

Patterns of Testosterone and Prolactin Concentrations and Reproductive Behavior of Helpers and Breeders in the Cooperatively Breeding Red-Cockaded Woodpecker (*Picoides borealis*)

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We examined the proximate causes of delayed breeding and alloparental behavior in the cooperatively breeding red-cockaded woodpecker by measuring plasma concentrations of testosterone (T) and prolactin (PRL) in female breeders, male breeders, and male helpers during different stages of the reproductive cycle. Male breeders and helpers have low T during the prebreeding period, highest T during copulation, and low concentrations of T during the egg-laying/incubation and nestling provisioning stages. Helpers appear physiologically capable of reproducing; their T concentrations equal that of male breeders. Helpers unrelated to the breeding female have higher T than helpers sharing a territory with their mother. Sexual inactivity by male helpers might be explained by behavioral suppression resulting from interactions of male helpers with the breeding pair that somehow differ in accordance with the helper's relatedness to the breeding female (e.g., female breeders are generally unreceptive to courtship from male helpers and least receptive to related helpers). Female breeder, male breeder, and male helper PRL levels did not differ and increased from the prebreeding stage through the copulation and egg-laying/incubation stages. During the nestling provisioning stage, male breeder and helper PRL declined, while female PRL continued to increase. Based on these results, we conclude that the physiological bases of alloparental behavior have not diverged

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Key Words: testosterone; prolactin; delayed breeding; helping behavior; red-cockaded woodpecker; *Picoides borealis*.

In many cooperative breeding systems among birds and mammals “helpers” delay breeding while they help raise young that are not their offspring (Emlen and Vehrencamp, 1983; Brown, 1987). Although the ultimate causes for delayed breeding and alloparental behavior in such species have been closely examined (Brown, 1987; Koenig and Mumme, 1990; Emlen, 1991; Cockburn, 1998), the proximate mechanisms underlying cooperative breeding behavior have not been thoroughly evaluated in many species. Reproductive behaviors in males of most avian and mammalian species are strongly correlated with elevated plasma testosterone concentrations (Wingfield and Farner, 1993) while low concentrations of reproductive hormones appear to be the proximate bases of sexual inactivity in mammalian (Creel, Creel, Wildt, and Monfort, 1992; Roberts, Zullo, Gustafson, and Carter, 1996) and some avian cooperatively breeding species (Reyer, Dittami, and Hall, 1986; Mays, Vleck, and Dawson, 1991; Schmidt, Bradshaw, and Follett, 1991; Schoech, Mumme, and Moore, 1991; Wingfield, Hegner, and Lewis, 1991; Schoech, Mumme, and Wingfield, 1996a; Poiani and Fletcher, 1994). Parental behavior in mammals, birds, and fish is correlated with

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elevated plasma prolactin concentrations (reviewed in de Vlaming, 1979; Buntin, 1996). Prolactin appears to play a role in the proximate control of alloparental behavior in cooperatively breeding species (Vleck, Mays, Dawson, and Goldsmith, 1991; Schoech, Mumme, and Wingfield, 1996b; Brown and Vleck, 1998). How these hormones are involved in the mediation of sexual behavior and alloparental behavior of helpers is an important question in cooperative breeding research.

Before hormonal data were available, various authors suggested several proximate causes of the sexual inactivity of helpers (Rowley, 1965; Brown, 1978). For example, it has been hypothesized that male helpers exhibit low testosterone concentrations and remain sexually inactive because they are (1) reproductively immature (delayed maturation, Brown, 1978), (2) in poor body condition (physiological suppression, Schoech, Mumme, and Wingfield, 1997; Wingfield *et al.*, 1991), or (3) not receiving appropriate stimulation from females (Poiani and Fletcher, 1994; Schoech *et al.*, 1996a). Alternatively, helpers may be reproductively capable, but may be prevented from reproducing because of social interference (behavioral suppression, Brown, 1978; Mumme, Koenig, and Pitelka, 1983; Emlen and Wrege, 1988). Endocrine studies of cooperatively breeding birds provide little support for the delayed maturation or physiological suppression hypotheses (Reyer *et al.*, 1986; Mays *et al.*, 1991; Schmidt *et al.*, 1991; Schoech *et al.*, 1991, 1997; Wingfield *et al.*, 1991; Poiani and Fletcher, 1994). Currently, the hypotheses that best explain the absence of sexual behavior by male helpers are behavioral suppression and absence of appropriate stimulation (Reyer *et al.*, 1986; Mays *et al.*, 1991; Poiani and Fletcher, 1994; Schoech *et al.*, 1996b).

The theoretical framework for research on the proximate causes of alloparental behavior is less complete than that for delayed breeding. The mediation of incubation and care of nestlings by prolactin involves a complex interplay with steroid hormones and environmental stimuli (Buntin, 1996). Work on other cooperatively breeding species indicates that prolactin concentrations of male helpers may rise prior to exposure to nestlings (Schoech *et al.*, 1996b), or they may differ from those of breeders by increasing only after stimulation from observing eggs and/or nestlings (Vleck *et al.*, 1991).

We measured plasma concentrations of testosterone and prolactin in female breeders, male breeders, and male helpers in the red-cockaded woodpecker (*Picoides borealis*) during different stages of the reproduc-

tive cycle. Red-cockaded woodpeckers are both socially and genetically monogamous (Haig, Walters, and Plissner, 1994). They live in groups consisting of a single breeding female, a dominant breeding male, and zero to four subordinate, nonbreeding helpers (Ligon, 1970; Walters, Doerr, and Carter, 1988; Khan and Walters, 1997). Seventy percent of breeding groups do not have helpers and of the 30% of groups with helpers, most have only a single helper (Walters *et al.*, 1988; Walters, Doerr, and Carter, 1992). Helpers are usually male offspring of the breeding pair that delay dispersal and remain on their natal territory (Walters, 1990). Because turnover of breeding females is higher than that of breeding males (Walters *et al.*, 1988), helpers often are related to the male breeder but are unrelated to the female breeder. Occasionally they help male breeders to which they are unrelated (5%). Helpers that inherit breeding status on their natal territory (14% per year Walters *et al.*, 1992) will pair with the resident female only if she is unrelated to them (Walters *et al.*, 1988; Daniels and Walters, 2000). The individuals in a group forage peaceably together and defend the same territory. Helpers participate in defending territories, constructing and maintaining nest and roost cavities, incubating eggs, feeding and brooding nestlings, removing fecal sacs from the nest cavity, and feeding fledglings (Walters, 1990; Jackson, 1994).

