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## Spring Arrival, Aggression and Testosterone in Female Red-winged Blackbirds (*Agelaius phoeniceus*)

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Female-female aggression among polygynously breeding Red-winged Blackbirds (*Agelaius phoeniceus*) has been reported by many researchers (e.g. Nero 1956, Roberts and Searcy 1988), but there is little agreement as to its function. A female Red-winged Blackbird might behave aggressively towards other females to deter or delay the settlement of potential competitors (Hurly and Robertson 1985, but see Searcy 1988), or to defend nest sites or subterritories from other females sharing her mate's territory (Hurly and Robertson 1984, but see Searcy 1986). These putative functions of female-female aggression are not mutually exclusive, and aggression could also serve additional functions, such as protection against intra-specific brood parasitism.

Our study documents temporal variation in female-female aggression in a population of Red-winged Blackbirds to determine whether aggressive behavior was associated more closely with female settlement or with initiation of nesting. If aggressive behavior by established females coincides with the peak of arrivals of unmated competitors, then it may serve to deter prospecting females from attempting to pair with males. However, if aggression occurs primarily during the initiation of clutches it may function in the defense of nest sites or nest-related resources.

Also, since exogenous testosterone has been shown to increase aggressive displays in free-living female Red-winged Blackbirds (Searcy 1988), we examined the seasonal profile of plasma testosterone concentration (hereafter referred to as testosterone) and looked for correlations between temporal peaks in testosterone and timing of female aggression and arrival. Although other functions cannot be ruled out in a correlative study such as ours, describing the timing and endocrine basis of female-female aggression is a necessary step towards understanding the role of this behavior in a polygynous mating system.

*Methods.*—The study site was a cattail (*Typha* sp.) marsh at the north end of Yellowwood Lake, a 54-ha lake in Yellowwood State Forest, Brown Co., Indiana. Red-winged Blackbirds have been studied and color

banded at this site nearly every year since 1969, and the habitat remains as described by Yasukawa (1979). Unbanded females were captured in handmade Potter ground traps baited with corn. Traps were located on platforms in males' territories and on the ground in nearby feeding areas. Although it has been shown that supplemental food can influence timing of reproduction in this species (Wimberger 1988), it is unlikely to have affected our results because: (1) bait was available on every male's territory; (2) bait was provided in small enough quantities that there was never any available 12 h after baiting; and (3) the bait was available only to birds that were captured.

To determine the arrival dates of females, a thorough census was made every day (1990), or every other day (1991), from dawn to 0900 EST on foot and by canoe. In both years we began censuses several weeks before any females were sighted (8 February 1990; 5 February 1991) and continued them well into the nesting season (25 June 1990; 31 July 1991). The probability of detecting newly arrived females was very high because: (1) until late May the study site was only sparsely covered with residual vegetation; and (2) female Red-winged Blackbirds are usually vocal when disturbed. Each sighting was plotted on a large-scale map of the study site. Unbanded females were counted as separate individuals if they were recorded at the same site on two or more consecutive censuses. Since the pattern of arrivals (by 10-day period) was similar for 1990 and 1991, with the same median, mode and range, we combined arrival data from these two years and used the combined means in correlation analyses. Arrival data shown in figures were from 1991 only.

Aggressive interactions between females were recorded opportunistically during the morning censuses in 1991. Chases were the only behavior reported, as these have a clear initiator, winner, and loser (the displaced female). We did not include aggressive interactions that occurred on or near baited platforms. To maintain independence of observations, any dyad of females seen interacting aggressively was counted only once for a given day. Dyads of females that engaged in aggression were classified as either "familiar" (nesting on same male's territory in 1991), or "nonfamiliar" (not nesting on same male's territory in 1991). We assumed that the male that behaved aggressively towards an observer checking nest contents was the mate of the female at that nest.

