current study in 1987, following their immigration into the study site, or as nestlings. Birds which were on the site before the beginning of the study and those which immigrated into the tract during the study could be classified as being either older or younger than one year based on their plumage (Pitelka 1945, Bancroft and Woolfenden 1982, Pyle et al. 1987).

Although Florida Scrub Jays exhibit little sexual dimorphism, laparotomies were not conducted because Florida Scrub Jays are federally designated as a threatened species. Determination of sex within a breeding pair is readily accomplished because only females develop brood patches and incubate (Woolfenden and Fitzpatrick 1984). Sex of nonbreeders can usually be determined by one of the following methods: (1) Females exhibit a unique vocalization, a *hiccup*, which is given during territorial displays (Woolfenden and Fitzpatrick 1984). (2) Florida Scrub Jays observe a highly structured within-group dominance hierarchy: because males dominate females, sex can often be determined by observ-

TABLE 1.   Sample sizes in each category. Reproductive stages: build = nest building, lay = egg laying, incubate = incubating, and feed = feeding nestlings.

| Sex | Status | Reproductive stage | | | |
		Build	Lay	Incubate	Feed
Male	Breeder	9	5	9	16
	Nonbreeder	4	3	1	5
Female	Breeder	6	2	6	11
	Nonbreeder	5	6	2	8

ing the outcome of dominance interactions (Woolfenden and Fitzpatrick 1977). (3) Males are generally slightly larger than females, however, no one character reliably separates the sexes. When bill and tail length from birds of known sex (data accumulated over ten years by Woolfenden and co-workers) were considered, discriminant function successfully predicted the sex of 78% (179 of 229) of these birds (Schoech et al., unpubl. manuscript). Further, if only those birds that discriminant function analysis assigned a probability prediction of 0.7 or greater were considered, the accuracy of this method increased to 86% (133 of 155). In 25 of 28 cases, in the current study, the results obtained by discriminant function analysis were confirmed by one of the three other methods for sex determination. Seven birds were classified to sex using solely discriminant function predictions, however, one of these had a discriminant function probability prediction of 0.54. Therefore, this individual was classified as 'sex unknown' and excluded from further analyses. (4) During the subsequent breeding season (March–June 1990) blood samples were collected from thirteen of the birds used in this study. These samples were analyzed by flow cytometry to determine the sex of the individuals (Tiersch et al. 1989; Nakamura et al. 1990; Tiersch et al. 1991). Of the thirteen, one proved to have been classified incorrectly by one or more of the above techniques. For this individual, the sex determined by flow cytometry was used.

Blood samples used in the hormonal analyses were collected from male and female breeders and nonbreeders during the following reproductive stages: nest building (performed solely by the breeding pair), egg laying, incubation and feeding of nestlings (see Table 1 for sample sizes). All blood samples were collected between 09:00 and 12:00 e.s.t. Individuals were trapped in Pot-

ter traps baited with peanuts. Traps were watched continuously and individuals were removed from the trap and bled as rapidly as possible after capture. Time from capture to completion of blood sampling was 5.18 ± 0.11 min (mean $\pm$ SEM, limits 2.25 and 7.5 min). Blood samples were collected in heparinized micro-hematocrit tubes following brachial venipuncture with a 27 gauge needle. Samples were kept cool on ice until return to the laboratory (1–3 hr). Plasma was separated by centrifugation, aspirated and stored at $-20°C$ until shipment on dry ice to Arizona State University for analysis.