We compared plasma testosterone concentrations of male helpers to those of male breeders to determine whether red-cockaded woodpecker helpers had low plasma testosterone concentrations. We measured variables that reflect body condition to determine whether helpers were in poorer body condition than breeders. To assess the physiological bases of alloparental behavior, we compared plasma prolactin concentrations of breeders with those of male helpers and examined the correlation between prolactin concentration and contribution to nestling care by individual birds.

METHODS

Study Area and Study Species

The study population of red-cockaded woodpeckers is located in the Sandhills region of south-central North Carolina and contains approximately 250 groups of birds. The Sandhills population inhabits roughly 110,000 ha, which includes portions of Fort Bragg Military Reservation, the Sandhills Gamelands,

TABLE 1Date and Mean Number of Days (Mean \pm SE) before/after Clutch Initiation for Each Sampling Stage

| Stage | | 1997 | 1998 | 1999 |
|-----------------------|-------------------------------|----------------|----------------|------------------|
| Prebreeding | Dates | 17–22 February | 9–12 February | |
| | Days before clutch initiation | -65 ± 1.7 | -76 ± 2.4 | |
| Copulation | Dates | 14–25 April | 24–28 March | 31 March–5 April |
| | Days before clutch initiation | -11 ± 1.8 | -30 ± 1.1 | -30 ± 1.7 |
| Lay/incubation | Dates | 26 April–8 May | 23 April–6 May | |
| | Days after clutch initiation | 9 ± 0.9 | 6 ± 0.7 | |
| Nestling provisioning | Dates | 8–21 May | 6–20 May | |
| | Days after clutch initiation | 26 ± 0.5 | 26 ± 0.5 | |

Note. Blood samples were collected during the following stages: (1) prebreeding, when no breeding activity was evident; (2) copulation, the 5–6 weeks preceding clutch initiation (Jackson, 1994); (3) lay/incubation, time period between clutch initiation and hatching; and (4) nestling provisioning, 10–16 days after hatching.

and the resort towns of Southern Pines and Pinehurst. The birds are color-banded and a census is conducted annually during the breeding season. Each year all unbanded immigrant adults and nestlings are caught and banded. Reproduction is monitored by visiting trees with nest cavities every 9–14 days during the breeding period. Detailed descriptions of the study area and monitoring methods are found in Carter, Stamps, and Doerr (1983) and Walters *et al.* (1988).

Blood Sampling and Field Measurements

We collected blood samples from female breeders, male breeders, and male helpers during the prebreeding, copulation, egg-laying/incubation, and nestling provisioning stages of the annual cycle in 1997 and 1998 and from the copulation stage in 1999 (Table 1). In this study, the status (i.e., helper or breeder) assigned to an individual was based on several criteria described in detail in Walters *et al.* (1988). These criteria include status in the previous year, age, kin relationships among males in a group, dominance status, and interactions with the breeding female. DNA fingerprinting of a sample of 224 individuals confirmed that only one male in a group breeds with the female (Haig *et al.*, 1994) and verified the accuracy of the behavioral criteria used to assign status. Prebreeding and copulation stages were determined from previous data from the Sandhills red-cockaded woodpecker population. Prebreeding is assigned to any sample collected greater than 5 weeks prior to egg-laying. Copulation typically begins 5–6 weeks prior to egg-laying (Jackson, 1994) and egg-laying usually begins the third week (± 1 week) of April in the Sandhills population (Walters *et al.*, 1988). We lumped the egg-laying and incubation stages because the majority (35/

40) of the copulation stage samples were collected more than 20 days prior to clutch initiation, whereas egg-laying and incubation occur within a short window of time (4 days for egg-laying, 10–11 days of incubation). The egg-laying/incubation and nestling provisioning stages were determined by locating nests and checking nest contents with a mirror and light every 9–14 days. We located nests by checking for birds flushing from tree cavities during the day. If a bird flushed, we climbed the tree and checked the cavity for the presence of eggs. Clutch size is 3–4 eggs (mean = 3.27 eggs, LaBranche and Walters, 1994). The estimated date the first egg was laid was calculated by aging nestlings using the criteria in Ligon (1970). We assumed that a single egg is laid each day and the eggs are incubated for 11 days following laying of the last egg (LaBranche and Walters, 1994).

Birds were captured in their roosting cavities by placing a net attached to a telescoping pole over the cavity entrance and banging the trunk of the tree to flush the bird into the net. To avoid diel variation in hormone concentrations, all birds were sampled in the evening from dusk to approximately 3 h after dusk. In February sampling occurred between 1800 and 2100, and from April through May sampling occurred between 1900 and 2200. Handling time was measured from the time the net was placed over the cavity entrance to the time a blood sample was collected: handling time was 11 ± 0.31 min (mean \pm SE; $n = 171$).

Blood samples (approximately 300 μ l) were collected in heparinized microhematocrit tubes from wing veins (vena ulnaris), punctured with a 26-gauge needle. Hematocrit tubes were sealed with S/P Miniseal (Baxter) and placed on ice in a cooler. Samples were centrifuged within 5 h of collection, and the

plasma was separated from the red blood cells and frozen in O-ring sealed vials. Frozen samples were transported to Virginia Tech and stored at -80°C until analysis. To assess body condition we measured wing-chord length (millimeters) and body mass (grams). We noted the presence or absence of a brood-patch because brood-patch development is dependent on both circulating steroid hormones and prolactin in most avian species (Buntin, 1996). We qualitatively correlate our observations of brood-patches with observed seasonal patterns of plasma testosterone and prolactin concentration.