In each year of the study over 90% of the breeding

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females were uniquely color banded by the time that the last female arrived. Seven (24%) of the 29 females involved in aggressive interactions had been captured for another study shortly after they arrived at the study site. These seven females had been held in a large outdoor aviary with other female Red-winged Blackbirds for an average of seven days (range 9–26 days). Of the dyads involved in aggressive interactions, 52% involved one of these experimental females. Manipulated females were involved in 55% of observed aggressive interactions and, thus, appeared to be involved in more interactions than expected from their proportion in the study population. However, since this difference did not approach significance ( $X^2 = 1.2$ ,  $df = 1$ ,  $P > 0.2$ ), and more detailed comparisons between the manipulated and unmanipulated females involved in this study indicate that they did not differ in several indicators of condition (e.g. mass, egg volume and clutch size; Cristol unpubl. data), data for these birds have been combined with those for unmanipulated females.

All data on testosterone were gathered in 1987 and 1988 (whereas, aggression data were gathered in 1991). Blood samples were taken from each female's alar vein within 10 min of the time she entered a trap. We collected samples only during the period 0700–1000 to avoid the possibility of diel fluctuations in hormone titers (Joseph and Meier 1973). We collected blood in heparinized capillary tubes and stored it on ice for up to 4 h before the plasma was separated with a clinical centrifuge. Plasma samples were then stored at  $-20^{\circ}\text{C}$ . Testosterone was measured in a single radioimmunoassay after purification of plasma on a diatomaceous earth/glycol microcolumn (Wingfield and Farner 1975, Johnsen in press). Plasma volumes ranged from 50 to 150 ml, and the range of detectable testosterone was 1.9 to 500 pg. The assay detected an average of 202 pg of testosterone in standard tubes containing 250 pg.

Searches for nests were conducted one to three times each week and, once nests were located, they were inspected every one to three days. Females were associated with particular nests by noting color bands of all individuals seen incubating or carrying food or nesting materials. Nest locations were plotted on maps, and dates of the first egg were recorded as eggs were laid or were determined by backdating from hatching date. In most cases commencement of nest building could not be determined precisely because the period of nest building in female Red-winged Blackbirds is highly variable early in the breeding season (3–12 days; Cristol unpubl. data; see also Teather et al. 1988). Because of the openness of the habitat it is unlikely that any nests remained undiscovered, except late in the season when vegetation was very dense.

We defined aggression rate as the frequency of aggressive interactions per 10-day period divided by the mean number of birds present during that period. The use of rate takes into account variation in the

number of females available to engage in aggressive interactions. We also compared aggression by stage of reproduction of the initiating female, and for this comparison used the frequency of aggressive interactions, as aggression rates cannot be calculated for females initiating breeding during different 10-day periods.

Variance in the seasonal profile of testosterone was tested with a Kruskal-Wallis one-way ANOVA (Siegel and Castellan 1988). The titers of many females fell below the detectable level for the assay, so to make our analysis more conservative these females were assigned the largest testosterone value that could have remained undetected.

The date that the first egg was laid in any year was treated as day 0, and all subsequent dates have been given an appropriate Julian number. For some analyses the nesting season was divided into 10-day periods. For analyses of aggression and testosterone by reproductive stage, each female's breeding season was divided into six comparable 12- to 14-day periods as follows: arrival (28–15 days prior to clutch initiation), pre-nesting (14–1 days prior to clutch initiation), eggs (0–13 days after clutch initiation), nestlings (14–25 days after clutch initiation), fledglings (26–39 days after clutch initiation), and late (40–53 days after clutch initiation). The reproductive status of the initiating female was used for classifying aggressive interactions by reproductive stage.

All correlations were tested with Kendall partial rank-order correlation coefficients (Siegel and Castellan 1988). We used a significance level of  $P < 0.05$ , except in the case of the correlation coefficients, where a tablewide sequential Bonferroni procedure was used to adjust for multiple tests (Rice 1989). All means are presented as  $\bar{x} \pm \text{SD}$  in the text; standard errors are indicated in figures.

