The Condor 96:1055–1063

MATE GUARDING AND EXTRA-PAIR PATERNITY IN NORTHERN CARDINALS[1]

GARY RITCHISON AND PAUL H. KLATT[2]
Department of Biological Sciences, Eastern Kentucky University, Richmond, KY 40475

DAVID F. WESTNEAT
Center for Ecology, Evolution, and Behavior, T.H. Morgan School of Biological Sciences, 101 Morgan Building, University of Kentucky, Lexington, KY 40506-0225

Abstract. We studied patterns of mate guarding and paternity in 21 pairs of Northern Cardinals (*Cardinalis cardinalis*) nesting in central Kentucky. DNA fingerprinting revealed that five of 37 nestlings (13.5%) resulted from extra-pair fertilizations (EPFs). Of 19 broods sampled, three (16%) had at least one extra-pair young. Although our observations of male cardinals making extra-territorial movements suggest that some males in the population may actively pursue EPFs, the percentage of extra-pair young in our study was lower than reported for many other passerines. Three non-exclusive factors may have contributed to this low percentage. 1. Male cardinals may gain more from parental efforts than from pursuing extra-pair copulations (EPCs). 2. Females in resident species such as Northern Cardinals probably have more opportunities to assess the quality of prospective mates prior to pairing than do females in migratory species and so EPCs may be less likely to be beneficial to females. 3. Most male cardinals exhibited mate guarding behavior. Males maintained contact with fertile mates 72.8% of the time during initial nesting attempts and, in addition, males followed females more often than females followed males in nine of 10 pairs.

Key words: Mate guarding; extra-pair fertilizations; Northern Cardinals; DNA fingerprinting; Cardinalis cardinalis.

INTRODUCTION

A mixed reproductive strategy is one in which an individual gains reproduction by behaving in two different ways during the same period of time. Trivers (1972) coined this phrase and predicted males would be expected to try to pair with one female and then pursue additional promiscuous matings once paired. Trivers' prediction has been confirmed for many bird species; already-paired males often purse extra-pair copulations (EPCs) with the mates of other males (McKinney et al. 1984, Westneat et al. 1990, Birkhead and Møller 1992). In at least some species, females also appear to follow a mixed reproductive strategy by pairing with one male and pursuing EPCs with other males (e.g., Smith 1988, Kempenaers et al. 1992, Wagner 1992). Genetic studies have confirmed in many species that EPCs result in fertilizations, sometimes at surprisingly high frequencies (30–70%; Birkhead and Møller 1992, Westneat and Webster, in press).

Although EPCs appear to be a major component of the reproductive behavior of males and females in many avian species, there is considerable variation in how EPCs occur and in their frequency (e.g., McKinney et al. 1984, Westneat et al. 1990, Birkhead and Møller 1992). Some ecological and social factors have been proposed as explanation for this variation. Opportunities for EPCs may be influenced by density (Birkhead 1978), the degree of breeding synchrony (Birkhead and Biggins 1987, Westneat et al. 1990), and features of the habitat (Sherman and Morton 1988). EPCs might also be more common when females choose mates indirectly via choice of nesting site rather than directly (Westneat et al. 1990). Within populations, some individuals might be involved in EPCs more frequently than others. Westneat et al. (1990) and Møller (1992) predicted that late-settling females would be more likely to pursue EPCs with better quality, but already paired, males. Similarly, mates of males that face conflicting demands between mate guarding and other activities, such as foraging (Westneat 1994) or taking care of fledglings, might experience more EPC attempts (e.g., Westneat et al. 1990).

Currently, there are too few data on a sufficient

[1] Received 11 March 1994. Accepted 28 June 1994.
[2] Present address: Department of Zoology, University of Alberta, Edmonton, Alberta T6G 2E9, Canada.

variety of species to allow a comparative analysis of these predicted relationships. Some analyses of observed rates of copulations have been attempted (e.g., Birkhead and Møller 1992), but these suffer from biases in the ease of observing behavioral events. At present, data on rates of extra-pair fertilizations (EPFs) have been gathered for too few species, thus preventing comparative analyses using measures less prone to observational bias. A growing set of studies of variation within populations has led to some new insights into the factors affecting EPCs (reviewed by Westneat and Webster, in press), but it is clear that differences among species in how these factors interact abound.

Here we present data from a behavioral and genetic study of Northern Cardinals (*Cardinalis cardinalis*) that was initiated to add to the data available for comparative analyses and test some hypotheses that have not often been addressed in other studies. Cardinals are a socially monogamous species (Bent 1968; Ritchison, unpubl. data) and are year-round residents in eastern North America. Cardinals breed in scrubby secondary growth common to "edge" habitat and residential areas. Pairs often can raise two broods in a season, yet predation of nest contents is high, so breeding is often asynchronous. Males provide substantial parental care to nestlings, and females often will renest while the male is providing for fledglings.