Hormone Analyses

Plasma testosterone (T) concentrations were measured by radioimmunoassay (RIA) following extraction in diethyl ether, drying under nitrogen gas, reconstitution in 10% ethyl acetate in iso-octane, and chromatographic separation on diatomaceous earth/glycol/water microcolumns. Column chromatography was used to remove neutral lipids and separate steroid hormones (methods from Wingfield and Farner, 1975; Ball and Wingfield, 1987). Neutral lipids were removed in 1.5 ml of 100% iso-octane, 5- α -dihydrotestosterone was removed in 2.0 ml of 10% ethyl acetate in iso-octane, and testosterone was collected in 2.7 ml of 20% ethyl acetate in iso-octane. The testosterone fraction was dried under nitrogen gas and reconstituted in 500 μl of buffer. To determine hormone recovery, 2000 cpm radiolabeled testosterone was placed in each sample prior to extraction and chromatography.

The reconstituted testosterone fraction was divided into duplicate samples in the RIA, and a 50- μl aliquot was counted to measure recovery of testosterone. Testosterone concentrations determined in the RIA were then corrected for individual column recovery ($91.9 \pm 0.52\%$, mean \pm SE). The standard curves ranged from 0.02 to 0.50 ng/ml. Sample plasma testosterone concentrations were measured in seven assay runs. Each assay run included four distilled water blanks and six replicates of the 0.10 ng/ml standard. If the water blanks were not equivalent to the standard without hormone in the RIA, the blank measurements were averaged and subtracted from each sample value before measurements were adjusted for recovery and sample volume. The inter- and intra-assay coefficients of variation for testosterone were 16.9% ($n = 7$ assay runs) and $12.8 \pm 2.9\%$ (mean \pm SE; $n = 7$ runs, six standard replicates within each run), respectively.

Radiolabeled testosterone was purchased from Du-

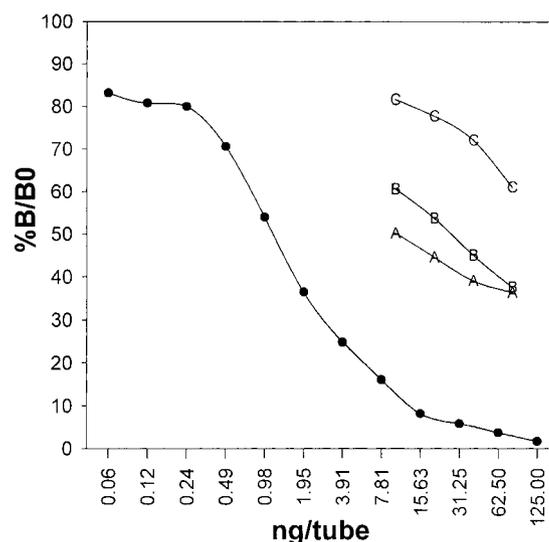


FIG. 1. Displacement of the binding of ^{125}I -labeled-recombinant-derived dove prolactin to anti-dove prolactin antibody by recombinant dove prolactin standard (filled circles), and red-cockaded woodpecker plasma pools with high (A), intermediate (B), and low (C) concentrations of prolactin.

Pont New England Nuclear (Wilmington, DE; T specific activity = 121 Ci/mmol). Acid-washed diatomaceous earth was purchased from Sigma (Celite D-5384). Testosterone standards were generously donated by M. S. Khan (St. Luke's Roosevelt Hospital, New York, NY). Goat antibody raised against rabbit testosterone was purchased from Wien Laboratories (Succasunna, NJ).

Plasma prolactin (PRL) was measured with a dove prolactin radioimmunoassay (P. J. Sharp and R. T. Talbot, unpublished results). A cDNA encoding ring dove (*Streptopelia risoria*) prolactin (Howarth, Goldsmith, and Sharp, 1994) was used to make recombinant-derived dove prolactin and to develop a radioimmunoassay as described by Talbot and Sharp (1994). The assay showed parallelism with three pools of woodpecker plasma selected for predicted high, intermediate, and low concentrations of prolactin (Fig. 1). Woodpecker samples were run in duplicate. Individual sample volumes varied, but were brought up to a constant volume of 100 μl with RIA buffer. The standards ranged between 0.06 and 125 ng/tube. All samples were run in a single assay with intra-assay variation of 6.2% ($n = 3$).

Nestling Provisioning Behavior

To examine the relationship between an individual's contribution to nestling care and its plasma pro-

lactin concentration, nests with 10- to 16-day-old young were observed for 2 h on the afternoon (1200–1800) of the evening capture and sampling. We observed 13 nests in 1997 and 17 nests in 1998. Individuals were identified by their unique color band combination using a spotting scope from a distance of approximately 20 m. The rate that an adult fed nestlings (nestling provisioning rate) was measured as the number of times an individual fed nestlings per hour. A nest visit was tallied as a feeding event if the individual entered the nest cavity with food in its bill and left the cavity with an empty bill.

Statistical Analyses

Seasonal variation of plasma hormone concentrations was analyzed with analysis of variance (ANOVA), followed by Tukey pairwise comparisons when appropriate. Mann–Whitney *U* test and Spearman rank correlation were used where indicated. Hormone concentrations and nestling provisioning data were transformed to meet assumptions of normality (Zar, 1996). Nonparametric statistical analyses were performed using Minitab 10.5; SAS 6.12 statistical software was used for all parametric tests. Significance level for all tests was set at $P \leq 0.05$. Means are shown with standard errors unless otherwise noted.

RESULTS

Body Condition

The linear regression model of body mass as a function of wing-chord length was significantly different from zero [mass = 0.272 (chord length) + 16.22; $F_{1,165} = 8.17$, $P = 0.005$]. Body condition measured as residuals did not differ among male helpers, male breeders, and female breeders.

