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Extrapair Paternity Associated with Renesting in the American Goldfinch

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Copulations outside of the social pair bond occur in a wide range of bird species (Westneat et al. 1990, Birkhead and Møller 1992). However, the fitness consequences of this behavior have been established only recently (e.g. Westneat 1990, Kempenaers et al. 1992, Lifjeld et al. 1993, Dunn et al. 1994, Hill et al. 1994). Failing to account for extrapair copulations (EPCs) can result in measures of reproductive success that are overestimated for some individuals and underestimated for others (Gibbs et al. 1990). Therefore, to better understand the adaptive significance of extrapair mating behavior, the degree to which EPCs result in fertilization must be assessed. This is possible with the use of molecular techniques such as DNA fingerprinting (Jeffreys et al. 1985a,b). The present study was undertaken to determine the prevalence and correlates of extrapair paternity (EPP) in a population of American Goldfinches (*Carduelis tristis*) by combining behavioral observations with DNA fingerprinting.

The American Goldfinch is a small (ca. 12 g), sexually dimorphic cardueline finch that exhibits social monogamy (Middleton 1993; but see Middleton 1988). Because of the late initiation of breeding and the occurrence of a postnuptial molt, egg laying in southern Ontario is confined primarily to July and August (Middleton 1993). The result is a relatively synchronous breeding season in which the majority of clutches are started in the latter half of July (Middleton 1979). Both male and female goldfinches provide high levels of pre- and posthatching parental care. During the incubation period, males contribute indirect parental care by feeding the incubating female. Initially, adult males and females feed the nestlings in approximately equal proportions, but males gradually assume the predominant role and contribute most of the parental care after fledging (Middleton 1993). Thus, the fitness costs of cuckoldry to a pair-bonded male are high, and males guard their mates immediately before and during egg laying (Middleton 1993).

According to Westneat et al. (1990), this pattern of reproduction (i.e. synchronized breeding, mate guarding by males) should reduce the potential for extrapair copulations. However, behaviors such as mate guarding are not necessarily effective and have been found to be inversely related to levels of paternity (Gowaty and Bridges 1991). In conjunction with factors such as a male-biased operational sex ratio in American Goldfinches (Middleton 1993), this suggests that the potential for EPCs may be high in this species.

Methods.—This study was conducted on portions of the campus, arboretum, and nature reserve of the University of Guelph, Guelph, Ontario, Canada (see Middleton 1979). Field research was conducted from 1 June to 15 September 1992. Mist netting at baited stations was initiated before the onset of nesting activity (15 June). Trapped birds were banded with a unique combination of colored and aluminum bands, aged (Middleton 1974), and weighed to the nearest 0.1 g. In addition, blood samples (50 to 75 μL) were taken for DNA analysis.

Once located, nests were checked daily to monitor progress and to determine the onset of laying. To establish social parentage, nests were observed from blinds using spotting scopes. Because female goldfinches build the nest unaided, female social parentage (as opposed to genetic parentage) was assigned by recording band colors during the early stages of the nesting cycle and confirmed throughout the observation period. Male social parentage was assigned only if a focal male consistently was associated with the female during nest construction and was observed feeding the female and / or the nestlings at the nest.

For DNA fingerprinting, genomic DNA (2 to 5 μg) was restriction-digested with *Alu*I and electrophoretically separated on 1.0% w / v agarose gels in 1 × TBE buffer (90 mM Tris, 90 mM boric acid, 2 mM EDTA, pH 8.0). Gels were run at 45 V for 48 h or until the 2-kilobase Lambda size marker had traveled approximately 25 cm. The DNA was then transferred onto neutral nylon membranes (Hybond-N) using a vacuum transfer apparatus (LBK-Vacugene, Pharmacia). The DNA subsequently was fixed to the membranes by baking (65°C for 4 h) and hybridized with a nonisotopic, digoxigenin-labeled, minisatellite probe (Jeffreys 33.15; Jeffreys et al. 1985a,b). The nonisotopic DNA fingerprint detection methods that we used are described in detail elsewhere (Gissing and Crease 1997).