EXTRACTION, CHROMATOGRAPHY AND RADIOIMMUNOASSAY

Plasma levels of testosterone, estradiol, corticosterone and progesterone were measured by radioimmunoassay following chromatographic separation on celite microcolumns (Wingfield and Farner 1975, Moore 1986). In brief, the following protocol was used: plasma samples were combined with 2,000 cpm of each radioactive steroid hormone (this allows individual recovery percentages to be calculated). Following overnight equilibration, the plasma/radioactive steroid hormone mixtures were extracted twice with 2 ml of diethyl ether. The ether phase was aspirated, dried under nitrogen gas and resuspended in 0.5 ml isooctane saturated with ethylene glycol. These samples and another 0.5 ml wash of isooctane saturated with ethylene glycol were transferred to columns packed with 1.5 ml of a mixture of celite, propylene glycol and ethylene glycol (4 g celite, 1 ml propylene glycol and 1 ml ethylene glycol) which were fitted with 0.5 ml water traps (3 g celite and 1 ml water). The chromatography effectively separates the steroid hormones from one another as well as removing neutral lipids that may interfere with the radioimmunoassay. Neutral lipids were eluted with 3.0 ml of isooctane and discarded: progesterone was eluted with 4.5 ml of 10% ethyl acetate in isooctane, testosterone with 4.5 ml of 20% ethyl acetate in isooctane, estradiol with 4.0 ml of 40% ethyl acetate in isooctane, and corticosterone with 4.0 ml of 50% ethyl acetate in isooctane. This separation procedure yields purified hormone with less than 3% overlap between adjacent fractions. The elutions were dried under nitrogen gas, resuspended in buffer and assayed using competitive binding techniques (Wingfield and Farner 1975). All resulting values were corrected for individual recovery and plasma volume. All

samples were measured in a single assay: the intra-assay coefficient of variation ($n = 6$) in this system is 7.5% for progesterone, 3.2% for testosterone, 6.6% for estradiol and 12.9% for corticosterone.

STATISTICS

Data were analyzed using two-way analysis of variance (ANOVA) with the status (breeder or nonbreeder) as factors and the four reproductive periods as treatments. However, because the sample size for male nonbreeders during incubation was one (Table 1), this period is omitted from statistical analyses of males. However, all four treatment groups are included in the graphical analyses. Data were tested for homogeneity of variance by Bartlett's test and for normality of distribution by the Kolmogorov-Smirnov test. Data were logarithm transformed as required to control variance (Sokal and Rohlf 1981).

Plasma levels of corticosterone increase rapidly during stress in many vertebrates (Moore and Miller 1984; Greenberg and Wingfield 1987; Wingfield 1988; Moore et al. 1991). To correct for the temporal increase in plasma levels of corticosterone due to the stress of handling, data were analyzed by analysis of covariance (ANCOVA) with time from capture until completion of bleeding as the covariate (Sokal and Rohlf 1981, Neter et al. 1985). The ANCOVA model assumes equivalence of slope for all treatment regression lines (Neter et al. 1985). Therefore, the four treatments (male breeders, male nonbreeders, female breeders and female nonbreeders) were tested for equivalence of slopes when corticosterone was regressed on time (by method described in Zar 1974). Since the slopes of the regression lines for the four treatment groups were not different ($F = 1.46, 0.1 < P < 0.25$), ANCOVA was used for statistical analyses and the residuals from the common regression line were used for graphical analyses.

To determine whether circulating levels of testosterone and estradiol in male and female breeders, respectively, paralleled each other, log transformed hormone values were regressed on time (days from the initiation of the clutch). The slopes from the resultant first order regression lines were then tested for equivalence (Zar 1974).

Post hoc multiple pairwise comparisons were conducted only for differences suggested by ANOVA or ANCOVA (Sokal and Rohlf 1981, Neter et al. 1985). With the exception of the comparisons of equivalency of regression lines
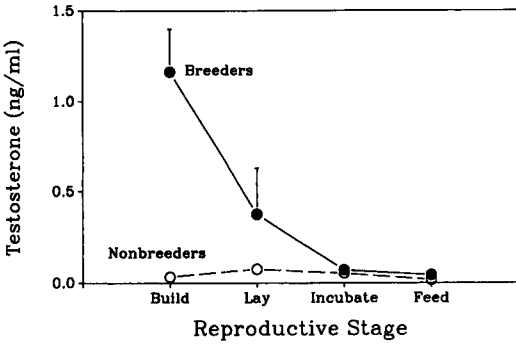
FIGURE 1. Comparison of plasma levels of testosterone in breeder and nonbreeder males throughout the breeding season. Values shown are means ± SEM.

slopes, all statistical analyses were carried out using Crunch Statistical Package (1987).