Testosterone

Year ($F_{1,127} = 17.41$, $P = 0.0001$), status ($F_{2,127} = 4.17$, $P = 0.0176$), and reproductive stage ($F_{3,127} = 18.77$, $P = 0.0001$) and an interaction between status and stage ($F_{6,127} = 2.69$, $P = 0.0171$) significantly influenced plasma testosterone concentrations. Female testosterone concentrations were significantly lower than those of male breeders and helpers (Tukey's, $P < 0.05$). Therefore, in subsequent tests, males and females were analyzed separately.

Males. Male breeders and male helpers had equivalent plasma concentrations of testosterone. Plasma testosterone concentrations were significantly influenced by year ($F_{2,100} = 6.69$, $P = 0.002$) and reproductive stage ($F_{3,100} = 16.32$, $P = 0.0001$; Fig. 2). Plasma testosterone concentrations were higher in 1997 than in 1998 and 1999 ($P < 0.05$, Tukey's). Among male breeders and male helpers plasma testosterone concentrations were significantly higher during the copulation stage than during the prebreeding, egg-laying/incubation, and nestling provisioning stages ($P < 0.05$, Tukey's). Interactions between status, stage, and year were not significant. Male helpers unrelated to the breeding female had higher plasma testosterone concentrations than helpers related to the breeding female (Mann–Whitney two-tailed $U = 173.0$, $P = 0.03$; Fig. 3).

Females. Female plasma concentrations of testosterone did not vary across breeding season stages ($F_{3,42} = 1.85$, $P = 0.1526$) but varied between years ($F_{1,42} = 8.88$, $P = 0.0048$; Fig. 2). Concentrations in samples collected in 1997 were higher than those in samples collected in 1998 (Tukey's $P < 0.05$).

Annual variation in testosterone. Between-year differences in testosterone concentration were the results of a few high values from samples collected from male breeders and helpers during the copulation stage in 1997 (Fig. 2). Testosterone concentration might change in relation to the time of laying and birds were sampled closer to egg-laying in 1997 than in 1998 and 1999 (Table 1). However, the data we collected in 1999 to test this idea do not support it. The highest testosterone concentration measured in 1997 occurred 20–25 days prior to egg-laying (Fig. 4). Samples collected 20–25 days prior to egg-laying in 1999 did not exhibit similarly high concentrations of testosterone.

Prolactin

Plasma prolactin concentrations did not vary between years; therefore, all subsequent analyses pooled samples from 1997 and 1998. Prolactin concentrations increased as the breeding season progressed ($F_{3,111} = 102.78$, $P = 0.0001$). Prolactin concentrations were lowest during the prebreeding stage and increased through the copulation and egg-laying/incubation stages (Tukey's test: prebreeding < copulate < lay/incubation, $P < 0.05$ for all comparisons; Fig. 5). During the nestling provisioning stage, female prolactin concentration continued to increase, while the prolactin concentrations of male breeders and helpers declined (Fig. 5). Because the interaction between sta-

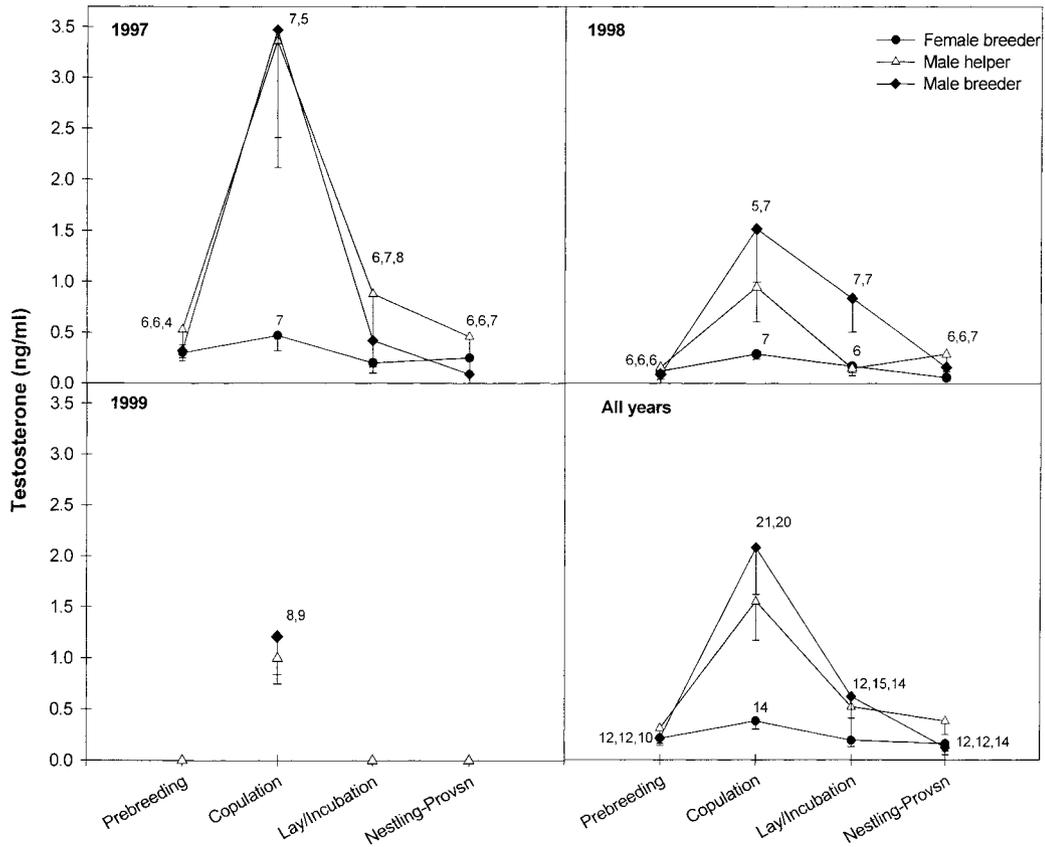


FIG. 2. Plasma testosterone concentrations (mean – SE) of female breeders, male breeders, and male helpers in 1997, 1998, 1999, and all years combined. Sample sizes are indicated on the plots; where means are too close to distinguish visually, sample sizes are listed in the following sequence: female breeder, male breeder, male helper; e.g., 6, 6, 4 during 1997 prebreeding stage represents 6 female breeders, 6 male breeders, and 4 male helpers. Note: only males were sampled in 1999 during the copulation stage.

tus and reproductive stage approached significance ($F_{6,111} = 2.18, P = 0.0502$), we conducted a post hoc ANOVA of data from the nestling provisioning stage. This analysis revealed that females had significantly higher prolactin concentrations than either male breeders or helpers ($F_{2,31} = 10.61, P < 0.0001$; female breeders > male breeders = male helpers, Tukey's, $P < 0.05$).