We predicted that cardinals would engage in EPCs. Because of the visually-occluded habitat, asynchronous breeding, and nature of male parental care, we suspected that males might have difficulty closely guarding their mates, particularly during second breeding attempts. Because cardinals are resident year-round, females might have information on male quality through social interactions in winter flocks (Ritchison and Omer 1990). Thus, we also predicted that at least some female cardinals might behave as do female Black-capped Chickadees (*Parus atricapillus*; Smith 1988) and Blue Tits (*P. caeruleus*; Kempenaers et al. 1992) and pursue EPCs with particular males.

METHODS

FIELD METHODS

Cardinals were studied at the Central Kentucky Wildlife Management Area, located 17 km southeast of Richmond, Madison County, Kentucky. During February through August 1992, cardinals were captured in mist nets and banded with unique combinations of three colored plastic bands and a FWS numbered aluminum band. Each cardinal was also marked with a uniquely numbered strip of white or yellow plastic tape attached to the tail (Ritchison 1984). No attempts were made to capture either member of a pair when the female was thought to be fertile. Nestlings were banded at 5 to 8 days post-hatching.

Blood samples were collected from each captured cardinal with 250-microliter capillary tubes after puncturing the brachial vein. Between 0.1 and 0.4 ml of blood was taken and placed in two or three vials containing 0.1 ml of TNE (10 mM Tris-10 mM NaCl-2 mM EDTA, pH 8.0) buffer (Quinn and White 1987). We took blood from nestlings at 5–8 days post-hatching.

Northern Cardinals are multi-brooded and pairs typically produce (or attempt to produce) two to four broods during the breeding season (Kinser 1973; Ritchison, pers. observ.). Each breeding attempt was divided into three periods. The fertile period was defined as lasting from seven days before the first egg of a clutch was laid until the day the penultimate egg was laid. The pre-fertile period was the period prior to the fertile period (through eight days before the first egg of a clutch was laid). After the first nesting attempt of the season, the pre-fertile period began the day after a nest was lost to a predator or, if the preceding nesting attempt was successful, the day after the young fledged. The post-fertile period began on the day the last egg of a clutch was laid and continued through the incubation and nestling periods (or until a nest containing eggs or young was lost to a predator).

Nests were found by following females or males, and by checking likely nest sites. For nests located during nest-building or during the egg-laying period, we were able to determine the exact date on which a clutch was initiated. For nests located after the clutch was complete, the date of clutch initiation was determined by back-dating from either the date of hatching or the date of fledging. When back-dating, the incubation period for cardinals was assumed to be 12 days (Bent 1968; Ritchison, pers. observ.) while the nestling period was assumed to be 10 days (Bent 1968; Ritchison, pers. observ.).

Observations of focal pairs ($n = 21$) began on 20 April and continued through 31 August. Typ-

ically, pairs were observed at least twice a week if their breeding status was unknown or if the female was known to be fertile. Most observations were made between sunrise and 11:00 hr, and these observation periods ($n = 256$) averaged 65 min in duration (range = 25 min–3 hr). During each period, we attempted to maintain contact with the focal individual for one hour. However, observation periods were sometimes shortened (e.g., when neither member of a pair could be located or because of inclement weather) or lengthened (e.g., when the breeding status of a pair was unknown). Additional time (approximately 200 hr) was spent looking for nests in territories where the female was thought to be nesting and in territories where the breeding status of the pair was unknown. During all focal pair observations, we attempted to follow the focal female. However, if unable to locate the female, we followed the male. If unable to locate either member of the focal pair, we searched the territory in an attempt to locate either the male or female.

During focal pair observation periods, we attempted to note: (1) Any within-pair or extra-pair copulations. (2) Extra-territorial movements by members of the focal pair or intrusions into the focal territory by non-focal conspecifics. (3) Intrapair contact, measured as the percent time the male was within 15 m of the female. We noted this either when certain of the location of both individuals or when certain of the location of one member of the pair and were equally certain that the other individual was more than 15 m distant. For example, suppose we could see both members of the pair and they were within 15 m of each other. If one member of the pair then flew 30 m across a field and out of sight, we counted the pair as being out of contact even though we only knew the precise location of one member of the pair. The dense vegetation of the study area and secretive nature of cardinals, especially females, often prevented us from maintaining contact (either visual or vocal) with both pair members. (4) Intrapair distance, the estimated distance between pair members. This was estimated at 1 min intervals and only when we were certain of the location of both birds. (5) Movements, defined as any flights longer than 10 m made when the pair was in contact (within 15 m). We noted which member of the pair initiated such flights and whether or not the other pair member followed.