Fingerprints were scored by overlaying acetate sheets and marking bands at their most intense point. Bands in offspring were scored as maternally derived, paternally derived, or novel (i.e. occurring

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in neither parent). The background band-sharing coefficient (D) was calculated by comparing fingerprints of mates within nests and non-mates across nests when multiple nests were run on the same gel. Band-sharing coefficients were calculated using the statistic:

$$D = 2N_{AB}/(N_A + N_B), \qquad (1)$$

where N_A and N_B are the number of scored fragments in individuals A and B, and N_{AB} is the number shared by both (Wetton et al. 1987). To account for nonindependence when using multiple pairwise comparisons, Lynch's (1991) modified formula for variance was used. The same scoring criteria and formulas were used for all parent/offspring comparisons within nests.

Results.—In 1992, nesting followed the pattern observed previously for the American Goldfinch population at Guelph (Middleton 1978, 1979, 1993). Egg laying was initiated on 10 July. The bulk of reproductive activity (clutches started, $n = 25$) occurred during July ($n = 16$, 64%), with 40% ($n = 10$) of all clutches being started between 16 and 31 July and 24% ($n = 6$) between 1 and 15 July. Most of the clutches started between 1 and 31 August ($n = 9$) were renesting attempts ($n = 5$) following nest failure. Thus, as in previous years (Middleton 1979), nesting in 1992 was relatively synchronous. Of 39 nesting attempts, 19 reached clutch completion with an average clutch size of 5.1 ± SE of 0.19 eggs. A total of 157.9 h was spent monitoring nests ($\bar{x} = 7.9 \pm 0.94$) to ensure the accurate assignment of social parentage. Behavioral observations confirmed social monogamy at all nests. Although alternate males occasionally were seen near nest sites, no extrapair copulations were observed.

For DNA fingerprinting, blood samples were obtained from the putative father, putative mother, and all nestlings ($n = 70$) at 15 nests. The background band-sharing coefficient for this population was 0.168 ± 0.021. The transmission of minisatellite fragments followed Mendelian inheritance patterns with an overall parent-to-offspring transmission frequency of 0.490 ± 0.047, which is close to the expected value of 0.5. No band was present in all offspring; hence, all detected minisatellite loci were heterozygous.

In true genetic offspring, all bands in a fingerprint should be traceable to either parent except for occasional mutations (i.e. novel fragments). In this study, most offspring had a low number of novel fragments: 42 (65.6%) had no novel fragments, 10 (15.6%) had one novel fragment, 2 (3.1%) had two novel fragments, and no offspring had three novel fragments. The remaining 10 nestlings had more than three novel fragments. We followed Westneat's (1990) approach of examining the frequency of novel fragments in relation to band-sharing coefficients to determine criteria for the exclusion of a parent.

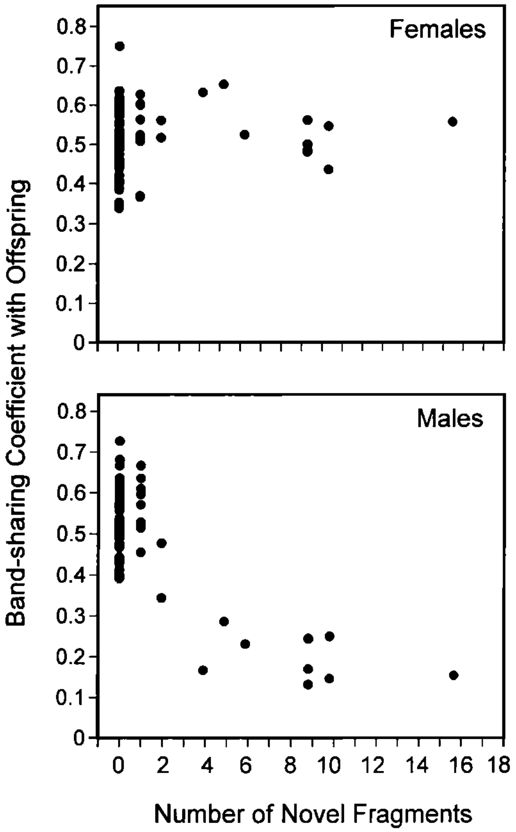

FIG. 1. Relationship between band-sharing coefficients of putative parents with offspring and number of novel fragments in nestling DNA fingerprints for the American Goldfinch.