Brood-Patch

Each member of a group developed a large brood-patch during the breeding season. During the copulation period, while prolactin concentrations were increasing and testosterone was peaking (in males), female breeders, male breeders, and male helpers were observed with completely defeathered and vascularized brood-patches that extended from the neck to the cloaca. The brood-patch was edemous during the incubation stage when plasma prolactin concen-

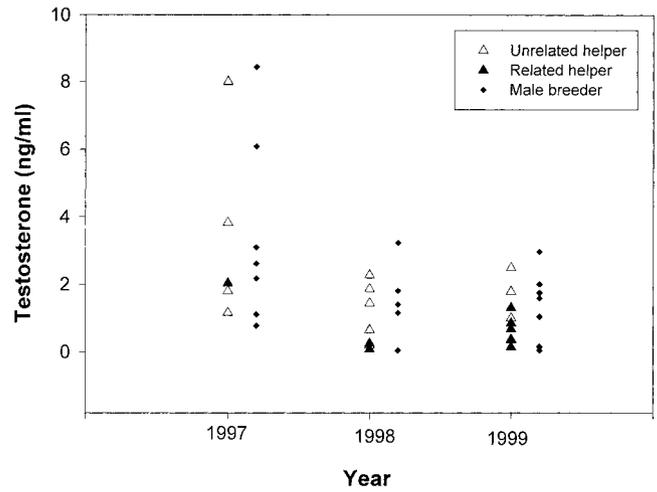


FIG. 3. Plasma testosterone concentrations of helpers related and unrelated to the female breeder during the copulation stage. Plasma testosterone concentrations of male breeders are plotted to the right of the male helper values. Each point represents an individual.

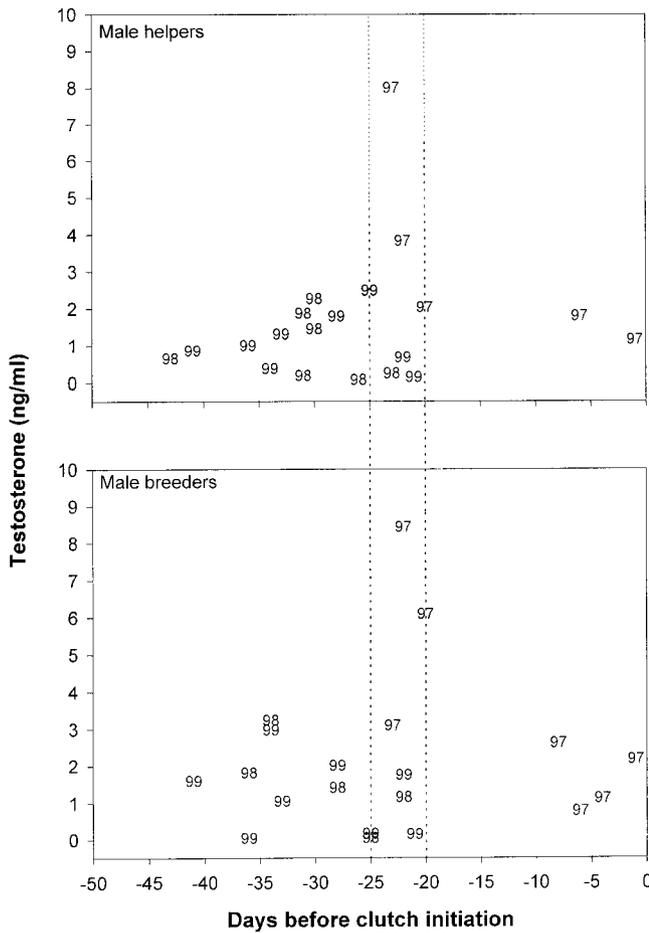


FIG. 4. Plasma testosterone concentrations of male breeders and male helpers plotted as a function of days before clutch initiation during the copulation stage. Symbols indicate the year in which the sample was collected. The time period between 20 and 25 days before egg-laying is highlighted to illustrate that high testosterone concentrations measured at that time in 1997 were not duplicated in other years.

trations were high and brood-patches of helpers appeared identical to those of breeders.

Prolactin and Nestling Provisioning Behavior

Whether an individual was a breeder or helper significantly affected the rate at which an individual provisioned nestlings ($F_{2,86} = 6.73, P = 0.002$; Fig. 6). Male and female breeders fed nestlings at equivalent rates, whereas male helpers provisioned nestlings at significantly lower rates than breeders of either sex (Tukey's, $P < 0.05$). During the nestling provisioning stage, when plasma prolactin concentrations are high in all group members, the rate that each adult fed nestlings did not correlate with its prolactin concentration (Fig. 7): female breeders (Spearman's $\rho =$

$-0.540, n = 11, P > 0.05$), male breeders (Spearman's $\rho = -0.301, n = 9, P > 0.20$), and male helpers (Spearman's $\rho = 0.073, n = 11, P > 0.50$). In fact, the trend among breeders was toward less feeding by individuals with higher prolactin concentrations.