RESULTS

TESTOSTERONE

Males. Plasma testosterone levels in breeder males were significantly higher than those in nonbreeder males (Fig. 1, $F = 25.81$, $P < 0.0001$). There were also highly significant differences in plasma testosterone levels between reproductive stages ($F = 18.30$, $P < 0.0001$). The between factor interaction was also highly significant ($F = 7.82$, $P < 0.002$). However, this interaction is easily interpreted as the result of the nonparallel changes in plasma levels of testosterone between nonbreeders and breeders and, therefore, does not confound interpretation of the main treatment effects (Fig. 1, see Neter et al. 1985). Multiple pairwise comparisons found that plasma titers of testosterone in breeding males were significantly higher during the nest building stage than while nestlings were present ($P < 0.01$,
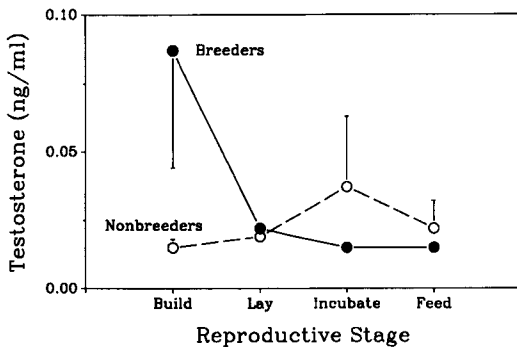

FIGURE 2. Comparison of plasma levels of testosterone in breeder and nonbreeder females throughout the breeding season. Values shown are means ± SEM.
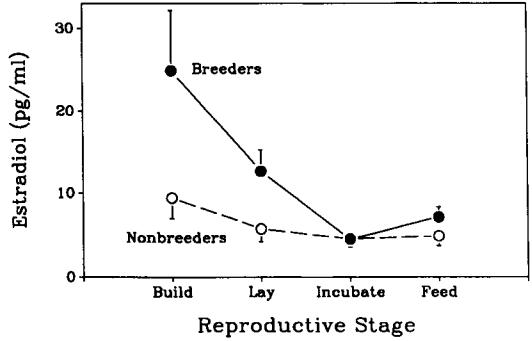

FIGURE 3. Comparison of plasma levels of estradiol in breeder and nonbreeder females throughout the breeding season. Values shown are means ± SEM.

Newman-Keuls). Breeders' plasma levels of testosterone during the nest building stage were higher than those of nonbreeders during the same reproductive stage ($P < 0.01$, Newman-Keuls).

Females. Plasma levels of testosterone did not differ between female breeders and nonbreeders (Fig. 2, $F = 0.81$, $P = 0.38$) nor did levels differ with reproductive stage ($F = 1.67$, $P = 0.18$). There was, however, significant between factor interaction ($F = 3.46$, $P = 0.026$). This interaction is similar to that found for males and is probably due to the decrease in plasma levels of testosterone in female breeders between the nest building and egg laying stages.

ESTRADIOL

Males. Circulating levels of estradiol in all males were below limits of detectability in our assay. Depending on the volume of plasma used and the recovery of estradiol for each individual sample, the limits of detectability for each individual sample ranged from 2.3 to 14.6 pg/ml.