*Results.*—We captured or sighted 65 females in 1991, of which 49 remained at the study site and laid at least one clutch of eggs. An additional 36 unbanded females were recorded, of which seven remained at the study area and laid at least one clutch of eggs. The number of females arriving in each 10-day period (including unbanded females) decreased sharply in the 10-day period before day 0 (Fig. 1A). The cumulative number of females present, calculated as the sum of the number present at the middle of each 10-day period, rose sharply until just after day 0 (Fig. 1A).

Independent instances of aggressive behavior were observed 27 times and involved 21 dyads composed of 29 females. The interactions took place on the territories of 13 males between day  $-22$  and day 25, and aggression rate peaked during the 10-day period prior to day 0 (Fig. 1B). According to reproductive stage, females were most aggressive during pre-nesting, but some females behaved aggressively during egg laying and incubation (Fig. 2). No aggression was observed during the nestling and fledgling stages despite con-

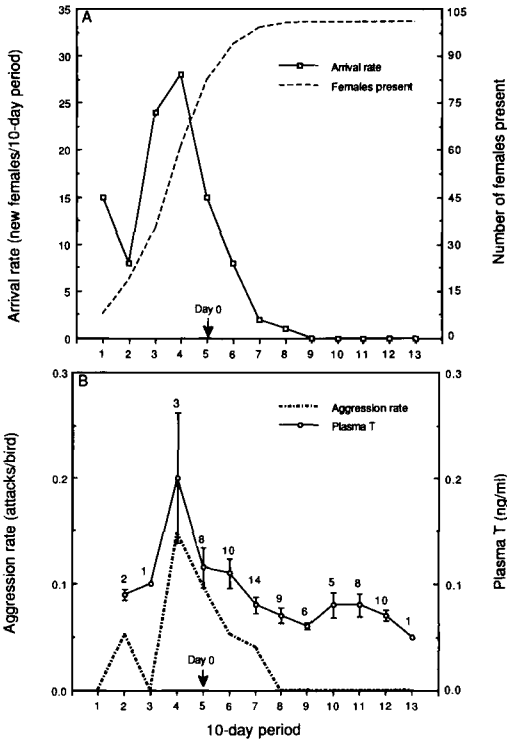


Fig. 1. (A) Number of female Red-winged Blackbirds at study site (dashed line), and number of newly arriving females (solid line) during each 10-day period of breeding season. (B) Rate of intrasexual aggression by female Red-winged Blackbirds (dashed line; number of interactions per bird present), and mean ( $\pm$ SE) plasma testosterone concentration (solid line, sample sizes indicated) during each 10-day period of breeding season. Day 0 is date when first female initiated a clutch.

tinued monitoring. The frequency of aggression differed significantly by stage of reproduction ( $G = 50.64$ ,  $df = 5$ ,  $P < 0.001$ ).

The female initiating a chase was the winner (i.e. displaced the loser) in all but one interaction, and there were no reversals when repeated encounters within a single dyad were considered. At the time of the first interaction of each dyad, initiators had been present at the study site for more than twice as long as the birds that they attacked (winners,  $\bar{x} = 26 \pm 12$  days present; losers,  $\bar{x} = 11 \pm 13$  days present; Wilcoxon signed ranks  $T_s = 188$ ,  $n = 20$ ,  $P = 0.0018$ ). In most cases only a single chase was observed, but occasionally one female chased another repeatedly for several hours. Seven (33%) of the dyads interacted on more than one day.

Considering the study site as a whole, there were 60 potential dyads of familiar females, and at least 1,540 possible unfamiliar dyads (including only

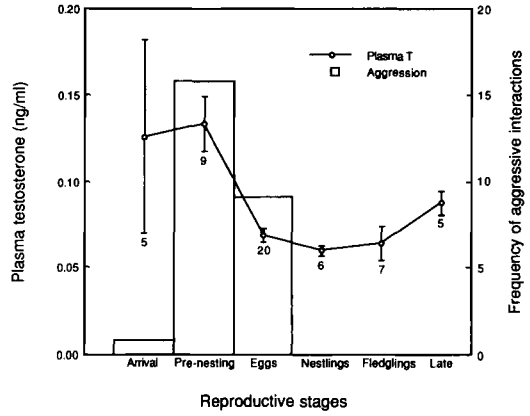


Fig. 2. Frequency of intrasexual aggression by female Red-winged Blackbirds during each stage of aggressor's reproductive cycle (bars; see text for definitions of stages), and mean ( $\pm$ SE) plasma concentration of testosterone of female Red-winged Blackbirds at each reproductive stage (solid line; sample sizes indicated).