First, using the average number of novel fragments over the categories zero, one, two, and three (0.259/offspring), the expected Poisson distribution was calculated and compared with the observed distribution (Westneat 1990). The observed number of offspring across these categories was not different from that expected under a Poisson distribution ($\chi^2 = 0.21$, df = 1, $P > 0.5$) for rare events. Under this distribution, the probability of a nestling containing three or more novel fragments arising through mutation alone was considerably less than 0.01. We concluded that nestlings with more than three novel fragments were genetically mismatched to one or both of their putative parents. Second, to differentiate between these two possibilities, band-sharing coefficients between putative parents and offspring were analyzed. Two patterns emerged. Average female band-sharing coefficients with offspring remained high ($\bar{x} = 0.537 \pm 0.021$) as the number of novel fragments increased, indicating that in all cases putative mothers were the true genetic parents of offspring (Fig. 1).

TABLE 1. Band-sharing coefficients and paternity conclusions for the four American Goldfinch nests with extrapair paternity.

Nestling	Band-sharing coefficient[a]		Conclusion[b]
	Female	Male	
	Nest 92-08		
1	0.632	0.167	EPC
2	0.652	0.286	EPC
3	0.558	0.491	M
4	0.435	0.250	EPC
5	0.524	0.231	EPC
	Nest 92-14		
1	0.429	0.634	M
2	0.383	0.696	M
3	0.500	0.255	EPC
4	0.522	0.178	EPC
5	0.625	0.511	M
	Nest 92-91		
1	0.370	0.610	M
2	0.556	0.154	EPC
3	0.524	0.518	M
4	0.560	0.132	EPC
	Nest 92-22		
1	0.485	0.244	EPC
2	0.600	0.526	M
3	0.545	0.146	EPC

[a] EPC, offspring sired through extrapair mating; M, offspring genetically matched to both putative parents.

[b] Between offspring and male or female parent.

Second, male band-sharing coefficients with their putative offspring dropped markedly beyond the critical value of three novel fragments (Fig. 1). The average band-sharing coefficient between putative fathers and offspring with zero, one, and two novel fragments was 0.531 ± 0.011, significantly higher than that for offspring with more than three novel fragments ($\bar{x} = 0.202 \pm 0.017$; $t = 12.21$, $P < 0.01$). Thus, we concluded that nestlings with more than three novel fragments were sired by extrapair males. In summary, 10 of 70 (14.3%) nestlings in 4 of 15 (26.7%) broods had fingerprint profiles inconsistent with putative parents, and all such nestlings resulted from extrapair copulations. The proportion of EPP offspring within nests was high, ranging from 40 to 80% (Table 1).

There was no difference in the date of first egg between broods with and without EPP offspring (Mann-Whitney *U*-test, $U = 24$, $P > 0.05$). There was also no significant difference in body mass between males that attended nests with ($\bar{x} = 13.1 \pm 0.24$ g) and without ($\bar{x} = 13.3 \pm 0.27$ g) EPP offspring ($t = 0.52$, $P > 0.6$). Of the four nests with EPP offspring, three of the attendant males were in their first breeding season (i.e. were inexperienced), and one was of unknown age. However, seven inexperienced males and four experienced males had no extrapair young in their broods. Because the age of one male was unknown, we performed these tests twice, classifying the male as experienced and then inexperienced. Neither test was significant (Fisher exact test, $P = 0.24$ and $P = 0.07$, respectively). Because age determination is difficult in female goldfinches, we did not perform this test for females.

Of the four nests that contained EPP offspring, three were renesting attempts after a previous nest failed due to predation during incubation. The proportion of nests with EPP offspring was significantly higher for such renests than for single nesting attempts (Fisher exact test, $P = 0.01$). The other nest that contained extrapair offspring also was considered anomalous in that there was a protracted delay between nest completion and clutch initiation; both birds were not seen for more than one week after the nest bowl was flooded before egg laying. This nest originally was categorized as abandoned, but the same banded birds returned and resumed breeding activity.

Discussion.—Based on DNA fingerprinting and behavioral observations, we found that 10 of 70 (14.3%) offspring in 4 of 15 (26.7%) broods resulted from EPCs in a socially monogamous population of American Goldfinches. The use of DNA fingerprinting also facilitates tests for correlates of paternity including phenotypic and ecological variables. In birds, correlates such as the presence of infertile eggs (Wetton and Parkin 1991), tarsus length and annual survival (Kempenaers et al. 1992), mate guarding and food abundance (Westneat 1994), and body mass (Whittingham and Lifjeld 1995) have been confirmed. However, other studies have failed to detect such correlates (e.g. Dunn et al. 1994), and correlates of paternity appear to differ across species. Moreover, levels of extrapair paternity have been found to vary across populations (Lifjeld et al. 1991, Gelter and Tegelstrom 1992) and years (Dunn et al. 1994, Hill et al. 1994) within the same species. The causal factors for such variation remain poorly understood.