Females. Plasma levels of estradiol in breeding females were significantly higher than in nonbreeder females (Fig. 3, $F = 8.07$, $P = 0.007$). There were also significant differences between reproductive stages ($F = 6.50$, $P = 0.001$). There was no significant between-factor interaction ($F = 1.17$, $P = 0.33$). Breeder females' plasma levels of estradiol were higher during nest building than while they were incubating or feeding nestlings ($P < 0.05$, Newman-Keuls). Breeder females' plasma levels of estradiol were nearly significantly higher than those of helpers during nest building, when levels in both classes of birds were at their peak ($0.05 < P < 0.1$, Newman-Keuls). However, despite the highly significant ANOVA, the conservative multiple pairwise comparisons
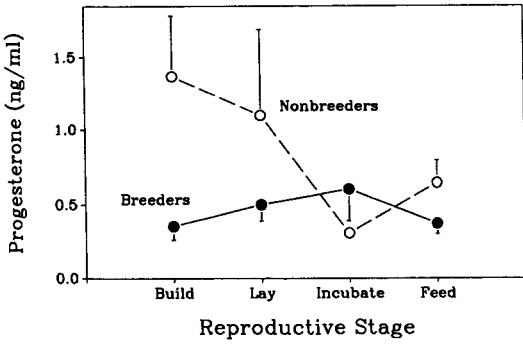
FIGURE 4. Comparison of plasma levels of progesterone in breeder and nonbreeder males throughout the breeding season. Values shown are means ± SEM.
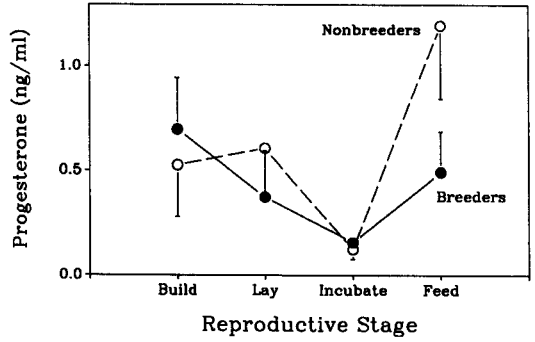

FIGURE 5. Comparison of plasma levels of progesterone in breeder and nonbreeder females throughout the breeding season. Values shown are means ± SEM.

revealed no statistically significant differences between breeders and nonbreeders during any reproductive stage.

There were no differences in plasma levels of estradiol during nest building (when levels were at their peak) between first year nonbreeders and older nonbreeders ($t = 0.47, P = 0.72$). However, small sample sizes ($n = 2$ and 3, respectively) limit the power of this test.

PROGESTERONE

Males. Nonbreeder males had significantly higher plasma levels of progesterone than did breeder males (Fig. 4, $F = 4.80, P = 0.035$). There were no differences in progesterone levels between reproductive stages ($F = 1.31, P = 0.28$). Neither was there a between-factor interaction ($F = 0.095, P = 0.91$). Despite the significant difference detected by ANOVA, the conservative post hoc tests revealed no statistically significant differences between breeders and nonbreeders during any reproductive stage.

Females. There were no differences in plasma levels of progesterone for any of the two-factor ANOVA comparisons (Fig. 5): between status ($F = 0.13, P = 0.72$), between reproductive stages ($F = 2.05, P = 0.12$), nor was there between-factor interaction ($F = 1.36, P = 0.27$).

CORTICOSTERONE

Males. The effect of time on plasma levels of corticosterone was highly significant (Fig. 6, $F = 9.50, P = 0.004$). There was no difference in plasma levels of corticosterone: between breeders and nonbreeders (Fig. 7, $F = 0.59, P = 0.45$); between reproductive stages ($F = 2.71, P = 0.08$); nor was

there a between-factor interaction ($F = 0.78, P = 0.47$).

Females. As was true for males, handling time as a covariate significantly affected plasma levels of corticosterone (Fig. 6, $F = 20.04, P < 0.001$). Breeder females had significantly higher plasma levels of corticosterone than nonbreeder females (Fig. 8, $F = 5.09, P = 0.03$). There were no significant differences between reproductive stages ($F = 1.31, P = 0.28$) nor was there interaction between factors ($F = 0.40, P = 0.76$).

COMPARISON OF MALE TESTOSTERONE AND FEMALE ESTRADIOL LEVELS IN BREEDERS

Inspection of the temporal pattern of plasma testosterone and estradiol in breeder males and females, respectively, reveals that they were highly synchronous (Fig. 9). The slopes of the first order
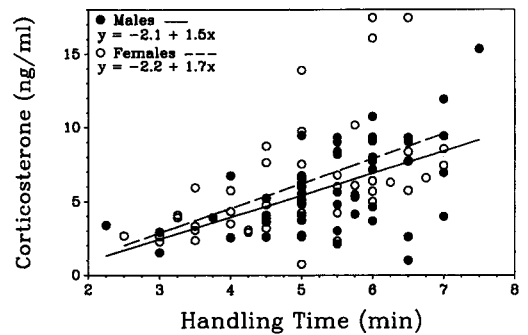