breeding females). In 11 (52%) of the 21 dyads that engaged in aggressive interactions the females were familiar, which is a significantly larger proportion than expected by their representation in the population of potential dyads ( $X^2 = 63.5$ ,  $df = 1$ ,  $P < 0.001$ , but see below). Aggression between familiar females was initiated on the same date, relative to the date of the initiator's first egg (hereafter clutch initiation), as aggression between unfamiliar birds (familiar,  $\bar{x} = -1.5 \pm 12.6$  days from clutch initiation; unfamiliar,  $\bar{x} = -1.3 \pm 7.5$  days from clutch initiation; Mann-Whitney  $U = 51.5$ ,  $n = 21$ ,  $P > 0.8$ ).

The number of females arriving in each 10-day period (hereafter arrival rate) was highly correlated with aggression rate (Table 1), and this relationship was

TABLE 1. Kendal simple rank-order correlations (upper right) and partial rank-order correlations (lower left) for rate of aggressive interactions, rate of arrival of new birds, rate of initiation of first clutches, and testosterone levels by 10-day periods ( $n = 12$ ).

|              | Aggression              | Ar-rival          | Clutch | Testos-terone |
|--------------|-------------------------|-------------------|--------|---------------|
| Aggression   | —                       | 0.72*             | 0.45   | 0.73*         |
| Arrival      | 0.68*/0.42 <sup>b</sup> | —                 | 0.33   | 0.73*         |
| Clutch       | 0.32 <sup>c</sup>       | 0.01 <sup>d</sup> | —      | —             |
| Testosterone | 0.43 <sup>c</sup>       | 0.43 <sup>d</sup> | —      | —             |

\*,  $P < 0.02$  (using tablewide sequential Bonferroni adjustment).  $P > 0.1$  for all other values.

<sup>a</sup> Controlling for clutch.

<sup>b</sup> Controlling for testosterone.

<sup>c</sup> Controlling for arrival.

<sup>d</sup> Controlling for aggression.

independent of the number of females that initiated clutches during the same period (hereafter clutch initiation rate). Neither arrival nor aggression rates were correlated with clutch initiation rate (Table 1). Thus, the highest rates of aggression were temporally associated with the peak in arrival of prospecting females rather than with the initiation of egg laying.

Mean testosterone peaked early in the season and then decreased during the 10-day period prior to day 0 and remained undetectable for the remainder of the breeding season (Fig. 1B). Even at the seasonal peak of testosterone, females had much lower plasma titers (by at least an order of magnitude) than males from the same population (Johnsen 1991). Testosterone differed significantly among 10-day periods ( $H_1 = 21.959$ ,  $df = 8$ ,  $P = 0.005$ ). Consideration of the reproductive stage of each female, rather than date, reveals a similar profile (Fig. 2). Although the peak was lower in the analysis by reproductive stage, mean testosterone still differed significantly according to stage ( $H_1 = 25.652$ ,  $df = 5$ ,  $P = 0.001$ ).

Mean testosterone during each 10-day period was significantly correlated with aggression rate, but not when arrival rate was held constant (Table 1). Likewise, mean testosterone was significantly correlated with arrival rate, but not when aggression rate was held constant. Because aggression and arrival rates were highly correlated, it was not possible to determine which was associated more closely in time with testosterone. Examining testosterone and frequency of aggression with respect to female reproductive stage indicates that testosterone was high before the frequency of aggression increased (Fig. 2).