The number of nestlings did not correlate with prolactin concentration in female breeders, male breeders, or male helpers. Brood sizes ranged from three to four, from one to four, and from two to four for female breeders, male breeders, and male helpers, respectively. Nestling age was significantly correlated with prolactin concentration in female breeders (Spearman's $\rho = 0.647, n = 11, P < 0.05$) and tended to be in male breeders. No relationship between prolactin concentra-

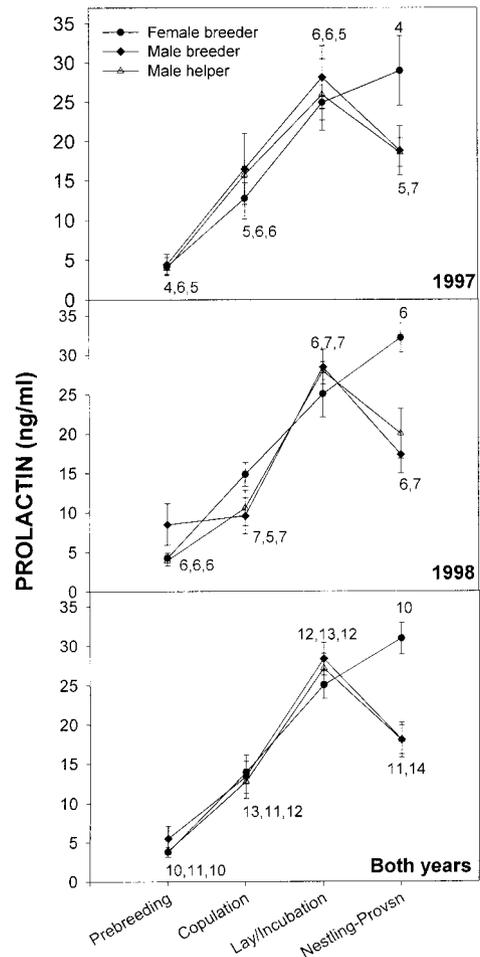


FIG. 5. Plasma prolactin concentrations (mean \pm SE) of female breeders, male breeders, and male helpers in 1997, 1998, and both years combined. Sample sizes are indicated on the plots; where means are too close to distinguish visually, sample sizes are listed in the following sequence: female breeder, male breeder, male helper; e.g., 4, 6, 5 during 1997 prebreeding stage represents 4 female breeders, 6 male breeders, and 5 male helpers.

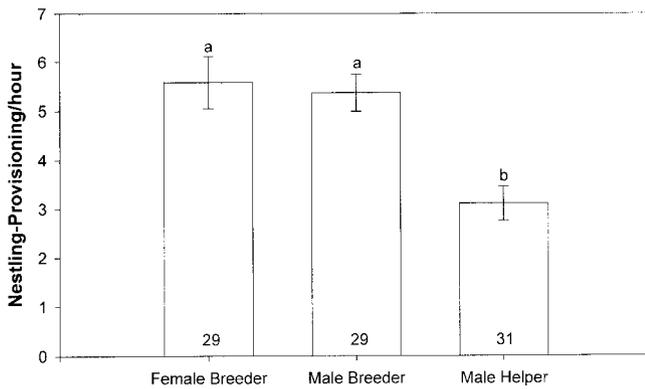


FIG. 6. Rates of nestling provisioning (mean \pm SE) by female breeders, male breeders, and male helpers in red-cockaded woodpeckers. Sample sizes are indicated at the bottom of each bar and lowercase letters indicate which comparisons were significantly different ($P < 0.05$).

tion and nestling age was evident among male helpers. Plasma prolactin concentration does not significantly correlate with the number of eggs in a clutch in female breeders, male breeders, or male helpers.

Hormone Concentration and Handling Time

Handling time did not influence plasma hormone concentrations of male or female breeders during any stage of the reproductive cycle. However, plasma testosterone concentrations showed small but significant decreases as handling time increased in male helpers sampled during the incubation stage [$\log T = -0.08$ (handling time) + 0.55; $F_{1,13} = 5.70$, $P = 0.03$]. Prolactin significantly increased with handling time in male helpers during the prebreeding stage [$\log PRL = 0.04$ (handling time) + 0.15; $F_{1,8} = 5.94$, $P = 0.04$] and incubation stage [$\log PRL = 0.02$ (handling time) + 1.08; $F_{1,11} = 8.01$, $P = 0.02$]. The most likely effect of handling time is to increase variability around the mean, thereby obscuring our ability to detect small differences between helpers and breeders or males and females. Since male breeders and male helpers exhibit equivalent hormone concentrations during reproductive stages in which no effects of handling time were detected, we conclude that the effects of handling time do not confound our overall interpretation of the hormone results.

DISCUSSION

Behavioral Suppression of Reproduction by Helpers

Because the delayed maturation, physiological suppression, and absence of stimulation hypotheses pre-

dict that helpers will exhibit testosterone concentrations lower than those of male breeders, our results are most consistent with the behavioral suppression hypothesis. Additionally, previous observations of helpers that assume a vacant breeding position and imme-