FIGURE 6. Changes in plasma levels of corticosterone in all individuals as a function of stress response to trapping, handling and bleeding. First order regression used to derive residuals.
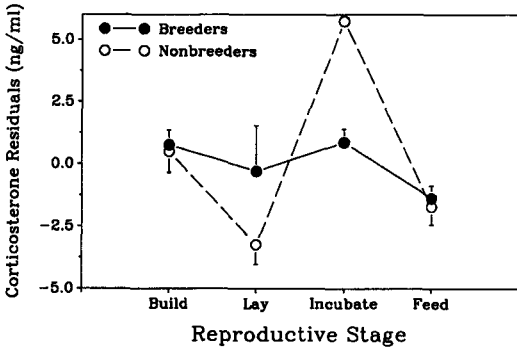
FIGURE 7. Comparison of corticosterone residuals of male breeders and nonbreeders throughout the breeding season. Residuals derived from regression line in Figure 6 correct for handling stress. Values shown are means ± SEM.

FIGURE 9. Plasma levels of testosterone and estradiol in male and female breeders, respectively, relative to the day each individual pair initiated their clutch (day 0). Third order regressions for each sex demonstrate the parallel responses within a pair.

regression lines derived from these hormone values (log$_{10}$ transformed) were nearly identical (b = −0.077, r^2 = 0.53, b = −0.057, r^2 = 0.62; Fig. 10). The equivalence of these slopes (t = −0.34, $0.7 < P < 0.8$) further demonstrates the degree of synchrony within a pair.

DISCUSSION

Nonbreeder male Florida Scrub Jays have much lower levels of circulating testosterone than breeder males. These low levels may be partially responsible for the lack of expression of full reproductive activities in nonbreeders. Low plasma levels of testosterone generally reflect low levels of spermatogenesis in birds (Wingfield and Moore 1987). Reyer et al. (1986) found that male

primary helpers (birds which assist their parents) in cooperatively breeding Pied Kingfishers (Ceryle rudis) had low plasma levels of testosterone and small aspermatogenic testes. In another cooperative breeder, White-browed Sparrow Weavers (Plocepasser mahali), Wingfield et al. (in press) also found that male helpers had significantly lower plasma levels of testosterone and smaller testes than breeder males. Thus, low plasma levels of testosterone throughout the breeding season suggest that in Florida Scrub Jays, male nonbreeders have nonfunctional testes.

In addition to direct physiological effects, testosterone also may mediate reproductive behaviors. Low plasma levels of testosterone could prevent the expression of sexual behavior in male Scrub Jay nonbreeders, as low testosterone levels have this effect in many vertebrates (Balthazart 1983; but see Moore and Kranz 1983; Crews
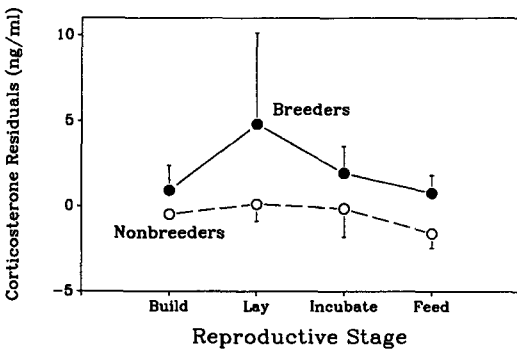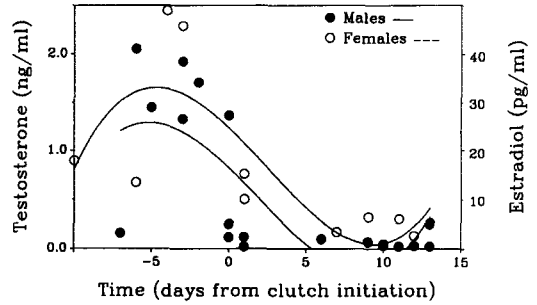