*Discussion.*—Aggression rate was correlated with the rate of arrival of females and not with the onset of egg laying. This suggests that the function of aggression may be to deter prospecting females, rather than defend nest-related resources. It is possible that the rate of aggression correlates with some other component of the nesting cycle, such as choice of nest sites, but we were unable to make such a comparison because of difficulty in accurately recording events in the nesting cycle prior to egg laying. Aggression might serve one function (detering prospective females) early in the season, and another function later (e.g. maintaining dominance hierarchies or defending nest-related resources among familiar females). If this were the case one might expect that aggressive interactions between residents (likely to be familiar) and incoming settlers (likely to be nonfamiliar) would tend to occur earlier than those between familiar females. However, the mean date of onset of aggression, relative to attacker's date of clutch initiation, did not differ for dyads of familiar and nonfamiliar females, failing to support the idea that aggression serves different functions in familiar and nonfamiliar dyads.

Although the disproportionately high incidence of aggression among familiar dyads might suggest that aggressive behavior was primarily important within

breeding groups, it should be remembered that non-familiar birds were usually present on any given territory for only a short time before being chased away, usually by the territorial male. Thus, although familiar dyads were far outnumbered by potential non-familiar dyads, familiar birds had a greater chance of being observed during aggressive interactions because of the far greater proportion of time spent in proximity.

Although the earliest-arriving females were unlikely to engage in aggressive interactions shortly after their return, the cost to a resident female of failing to respond aggressively to an unfamiliar, prospecting female could be very high if resources such as food or male parental care later become limited. This could explain why, in our analysis by reproductive stage, testosterone was high during the arrival stage, before the actual peak in aggressive behavior among females. Although testosterone is an important hormonal influence on aggressive behavior in birds (Wingfield et al. 1987, 1990), little is known of its role in aggression among breeding females (Harding 1983). The peak in testosterone early in the breeding season among polygynous female Red-winged Blackbirds is similar to that observed in females of the primarily monogamous White-crowned Sparrow (*Zonotrichia leucophrys*; Wingfield and Farner 1980). Analysis on a finer temporal scale and with larger sample sizes will be needed to determine whether female Red-winged Blackbirds arrive with testosterone already high, or whether levels rise shortly after they arrive. It is possible that female Red-winged Blackbirds maintain high testosterone in spring roosts and that testosterone declines throughout the breeding season.

The decrease in testosterone before the end of the period of heightened aggression could be explained if high testosterone were incompatible with nest construction, incubation or other aspects of female nesting behavior. In this regard, Searcy (1988) reported that of 12 female Red-winged Blackbirds implanted with testosterone, only one laid eggs, although all were present and exhibited aggressive behavior for at least 20 days. If high testosterone interferes with nesting behavior, female Red-winged Blackbirds with lower testosterone at the onset of nesting may have a fitness advantage. Finally, several studies suggest that female Red-winged Blackbirds might behave in a cooperative, rather than competitive manner while nesting, thereby reducing the probability of nest predation (Westneat 1992 and references therein). One possibility is that high testosterone might interfere with such cooperative behavior.

The challenge hypothesis predicts that testosterone will be high at times of social instability, and the seasonal profile of testosterone in males will reflect that of aggressive encounters (Wingfield et al. 1987, 1990). Our analysis by 10-day period shows that female Red-winged Blackbirds exhibited their highest

testosterone at the peak of arrival of prospective breeding females, which coincided with the peak of aggressive behavior. This is consistent with the challenge hypothesis because the peak of arrival of females should be the period of greatest social instability. However, our comparison of testosterone and frequency of aggression among females in each stage of reproduction indicates that testosterone was high before the peak of aggression. This suggests that for individual females testosterone does not correspond closely in time with aggression, or that there is a lag between the onset of high testosterone and its behavioral effects. A more detailed study in which hormone and behavioral data are gathered in the same years will be needed to more fully explain the relationship between aggression, arrival, and testosterone. Untangling the relationships between these variables for female Red-winged Blackbirds promises to provide a better understanding of the proximate and ultimate forces shaping female behavior in this well studied, but still puzzling, breeding system.

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