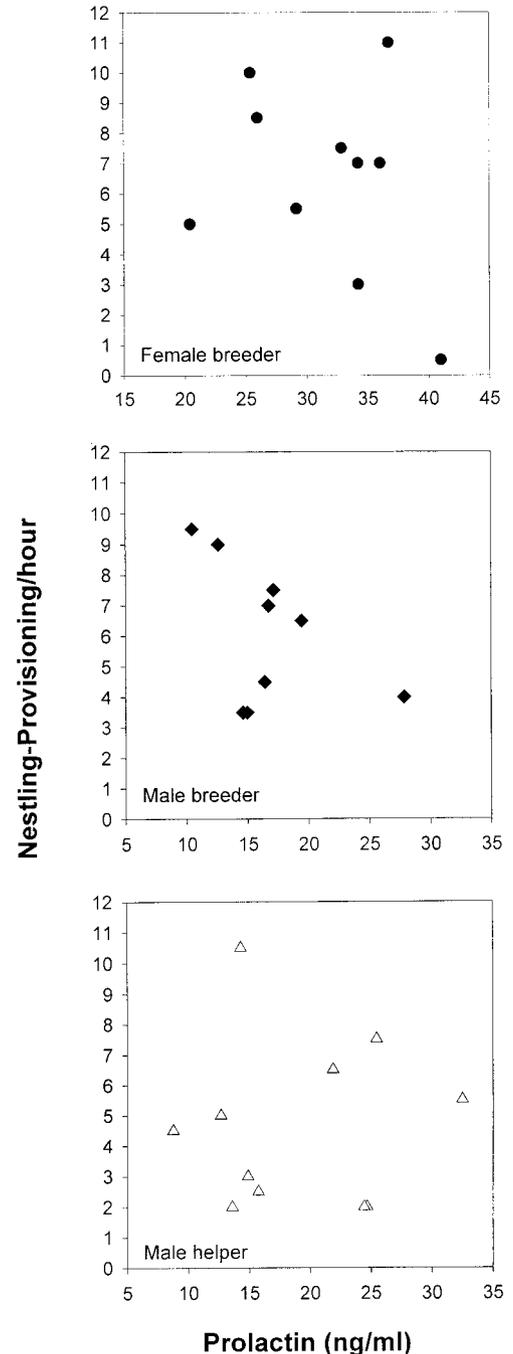


FIG. 7. Nestling provisioning rate of female breeders, male breeders, and male helpers do not vary as function of plasma prolactin concentration. Symbols represent individual sample values.

diately reproduce are inconsistent with the delayed maturation and physiological suppression hypotheses (Walters *et al.*, 1988). Observations of 1-year-old male red-cockaded woodpeckers successfully reproducing (Walters, 1990) support the conclusion that delayed maturation does not explain the absence of sexual activity by helpers in this species. Our data also do not support the physiological suppression hypothesis because helper and breeder body conditions were equivalent.

Our findings that unrelated male helpers have higher plasma testosterone concentrations than helpers that share a territory with their mothers suggest that this difference results from behavioral suppression. The absence of sexual activity by male helpers that are unrelated to the breeding female also might result from male–male interactions (challenge hypothesis; Wingfield, Hegner, Dufty, and Ball, 1990) and/or because the breeding female is not receptive to copulation attempts by the male helper. That male–male interactions may inhibit reproductive activity by helpers is supported by the observation that breeding males tend to follow helper males during the copulation stage and the two males sometimes interact aggressively (Lape, 1990). Female breeders also appear to be unreceptive to approaches by unrelated helpers during the copulation period. For example, Lape (1990) observed a subordinate male helper approach a female breeder and “nudge her rump.” In response, the female vocalized loudly and moved away. The effectiveness of the mechanism, which is still unknown, that prevents subordinate male red-cockaded woodpecker helpers from reproducing suggests female involvement rather than behavioral suppression through male–male interaction alone. Although numerous copulations between breeders have been observed (Lape, 1990), helpers have never been observed to copulate with a female breeder (in contrast to copulation of breeders, see below), despite extensive observational sampling (Lape, 1990; Walters, 1990). Furthermore, genetic studies confirm the lack of extrapair paternity in this species (Haig, Belthoff, and Allen, 1993; Haig *et al.*, 1994).

Our observation that male helpers that are related to the breeding female exhibit lower testosterone concentrations than unrelated males is consistent with the absence of stimulation hypothesis (also see Schoech *et al.*, 1996a). Helpers that are related to the breeding female may not be involved or are less involved in intragroup social interactions that are either sexually stimulating or agonistic. Although copulation in the red-cockaded woodpecker is conspicuous, prolonged

and frequent, and occurs for several weeks prior to egg-laying (Lape, 1990), just observing sexual behavior may not be sufficient to stimulate sexual activity by related helpers. That perceptual context has a tremendous effect on reproductive physiology was demonstrated by Friedman (1977), who found that female doves with a male directing his courtship behavior toward her had greater ovarian development than females that observed a male directing his courtship behavior away from her.

That the plasma testosterone concentration of related helpers is not different from that of breeding males suggests that absence of stimulation does not entirely explain our results. We propose that related helpers are behaviorally suppressed. The mechanism that prevents inbreeding between female breeders and male helpers is that female breeders do not exhibit sexual behavior when they interact with related male helpers. The social context in which female breeders make dispersal decisions supports this hypothesis. For example, when a male helper inherits a territory, if the female breeder is unrelated to the male helper she stays, but if she is his mother then she disperses (Walters *et al.*, 1988; Daniels and Walters, 2000).

Testosterone appears to mediate the expression of male sexual behavior in the other cooperatively breeding species in which plasma testosterone concentrations have been examined. For example, sexually active male breeders have testosterone concentrations that are consistently higher than those of reproductively inactive male helpers in the bell miner (*Manorina melanophrys*, Poiani and Fletcher, 1994), Florida scrub-jay (*Aphelocoma coerulescens*, Schoech *et al.*, 1991, 1996a), and white-browed sparrow weaver (*Plocepasser mahali*, Wingfield *et al.*, 1991). In contrast, presumably sexually active male helpers exhibit plasma concentrations equivalent to those of male breeders in the pied kingfisher (*Ceryle rudis*, Reyer *et al.*, 1986), Harris' hawk (*Parabuteo unicinctus*, Mays *et al.*, 1991), and Australian magpie (*Gymnorhina tibicen*, Schmidt *et al.*, 1991). In the Harris' hawk, subordinate male helpers unrelated to the breeding female have been observed to copulate, unsuccessfully, with the breeding female (Mays *et al.*, 1991). In the pied kingfisher, helper males that are unrelated to the breeding female are chased out of the territory by the breeding male when the female breeder is fertile (Reyer *et al.*, 1986). This suggests that unrelated helper males will mate with the breeding female if given a chance. Red-cockaded woodpecker helpers are unique in that they exhibit a combination of high plasma testosterone concentrations but no evidence of sexual activity.