FIGURE 8. Comparison of corticosterone residuals of female breeders and nonbreeders throughout the breeding season. Residuals derived from regression line in Figure 6 correct for handling stress. Values shown are means ± SEM.
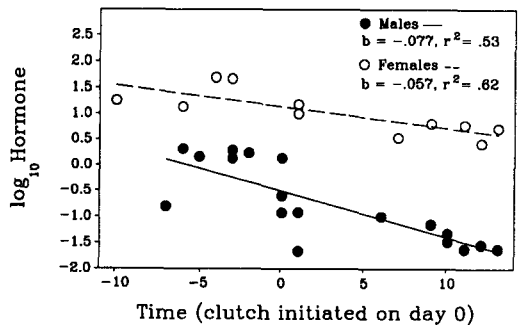

FIGURE 10. Comparison of first order regression lines derived from data presented in Figure 9.

1984; Crews and Moore 1986; Schoech and Matt, submitted). That breeder male Scrub Jays have high testosterone levels when females are receptive and, therefore, when the females are expressing sexual behavior is consistent with this suggestion.

Selection may have favored low levels of testosterone in helper Florida Scrub Jays for several reasons. First, because nearly all Florida Scrub Jay helpers assist at least one parent (Woolfenden and Fitzpatrick 1984), low levels of testosterone and the consequent inhibition of reproductive activity may minimize the probability of incestuous matings by helpers (cf. Mays et al., in press). Second, since dispersal success is low in this habitat-constrained species, nonbreeders may benefit by remaining in their natal territory (Woolfenden and Fitzpatrick 1984, for discussion of habitat-constraint and its role in the evolution of cooperativity see Selander 1964, Brown 1969, Emlen 1982). Elevated plasma levels of testosterone mediate the expression of aggression and, therefore, low levels of circulating testosterone may prevent nonbreeders from initiating or escalating aggressive interactions with the breeding male (cf. Wingfield and Moore 1987, Wingfield et al. 1987). Third, by remaining reproductively incompetent, an individual may not be perceived as a threat to supplant the breeder. By not threatening the breeding male's assurance of paternity the nonbreeding male can delay dispersal and remain within a territory. In the Pied Kingfisher reproductively competent secondary helpers are driven from the nest area by the breeding male until the breeding female has completed laying and is no longer receptive (Reyer et al. 1986).

Our primary interest was why nonbreeders do not breed. However, a related question is the degree to which alloparental behaviors are hormonally mediated. Progesterone plays a role in the initiation of parental behavior in many birds (Silver 1978). Unexpectedly, plasma levels of progesterone in male nonbreeders were elevated during the nest building (only breeders participate in nest building) and egg laying periods rather than when feeding nestlings, the period when nonbreeders express alloparental behavior by defending the nest or by feeding nestlings. This contradicts the idea that elevated progesterone stimulates alloparental behavior in male nonbreeders. Although Florida Scrub Jay male nonbreeders feed nestlings more frequently than female nonbreeders (Stallcup and Woolfenden 1978), the absence of elevated progesterone in breeders of either sex or in female helpers is also inconsistent with an alloparental (or parental) role for progesterone. However, an alloparental role for the elevated circulating levels of progesterone cannot be ruled out. Progesterone may synergize with prolactin to stimulate parental care (Silver 1978, Balthazart 1983). If so, elevated progesterone levels prior to expression of alloparental behavior might sensitize target tissues to the subsequent actions of prolactin. Male auxiliaries of cooperatively breeding Harris' hawks (Parabuteo unicinctus) have elevated prolactin levels when feeding nestlings (Vleck et al., in press), however, there are no differences in progesterone levels.

Alternatively, elevated titers of progesterone may contribute to breeding inhibition in male Florida Scrub Jay helpers. Testosterone-induced courtship behavior in male ring doves (Streptopelia risoria) is inhibited by exogenously administered progesterone (Erickson et al. 1967). Further, exogenous progesterone reduces circulating levels of gonadotropins and acts as an antispermatagen in many species (Ericsson et al. 1964, Ericsson and Dutt 1965, Brenner et al. 1975). Additionally, exogenous progesterone interferes with accessory gland function and thereby decreases semen volume in rams and rabbits (Ericsson et al. 1964, Ericsson and Dutt 1965). Also, progesterone is a testosterone precursor and elevated levels of progesterone may reflect inhibition of testosterone synthesis. It is clear that progesterone can affect sexual and parental behavior through multiple pathways, however, further research is required to elucidate its role in Florida Scrub Jays.