PATERNITY ANALYSIS—DNA FINGERPRINTING

Paternity was analyzed using standard DNA fingerprinting techniques (e.g., Westneat 1990, 1993). Blood samples collected from adults and nestlings were stored at −20°C to −80°C until analysis. DNA was extracted from the blood using a modification of the procedure described by Quinn and White (1987).

About 15 micrograms of DNA was digested with the restriction enzyme *Alu*I. Digested DNA was precipitated, pelleted, and washed twice with 70% ethanol, then resuspended in 24 microliters of TE (10 mM Tris-1 mM EDTA, pH 7.4). The concentration of digested DNA was determined using a spectrophotometer and the total amount of digested DNA in 20 microliters was then adjusted to within 1 microgram for all lanes (usually 6–8 micrograms of DNA). The samples were placed in 0.8% agarose gels and subjected to a voltage differential for 1,650 volt-hr (approximately 46 hr) in 1× TBE buffer (0.089 M Tris, 0.089 M borate, 0.002 M EDTA). A pump circulated the buffer solution during electrophoresis. Gels were treated as in Westneat et al. (1988), and DNA was transferred under vacuum to nylon membranes with 1 M ammonium acetate, 0.04 M NaOH. These membranes were then baked at 80°C for 2 hr.

Membranes were placed in Plexiglas tubes with 30 ml of prehybridization solution (NaPi) at 60°C for 48 hr (Westneat et al. 1988). Between 50 and 200 ng of probe DNA (either a fragment of M13 or the excised 2.5 kb fragment of mouse *per*; Shin et al. 1985) was labeled by random priming. Unincorporated nucleotides were separated from labeled probes using columns of Sephadex G-50 (medium) in TE. Labeled probe was added to fresh NaPi solution and placed in the Plexiglas tubes. Hybridization occurred at 60°C for 48 hr.

Washes depended on which of the two probes (M13 and Mouse 2.5 *per*) were used. All hybridized membranes, regardless of probe, were initially washed twice at room temperature and once at 60°C with 2× SSC and 0.5% sodium dodecyl sulfate (SDS). Membranes probed with M13 were then wrapped and placed on film (Kodak XAR). Membranes probed with *per* were washed an additional one or two times with 1× SSC at 65°C. Films were exposed without intensifying screens for 2 days to 3 weeks, depending on the strength of the signal. Membranes were then stripped using 0.4 N NaOH at 42°C, neutralized in 0.2 M

Tris, 0.5% SDS, 0.1× SSC, and washed in 0.5% SDS, 0.1× SSC at 65°C. If not reprobed immediately, the membranes were stored in 0.5× TBE at 4°C.

PATERNITY ANALYSIS—SCORING AND INTERPRETATION

Scoring followed the general methods described by Westneat (1990, 1993) and was done by one of us (PHK). A subset of the samples were also scored by DFW and the assignments of parentage agreed exactly. Scoring was not done blindly; nestlings were always run within a few lanes of each social parent (i.e., the male and female holding the territory at the time the nest was built and eggs were laid). Scoring consisted of placing acetate overlays on the developed autoradiograph, marking bands with colored pens, and judging whether or not bands had migrated sufficiently similar distances to be called the same. If the center of a band was within 0.5 mm of another band, they were judged the same (Westneat 1990, Smith et al. 1991). In all cases, scoring was conservative. If possible, bands in the nestlings were matched with bands in the social parents. Thus, bands in nestlings that were not present in a social parent had to be clearly different to be considered novel fragments. Data on novel fragments and proportions of bands shared between offspring and each social parent were used to determine exclusions (e.g., Westneat 1990).

RESULTS

FIELD OBSERVATIONS

Copulations and extra-territorial movements. We observed no copulations, either within-pair or extra-pair, in 276 hours of focal pair observations. On 14 occasions, conspecific intruders were observed in the territories of focal pairs. All observed intrusions were by males and most (10) occurred during the focal female's fertile period, and the rest (4) occurred during the pre-fertile period. All intruding males were subsequently chased out of the territory by the focal male.

Focal males ($n = 7$) were observed making 15 extra-territorial excursions. Eight of these excursions were into the territories of non-focal pairs and, as a result, the breeding status of the females in these territories was not known. Of the remaining seven excursions, five were made into territories in which the resident female was known to be fertile, one into a territory in which the female was incubating (post-fertile), and one into a territory in which the female had recently lost a nest (pre-fertile). No focal females were observed making extra-territorial excursions.