Seasonal and Annual Patterns of Testosterone Concentration

In breeder and helper male red-cockaded woodpeckers, the seasonal profile of testosterone concentrations (see Fig. 2) is similar to that of other monogamously breeding species in which males exhibit parental care (Wingfield and Farnar, 1993; Beletsky, Gori, Freeman, and Wingfield, 1995; Buntin, 1996). These results suggest that male helpers, regardless of their relatedness to the breeding female, respond to environmental cues (e.g., photoperiod). These cues initiate an endocrine cascade resulting in gonadal recrudescence and an initial increase of testosterone concentration comparable to that of male breeders. The seasonal pattern of testosterone secretion also resembles that of year-round territory holders such that the absolute increase in testosterone during the breeding season is of lower magnitude than in seasonal migrants. Seasonal migrants establish and defend new territories each year, resulting in high levels of male-male interactions and resulting in high plasma concentrations of testosterone (Wingfield *et al.*, 1990; Wingfield and Hahn, 1994).

The annual variation observed may be a result of the idiosyncrasies of the particular individuals sampled in 1997. Three of the four samples that had atypically high concentrations of testosterone (a breeder, a related helper, and an unrelated helper; Fig. 2) were collected from the same group (SGL E04). The birds in this group may have experienced some event that triggered increased production of testosterone just prior to sampling; perhaps intragroup or intergroup conflict. Another possibility is the wildfire that burned in the area of their roosting cavities in their territory that same day! It is interesting to note that the oldest unrelated helper ($T = 8.00$ ng/ml) was observed intruding in a neighboring group during the breeding season in 1997.

Prolactin, Parental Behavior, and Alloparental Behavior

Our results are consistent with the hypothesis that proximate control of alloparental behavior of red-cockaded woodpecker helpers is mediated by the same factor(s) that controls the parental behavior of breeders. Like male breeders, male helpers exhibit parental behavior after a decline in testosterone and an increase in prolactin, a pattern that matches the general avian profile for biparental, monogamous species (Ball, 1990; Goldsmith, 1990). Helpers, like breed-

ers, develop brood-patches and brood-patch development in male helpers is likely to be dependent on both prolactin and sex steroids, as it is in most breeding birds (Buntin, 1996). Helpers also incubate eggs and incubation bouts are divided equally among group members (Khan and Walters, unpublished data). During egg-laying/incubation, male helpers have concentrations of prolactin equivalent to those of male and female breeders. This is consistent with studies on other species that demonstrate that prolactin concentrations correspond to the amount of incubation performed (*Cygnus atratus*, Goldsmith, 1982; *Diomedea* spp., Hector and Goldsmith, 1985; *Sula capensis*, Hall, 1986; *Calidris pusilla*, Gratto-Trevor, Oring, Fivizzani, El Halawani, and Cooke, 1990). As with breeders, the rate of nestling provisioning by helper males was not directly related to prolactin concentration. Despite the lack of a one-to-one relationship between prolactin and parental or alloparental behavior, one cannot rule out the possibility that these behaviors occur whenever an individual whose prolactin has reached a threshold concentration is exposed to the appropriate stimuli (i.e., eggs, young, or both).

That female plasma concentrations of prolactin remain elevated during the nestling provisioning stage while male prolactin concentrations decline is perplexing. This pattern might be expected if the female breeder has more visual or physical contact with the nestlings than the male breeder or male helper. Brooding activity is known to be associated with elevated levels of prolactin (Lea and Sharp, 1991; Lormée, Jouvantin, Lacroix, Lallemand, and Chastel, 2000). If our results are explained by differential brooding behavior of females, this behavior must occur very early in the nestling stage (before 10 days after hatch) because distinct shifts in parental care by males and females have not been observed to occur at nests with young that are greater than 10 days old (Khan and Walters, unpublished data). Alternatively, our results may be explained by female red-cockaded woodpeckers being more sensitive to nest stimuli than males. In the ring dove, brief exposure to squabs stimulated more prolactin secretion in females than in males (Lea and Sharp, 1991). Determining the effect of nest contents on male and female prolactin concentrations would require experimental manipulation of nest stimuli. Finally, estradiol and progesterone, in addition to prolactin, are involved in parental care of females (reviewed in Buntin, 1996). Measurement of progesterone and estradiol would provide more information about what mediates parental care in female red-cockaded woodpeckers.

Evolution of Alloparental Behavior

Evolutionarily, helping behavior is a derivation of parental behavior (Brown, 1987). Cooperative breeding is thought to be a derived trait in red-cockaded woodpeckers because helping behavior is not obligatory (Walters, 1990) and cooperative breeding is represented only by this single species within the genus *Picoides*. Therefore, the results of our study may more accurately reflect the original physiological bases of alloparental behavior, prior to modification by natural selection, than studies of other cooperatively breeding species, in which helping behavior has existed within the species' lineage for a longer period of time. The finding that the physiological bases of parental behavior and alloparental behavior are the same is especially relevant to the debate over whether alloparental behavior is a nonadaptive trait maintained as a by-product of selection on parental behavior (Jamieson and Craig, 1987; Jamieson, 1989) or is an adaptive trait maintained by fitness benefits (Emlen, 1991). If helping behavior is a nonadaptive trait, then the physiological bases of alloparental behavior will not have diverged from that of parental behavior. However, if helping behavior is an adaptive trait, then the physiological bases of parental behavior are expected to be modified by natural selection to facilitate the expression of alloparental behavior (Vleck *et al.*, 1991).

In some cooperatively breeding species, the physiological bases of alloparental behavior may have diverged from that of parental behavior. For example, plasma prolactin concentration was positively correlated with provisioning rate among Florida scrub-jay helpers, but no relationship was evident among breeders (Schoech *et al.*, 1996b). Vleck *et al.* (1991) observed that the prolactin concentrations of Harris' hawk male and female breeders declined after hatch despite continued expression of parental behavior, while prolactin concentrations increased in male helpers during the nestling provisioning stage. These results suggest that natural selection had modified the physiological basis of parental behavior in helpers. If the origin of alloparental behavior is correctly represented by the proximate mechanism we observe in the red-cockaded woodpecker, and the mechanisms observed in Florida scrub-jays (Schoech *et al.*, 1996b) and Harris' hawks (Vleck *et al.*, 1991) are derived from these origins, then alloparental behavior has been retained despite decoupling from other elements of the proximate control of reproductive behavior. This suggests that helping behavior is adaptive.

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