The differences in circulating estradiol levels between female breeders and nonbreeders are similar to the differences in testosterone levels in males. Thus, breeding inhibition in female helpers also appears to be mediated, at least in part, by extremely low levels of a primary sex steroid. Low levels of estradiol in female helper Florida Scrub Jays suggest their ovaries are nonfunctional since active ovaries secrete estradiol. In the absence of sufficient levels of estradiol, neither follicular maturation nor vitellogenesis occur (Balthazart 1983). Elevated plasma levels of estradiol in female birds are also required for expression of courtship, nest building and copulatory behaviors (Moore 1982, Moore and Kranz

1983, Balthazart 1983, Wingfield and Moore 1987).

Two other studies of cooperatively breeding birds support the hypothesis that low levels of estradiol inhibit reproduction through either behavioral or physiological pathways. (1) Wingfield et al. (submitted) found that helper female White-browed Sparrow Weavers had much lower plasma levels of estradiol and significantly smaller ovarian follicles than did breeding females. (2) Female auxiliary Harris' Hawks also had low plasma levels of estradiol (Mays et al., in press). In both of these species breeder females had elevated plasma levels of estradiol during the nest building stage when ovarian growth, vitellogenesis and estradiol-dependent reproductive behaviors occur (Balthazart 1983, 1989).

One hypothesis to explain breeding inhibition in cooperative breeders is the "psychosomatic inhibition" (Carrick 1972) or "psychological castration" hypothesis (Brown 1978, Reyer et al. 1986). This hypothesis implies that the presence of a breeding pair causes nonbreeders to remain sexually quiescent. Wingfield et al. (submitted) suggest that "psychological gonadectomy" may be implemented through dominance interactions between breeders and helpers. Florida Scrub Jay breeders are completely dominant over helpers (Woolfenden and Fitzpatrick 1977). If breeding suppression occurs as a result of within-group dominance interactions, the "psychological castration" hypothesis can be used to predict that subordinate individuals would have higher plasma levels of corticosterone due to the stress of being dominated (Siegel 1980, Harvey et al. 1974, Wingfield 1988). Elevated plasma levels of corticosterone can lower concentrations of circulating testosterone and gonadotropins and inhibit the expression of sexual behavior (Wilson and Follett 1975; Moore and Miller 1984; Moore and Zoeller 1985; Wingfield 1988; Moore et al. 1991).

Contrary to the predictions we generated employing the "psychological castration" hypothesis, we found that nonbreeders had generally lower plasma levels of corticosterone. Female nonbreeders had significantly lower plasma levels of corticosterone than breeders and a similar trend occurred in males but was not significant. Neither Mays et al. (in press) nor Wingfield et al. (in press) found any differences in corticosterone levels between breeders and helpers in their studies of cooperative breeders. Thus, there is no hormonal evidence that glucocorticoid-me-

diated stress of intrasexual dominance is involved in breeding suppression in cooperatively breeding birds.

A second hypothesis for how breeding is inhibited in nonbreeding males of cooperatively breeding species is that reproductively competent males are prevented from sexual activities by breeding males who continuously guard their mates against extra-pair copulations. The findings by ourselves, Reyer et al. (1986, primary helpers only) and Wingfield et al. (in press) of low levels of reproductive hormones are not consistent with this explanation for most cases of cooperative breeding. However, this explanation may apply to secondary helpers in the Pied Kingfisher (Reyer et al. 1986). Secondary helpers are males that are not related to the breeders, have breeder-like levels of testosterone and functional testes. They are denied access to the female during egg laying by the breeding male and not allowed to help until the breeding female is no longer receptive. Additionally, in two other cooperatively breeding species, the Acorn Woodpecker (*Melanerpes formicivorus*) and the White-fronted Bee-eater (*Merops bulockoides*), male breeders mate guard extensively to minimize extra-pair copulations (Mumme et al. 1983, Emlen and Wrege 1986). However, in both of these species the threat of extra-pair copulations comes almost exclusively from other male breeders.