Nesting attempts and nesting success. Focal pairs ($n = 21$) initiated 55 nesting attempts and 18 of these attempts (32.7%) produced fledged young. The mean number of nests initiated by pairs present throughout the breeding season (April–August) was 2.94 ($n = 17$ pairs). Most unsuccessful nests were lost to predators during the egg stage ($n = 32$), while four were lost to predators during the nestling stage. One nest failed early in the nestling stage when the adult male was killed (hit by a vehicle along a nearby road) and the female abandoned the nest.

Duration and fate of focal pairs. Seventeen of 21 focal pairs remained together on their territories throughout the breeding season (April–August), including all three pairs with extra-pair young (see Paternity Analysis section). As noted above, one focal male was found dead in mid-May and his mate subsequently abandoned the territory. Two other pairs were last observed on their respective territories in mid-June after having failed in their first nesting attempt. The reason for the disappearance of one of these pairs was unknown. However, shortly before the other pair disappeared, the male was apparently injured (he had lost many feathers on his head and appeared to have trouble flying). A new pair was observed in this now vacant territory in early July and this pair initiated a nest in late July (and subsequently fledged three young). Both members of this new pair were marked individuals and had been previously observed in areas adjacent to their new territory. These observations, although limited, suggest that both individuals had been floaters (i.e., did not have a territory).

Intrapair contact and distance. The percent of time that pairs were in contact varied with breeding stage (Table 1). Although not quite significant (Wilcoxon test, $z = 1.77$, $P = 0.078$), pairs were typically in contact more during the fertile period than during the pre-fertile period. Pairs were in contact significantly less often during the post-fertile period than during the fertile period ($z = 3.83$, $P < 0.0001$). Although not quite significant ($z = 1.78$, $P = 0.075$), the average time pairs spent in contact during the fertile period was lower during later (second, third, and fourth nests) nesting attempts (Table 1).

The mean intrapair distance was significantly greater ($z = 2.07$, $P = 0.038$) during the pre-fertile

TABLE 1. The percent of one-minute intervals the members of Northern Cardinal pairs were within 15 m (pair contact) and the average intrapair distance between the members of the pair in the pre-fertile, fertile, and post-fertile stages (see text). Values are means plus or minus the standard deviation. Values in parentheses are the number of pairs and the mean number of minutes observed per pair (for pair contact) or number of observation intervals per pair (for pair distance).

	Pre-fertile	Fertile	Post-fertile
		Pair contact (% of minutes)	
All nests	42.7 ± 27.3 (n = 11, 43.2 min/pair)	61.4 ± 26.0 (n = 17, 32.8 min/pair)	16.6 ± 8.7 (n = 9, 48.4 min/pair)
First nests		72.8 ± 27.4 (n = 9, 31.6 min/pair)	
Later nests		49.5 ± 29.0 (n = 8, 17.1 min/pair)	
		Intrapair distance (m)	
All nests	32.8 ± 18.5 (n = 11, 24.4 obs./pair	19.0 ± 13.8 (n = 17, 20.2 obs./pair)	50.5 ± 24.1 (n = 9, 37.1 obs./pair)
First nests		8.8 ± 3.9 (n = 9, 19.1 obs./pair)	
Later nests		25.3 ± 13.6 (n = 8, 19.0 obs./pair)	

period than during the fertile period (Table 1). In addition, the mean intrapair distance was significantly greater ($z = 3.07$, $P = 0.002$) during the post-fertile period than during the fertile period. The mean intrapair distance during the fertile period was significantly greater ($z = 2.26$, $P = 0.024$) during later (second, third, and fourth nests) nesting attempts (Table 1).

Movements. Overall, 112 flights ($n = 17$ pairs; median = 7 flights per pair; range = 1–16) were observed when pairs were in contact, with females initiating 77 and males 35. Females initiated flights more often than males in 13 of 17 pairs, a significant trend (binomial test, $P < 0.05$). Among those pairs in which both members were observed initiating flights, males followed females more often ($\bar{x}$ = 84.7% ± 14.9% [SD]) than females followed males ($\bar{x}$ = 32.7% ± 32.5% [SD]) in nine of 10 pairs (binomial test, $P < 0.02$).

PATERNITY ANALYSIS

DNA fingerprints from both M13 and mouse *per* probes revealed a number of fragments exhibiting considerable variation among individuals (Table 2). We did not test for linkage or allelism among fragments; in the analyses of parentage described below we assume that both are negligible. Violations of that assumption sufficient to dramatically change our results are unlikely given data from other species and the distributions of band-sharing and novel fragments that we found.

We compared the fingerprints of 37 young with their putative parents (the male and female associated with the nest at egg-laying). If these offspring were truly descendant from the putative parents (1) all the fragments in their DNA fingerprints should be present in one or the other of the parents and (2) they should have substantially higher proportions of bands shared with each putative parent than with any randomly picked individual.