Our study provides the first evidence that one such factor may be renesting following nesting failure. Although the total sample of nests surveyed was limited, the association between the occurrence of EPP and disruptions in the nesting cycle was strong. If EPP is not correlated with nest disruption in the goldfinch, then the probability of all four disturbed nests containing EPP offspring would be less than 0.01, given that the overall frequency of such nests was 26.7%. Conversely, the probability of all 11 of the undisturbed nests containing no EPC offspring would be 0.03. Thus, among other potential causes, the possibility that nest disruption is a contributing factor to EPP deserves further consideration.

We observed no cases of forced copulation, so it seems unlikely that this accounted for extrapair offspring. Moreover, female passerines generally control the timing and success of copulations (Arvidsson 1992, Birkhead and Møller 1992). Thus, extrapair offspring in goldfinches appear to result from females actively soliciting EPCs or accepting unsolicited EPCs. Distinguishing between these alternatives was not possible in our study. However, the question remains: Why do disruptions in the nesting cycle lead to the involvement of females in EPCs? We present two possible hypotheses that lead to testable predictions.

First, from a male perspective, disruptions may reduce mate-guarding efficiency, providing opportunities for females to participate in EPCs. Because of the high cost of being cuckolded, males should employ paternity-assurance behaviors (Trivers 1972). Indeed, male goldfinches guard their mates intensively during the nest-building and egg-laying periods (Middleton 1993). Although mate-guarding behavior can be influenced by a male's age and physical condition, we found no significant effect of male age or body mass on the proportion of broods containing extrapair offspring. Alternatively, it is possible that males are less effective or show reduced levels of mate guarding at nests with anomalous temporal patterns, such as renestings following predation. When a nest is lost to predation, female goldfinches begin producing a new clutch within seven days (Middleton 1979). In such situations, a male may have little time to synchronize its behavior with that of its mate immediately after a nest loss, which could delay the resumption of mate guarding or reduce its intensity. This undoubtedly will depend on the behavioral plasticity of the male. However, female goldfinches generally start relaying within a period of time that corresponds to the duration of sperm storage in passerines (7 to 10 days; Birkhead et al. 1989), and even slight reductions in paternity-assurance behavior immediately after nest loss could result in cuckoldry. If such disruptions have a negative effect on mate-guarding behavior, then one would predict mate guarding to be less intense immediately following nest loss. In addition, because renesting necessarily increases the amount of effort (total seasonal time budget) expended by the paired male in both mate-guarding and copulatory behavior compared with males that nest successfully in their first attempt, renesting increases the probability that extrapair males will gain access to paired females. This may be particularly relevant if renesting occurs outside the peak breeding season (i.e. reduces synchrony) when extrapair males are less occupied with mate guarding and may be more able to actively pursue or participate in EPCs.

Second, from a female perspective, females may increase efforts to obtain, or be more accepting of, EPCs following a disruption in the first nesting attempt. This may be the case if, for example, females use nest failure as a cue to assess male quality. In such cases, even if males maintain the same level of mate guarding during renesting attempts, mate-guarding efficiency may be effectively reduced because of a modified female strategy. Under this hypothesis, females should be more likely to participate in EPCs during renesting attempts either by being less resistant to the advances of extrapair males or by soliciting EPCs more readily. If females do become more promiscuous during renesting attempts, such behavior may be accompanied by a reduction in copulation rates within pairs, an alteration in the timing of EPCs, or an active increase in the opportunity for EPCs.

In conclusion, our findings suggest that males attempt to protect paternity by mate guarding but are less effective at doing so when their first nesting attempt is disrupted. Whether this occurs because of reduced male mate-guarding behavior and/or increased female promiscuity remains unclear. The opportunistic employment of a mixed strategy by female American Goldfinches in association with a largely stochastic variable (predation) represents a potentially important factor in addressing the difficulty of predicting levels of EPCs based on ecological factors at the species level (see Westneat et al. 1990). That is, variation in nesting patterns among species and among populations within species may result in different potentials for extrapair copulation (see Lifjeld et al. 1991, Gelter and Tegelstrom 1992). Our findings suggest that one important and previously unexplored component of this variation is the frequency of nest failure due to predation. This may be particularly relevant in monogamous passerines in which males attempt to ensure paternity through mate guarding and females renest rapidly following nesting failure.

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