A third explanation for the sexual inactivity of nonbreeders is that due to the inadequate production of reproductive hormones these individuals are physiologically and behaviorally incapable of breeding. The general findings by ourselves and others (see above) of low levels of reproductive hormones and nonelevated levels of adrenal steroids are most consistent with this explanation. There are two possible explanations for how low levels of reproductive hormones occur in nonbreeders.

First, these may be young birds that cannot reproduce until they are older (Skutch 1961, Lack 1966, Rowley 1978, Brown 1985). This, however, does not apply to all species. For example, Reyer et al. (1986) found low plasma levels of testosterone in all ages of male primary helpers in Pied Kingfishers, thus demonstrating the independence of breeding status and age in this species. Wingfield et al. (in press) also conclude that the lack of gonadal maturation in helpers of both sexes in White-browed Sparrow Weavers cannot solely be attributed to age. Sim-

ilarly, we found no age related differences in plasma levels of estradiol in Florida Scrub Jay female nonbreeders. Unfortunately, due to a concurrent experiment that removed older nonbreeders from our study population, we could not make the same comparison for males. There is, however, other evidence that supports the view that age alone does not determine reproductive status in Florida Scrub Jays. First year Florida Scrub Jays (males and females) have bred successfully (Woolfenden and Fitzpatrick 1984; Webber and Cox 1987, unpublished data) and some individuals have bred one year and helped the next (Woolfenden and Fitzpatrick 1984). Although uncommon, these occurrences demonstrate that some Florida Scrub Jays are physiologically capable of reproducing in their first year posthatching and suggest that factors other than age determine breeding status.

A second explanation for low levels of reproductive hormones in nonbreeders is that they forego breeding either to prevent inbreeding or, alternately, to increase the likelihood that they will be allowed to share an already occupied territory (see discussion above). For convenience, we will refer to this explanation as the "assessment" hypothesis. An individual may "assess" its options (i.e., territory or mate availability) and either become reproductively active or remain reproductively quiescent depending upon the circumstances. The difference between this hypothesis and the psychological castration hypothesis is subtle but critical: the psychological castration hypothesis implies that an individual's inability to reproduce is extrinsically enforced via dominance interactions (see above and see discussion by Reyer et al. 1986). The assessment hypothesis implies that a helper may "choose" to remain reproductively incompetent in the presence of a breeding pair only as an alternate strategy in the absence of other options. The hormonal data collected in all species so far are most consistent with the assessment hypothesis since there are few indications of age-related correlations and few indications that nonbreeders are physiologically stressed. Furthermore, our data on the close synchrony between hormone levels of breeding males and females suggests a proximate explanation for this effect. Interactions among breeding pairs are known to be important in stimulating and synchronizing reproductive activity in many taxa (Harding 1981, also see review by Wingfield and Moore 1987). The synchrony of testosterone and estradiol lev-

els in males and females, respectively, suggests the same for breeder Florida Scrub Jays. Lack of participation in the formation and maintenance of the pair bond may prevent increases in reproductive hormones in nonbreeders.

Despite differences, many cooperatively breeding species share common life history traits that may promote the evolution and maintenance of this social system. Many cooperative breeders live under severe ecological constraints, hold year-round territories, are nonmigratory, and live in tropical or subtropical climates. The similarity in ecological conditions that promote cooperative breeding in otherwise phylogenetically diverse species has apparently promoted similarities in the underlying endocrine mechanisms. Nonbreeders generally have low levels of sex steroid hormones, hormone profiles of breeders are comparable to those of non-comparative species, age by itself is a poor predictor of breeding status, and nonbreeders have the same or lower levels of adrenal glucocorticoids as breeders. Thus, in all species in which the reproductive endocrine profiles have been studied, the inhibition of breeding appears to be mediated by low levels of reproductive hormones rather than by elevated glucocorticoids or by denial of breeding opportunities by nonhormonal mechanisms. This reproductive incompetence may increase the likelihood of nonbreeders being allowed to remain in their natal territory while decreasing the probability of potentially deleterious incestuous matings.

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