Both (1) and (2) are shown in Figure 1. Of the 37 offspring, 21 shared all of their fragments with the combination of the two putative parents. All of these had band-sharing proportions (both probes combined) with each putative parent that substantially exceeded the one-tailed, upper limit on band-sharing between random pairs of adult

TABLE 2. Average number of bands scored and average proportion of bands shared for both M13 and the mouse *per* probes in adult Northern Cardinals.

	Number of bands scored		Proportion of bands shared	
	M13	Per	M13	Per
Number of Individuals/dyads	41	41	53	53
Mean	29.6	38.1	0.15	0.21
SD	5.6	7.4	0.07	0.06

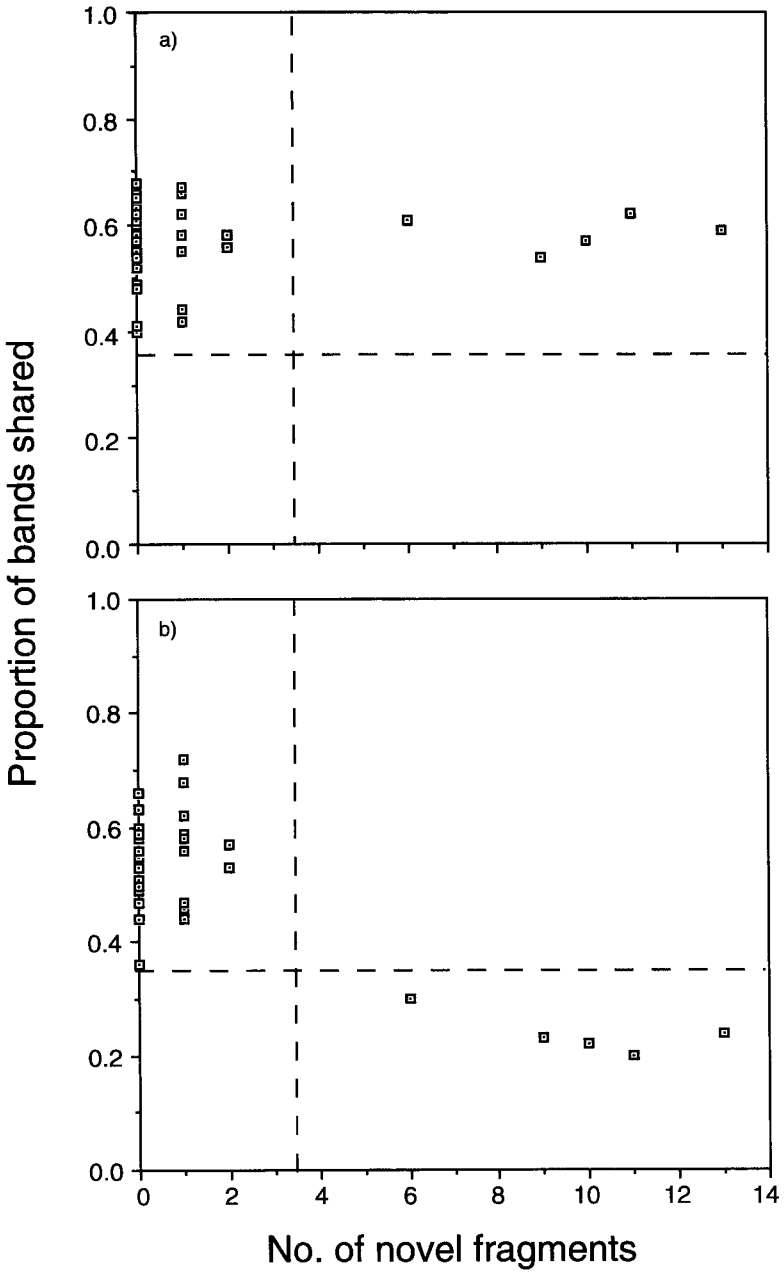

FIGURE 1. Proportions of bands shared by number of novel fragments for 37 cardinal nestlings. a) Band-sharing proportions between the nestling and the female at the nest. b) Band-sharing proportions between the nestling and the male associated with the nest. The horizontal dotted line in both represents the lower, one-tailed, 99% confidence limit on the parent-offspring distribution of band-sharing proportions, which was calculated using band-sharing proportions between nestlings with no novel fragments and the putative parents. The vertical dotted line in both (a) and (b) separates nestlings with few novel fragments (compatible with previously determined mutation rates in other species), and nestlings with many novel fragments.

individuals in the population ($\bar{x} = 0.19$, SD = 0.05, $n = 53$). Thus, it is unlikely that these nestlings were anything but offspring of both the male and female associated with the nest.

The remaining 16 offspring had at least one fragment not present in either putative parent. Such novel fragments could come from either mutation or from an adult other than the putative parent being the genetic parent. If we assume mutation rates comparable to other studies (0.001–0.01 per fragment per generation; e.g., Jeffreys et al. 1985; Burke et al. 1989; Westneat 1990, 1993), then mutation should result in only a few novel fragments. Indeed, the 16 nestlings with some novel fragments are divided into two groups; 11 have only one or two novel fragments, whereas five have six or more. Figure 1 shows that the nestlings with one or two novel fragments have high band-sharing with both putative parents, whereas those with six or more have band-sharing proportions with the male at the nest substantially below 0.38, the lower 99% confidence limit on the parent-offspring distribution (band-sharing between adults and nestlings with no novel fragments; $\bar{x} = 0.55$, SD = 0.074, $n = 41$) and no different from that of random pairs of individuals from the population at large. We concluded that these five were from EPCs, and all others were descendant from the putative parents. Thus, 13.5% of the nestlings surveyed came from EPCs and none came from intraspecific brood parasitism. The nestlings from EPCs were from three broods (16% of the 19 sampled); one each from a brood of one and a brood of two, and three from a brood of three.

We attempted to match the five excluded young with any of the males we had sampled. We did this by identifying fragments in each nestling's fingerprint that were not shared by the female (henceforth, paternal fragments). We then searched the fingerprints of all other males in the population ($n = 19$) for matches. None of the males had all of the paternal bands in each of the five nestlings. One male did match 9 of 10 paternal fragments in one nestling. Assuming that these fragments are independent, the probability that a given male would match 9 of 10 fragments by chance is exceedingly small (binomial probability using a proportion of bands shared of 0.19, $P < 0.00001$). The probability that one out of the 19 males surveyed would match by chance is larger, but also very slight ($P = 1 - (.99999)^{19}$, < 0.0002). Hence this male is the likely sire of one of the nestlings from an EPF. Indeed, this male defended a territory with a boundary 750 m from the focal nest. Interestingly, this male and his mate lost a nest containing three nestlings to predation on 29 July while the female in the territory with this male's apparent extra-pair young was fertile from 30 July through 5 August.

In the other four cases, no male came within four fragments of matching all paternal fragments. These four nestlings were from two broods, and in each case there was at least one neighboring male that had not been sampled.

Paternity and behavior. Although we attempted to quantify the mate guarding behavior of two of the three males whose nests contained extra-pair young, sample sizes were too small for meaningful analyses. In one pair, the female was observed for 31 min during her fertile period and her mate was in contact 19.4% of that time. In the second pair, the female was observed for 56 min during the fertile period and her mate was in contact 33.9% of that time. The relatively low level of mate guarding exhibited by the male in this second pair appeared to be due, at least in part, to the configuration of his territory. This male's territory was bissected by the territory of another male, and the centers of the two sections of his territory were about 250 m apart. Our observations suggest that, unlike the male, the activities of his mate were limited to one section of the territory. As a result, this male was often located in a different section of the territory, and was, therefore, often out of contact with his mate.

DISCUSSION

We found that 13.5% of the young cardinals in our study resulted from extra-pair fertilizations. Further, our observations of male cardinals intruding onto the territories of fertile females indicate that some males may actively pursue such extra-pair fertilizations. Trespassing males stayed low and remained quiet (Kinser 1973), typical behavior for male birds seeking EPCs (Møller 1985). Although Kinser (1973) observed females occasionally trespassing into the territories of neighbors, we did not witness this during our observations. Because females were generally difficult to observe, we cannot assess whether some or all EPFs were obtained by females pursuing EPCs.

As in other species, male cardinals would benefit from extra-pair fertilizations by increasing their reproductive success at the expense of other

males. Extra-pair young may be particularly valuable for some male cardinals because of the high rates of predation. Only about one-third of the nesting attempts in our study resulted in fledged young and six of the 21 pairs observed during our study produced no young. Thus, in addition to increasing the reproductive success of individual males, EPFs might also reduce the variance in male reproductive success when predation rates are high (e.g., Seger and Brockmann 1987).

Despite these potential benefits, the percentage of extra-pair young among cardinals in our study was lower than reported for many other passerines (e.g., Birkhead and Møller 1992; Westneat and Webster, in press). If our results accurately reflect the mating strategies of Northern Cardinals, three nonexclusive factors may contribute to the relatively low rate of extra-pair paternity. First, Westneat et al. (1990) noted that any gains in fitness a male might achieve by pursuing additional matings must be weighed against possible gains from other activities. During the nestling stage, male cardinals contribute substantially to the feeding of young (Laskey 1944; Bent 1968; Ritchison, pers. observ.) and, in fact, male contributions may be essential. For example, when a male cardinal in our study was killed, his mate apparently abandoned three nestlings. Male cardinals also care for fledglings while females initiate another nest. As a result, male cardinals may be less likely to pursue EPCs during the nestling and post-fledging periods even if fertile females are available. During the incubation period, male cardinals occasionally sit on the nest for short periods (Bent 1968), and also feed the female (Laskey 1944; Ritchison, pers. observ.). This pattern suggests that male cardinals may gain more from parental efforts than from pursuing EPCs.

Low gains of EPCs for males may also be due to a second factor; a reluctance of females to engage in EPCs. Unfortunately, the role of female Northern Cardinals in EPCs remains unclear because we observed no attempted EPCs and, thus, do not know if females actively solicited, passively accepted, or resisted EPCs. No indication of female pursuit of EPCs was observed in our study, although females were difficult to watch. Kinser (1973:68) noted that attempts by male cardinals to copulate were usually unsuccessful until "... the female reached a state in which she invited copulation." Such observations suggest that extra-pair fertilizations in cardinals may require the cooperation of females and, if so, the low percentage of extra-pair young in our study could indicate a low percentage of females that choose to participate in EPCs. We have no data that might distinguish between two possible reasons for a lack of female cooperation: (1) females have chosen mates directly and for most there is little to be gained from another male's genes, or (2) male responses to infidelity are costly to females so they avoid EPCs.

At least during the initial nesting attempt, most male Northern Cardinals exhibited mate guarding behavior, with males maintaining contact with fertile mates 72.8% of the time and usually following when their mates initiated flights. Male mate guarding may, therefore, have been another factor contributing to the low level of extra-pair paternity in initial nests. Male cardinals exhibited significant individual variation, with time spent in contact with fertile mates ranging from 19.4% to 100%. Sample sizes were too small for meaningful analysis, however, the one case of extra-pair paternity among first nesting attempts was found in the pair where the male maintained contact with his fertile mate only 19.4% of the time. Although anecdotal, this is consistent with the idea that mate guarding by males prevents EPCs.

Mate guarding by male cardinals declined during later nesting attempts. This change in behavior was most apparent among males with fledged young. Female cardinals are frequently unguarded while their mates are caring for fledged young, yet we found no extra-pair young in the second broods of pairs where males were so engaged. Kinser (1973) reported similar behavior and noted that male cardinals caring for fledged young rarely followed females involved in construction of a second nest. Few data on the extra-pair behavior of males and females from other double or multi-brooded species are available. However, Møller (1991) found that the intensity of mate guarding by male Barn Swallows did not change between first and second nesting attempts, suggesting that neither male nor female swallows altered their reproductive strategies. In contrast, Weatherhead and McRae (1990) suggested that females in double-brooded species may alter their reproductive strategies from the first to second broods. That is, males may have proven their quality by virtue of the first nest's success and, therefore, females will refrain from copulating with other males during a second nesting attempt. In our study, males exhibited

less guarding during second broods, but paternity was just as high. Thus, guarding alone cannot explain high paternity in second broods. Whether or not this additional factor is the behavior of female cardinals, as predicted by Weatherhead and McRae (1990), remains unclear. Our results do suggest that the dynamics of the cardinal's mating system are, as in other species, more complicated than previously imagined.

ACKNOWLEDGMENTS

We thank Sunni Hylton, Matthew Mays, and Michael Hill for assistance in the field, Herman Mays and Elizabeth Gray for considerable advice and assistance with the DNA analyses, and Geoffrey Hill and an anonymous reviewer for helpful comments on the manuscript. Funding was provided by a Kentucky NSF/EPSCoR grant to Gary Ritchison and an NSF/EPSCoR grant to the Evolutionary Ecology group at the University of Kentucky.

LITERATURE CITED

BENT, A. C. 1968. Eastern Cardinal, p. 1–15. *In* Life histories of North American cardinals, grosbeaks, buntings, towhees, finches, sparrows, and allies. U.S. Nat. Mus. Bull. 237, Smithsonian Institution Press, Washington, DC.

BIRKHEAD, T. R. 1978. Behavioural adaptations to high density nesting in the Common Guillemot *Uria aalge*. Anim. Behav. 26:321–331.

BIRKHEAD, T. R., AND J. D. BIGGINS. 1987. Reproductive synchrony and extrapair copulation in birds. Ethology 74:320–334.

BIRKHEAD, T. R., AND A. P. MØLLER. 1992. Sperm competition in birds: evolutionary causes and consequences. Academic Press, London.

BURKE, T., N. B. DAVIES, M. W. BRUFORD, AND B. J. HATCHWELL. 1989. Parental care and mating behavior of polyandrous dunnocks *Prunella modularis* related to paternity by DNA fingerprinting. Nature 338:249–251.

JEFFREYS, A. J., V. WILSON, AND S. L. THEIN. 1985. Hypervariable "minisatellite" regions in human DNA. Nature 314:67–73.

KEMPENAERS, B., G. R. VERHEYEN, M. VAN DEN BROECK, T. BURKE, C. VAN BROECKHOVEN, AND A. A. DHONDT. 1992. Extra-pair paternity results from female preference for high-quality males in the Blue Tit. Nature 357:494–496.

KINSER, G. W. 1973. Ecology and behavior of the cardinal in southern Indiana. Ph.D.diss., Indiana Univ., Bloomington, IN.

LASKEY, A. R. 1944. A study of the cardinal in Tennessee. Wilson Bull. 56:27–44.

MCKINNEY, F., K. M. CHENG, AND D. J. BRUGGERS. 1984. Sperm competition in apparently monogamous birds, p. 523–545. *In* R. L. Smith [ed.], Sperm competition and the evolution of animal mating systems. Academic Press, Orlando, FL.

MØLLER, A. P. 1985. Mixed reproductive strategy and mate guarding in a semi-colonial passerine, the swallow *Hirundo rustica*. Behav. Ecol. Sociobiol. 17:401–408.

MØLLER, A. P. 1991. Double broodedness and mixed reproductive strategies by female swallows. Anim. Behav. 42:671–679.

MØLLER, A. P. 1992. Frequency of female copulations with multiple males and sexual selection. Am. Nat. 139:1089–1101.

QUINN, T. W., AND B. N. WHITE. 1987. Identification of restriction-fragment-length polymorphisms in genomic DNA of the Lesser Snow Goose. Mol. Biol. Evol. 4:126–143.

RITCHISON, G. 1984. A new method of marking birds. North Amer. Bird Bander 9:8.

RITCHISON, G., AND M. K. OMER. 1990. Winter behavior of Northern Cardinals. Trans. Kentucky Acad. Sci. 51:145–153.

SEGER, J., AND M. J. BROCKMANN. 1987. What is bet-hedging? Oxford Surveys in Evol. Biol. 4:182–211.

SHERMAN, P. W., AND M. L. MORTON. 1988. Extra-pair fertilizations in mountain White-crowned Sparrows. Behav. Ecol. Sociobiol. 22:413–420.

SHIN, H. S., T. A. BARGIELLO, B. T. CLARK, F. R. JACKSON, AND M. W. YOUNG. 1985. An unusual coding sequence from a *Drosophila* clock gene is conserved in vertebrates. Nature 317:445–448.

SMITH, H. G., R. MONTGOMERIE, T. POLDMAA, B. N. WHITE, AND P. T. BOAG. 1991. DNA fingerprinting reveals relation between tail ornaments and cuckoldry in Barn Swallows. Behav. Ecol. 2:90–98.

SMITH, S. M. 1988. Extra-pair copulations in Black-capped Chickadees: the role of the female. Behaviour 107:15–23.

TRIVERS, R. 1972. Parental investment and sexual selection, p. 136–179. *In* B. Campbell [ed.], Sexual selection and the descent of man. 1871–1971. Aldine, Chicago.

WAGNER, R. H. 1992. The pursuit of extra-pair copulations by monogamous female Razorbills: how do females benefit? Behav. Ecol. Sociobiol. 29: 455–464.

WEATHERHEAD, P. J., AND S. B. MCRAE. 1990. Brood care in American robins: implications for mixed reproductive strategies by females. Anim. Behav. 39:1179–1188.

WESTNEAT, D. F. 1990. Genetic percentage in the Indigo Bunting: a study using DNA fingerprinting. Behav. Ecol. Sociobiol. 27:67–76.

WESTNEAT, D. F. 1993. Polygyny and extrapair fertilizations in eastern Red-winged Blackbirds. Behav. Ecol. 4:49–60.

WESTNEAT, D. F. 1994. To guard mates or go forage: conflicting demands affect the paternity of male Red-winged Blackbirds. Am. Nat. 144:343–354.

WESTNEAT, D. F., W. A. NOON, H. K. REEVE, AND C. F. AQUADRO. 1988. Improved hybridization conditions for DNA 'fingerprints' probed with M13. Nucleic Acids Res. 16:4161.

WESTNEAT, D. F., P. W. SHERMAN, AND M. L. MORTON. 1990. The ecology and evolution of extra-pair copulations in birds. Curr. Ornithol. 7:331–369.

WESTNEAT, D. F., AND M. S. WEBSTER. In press. Molecular analyses of kinship in birds: interesting questions and useful techniques, *In* R. DeSalle, B. Schierwater, B. Streit, and G. P. Wagner [eds.], Molecular approaches to ecology and evolution. Birkhauser, Basel.