

## EXTRA-PAIR PATERNITY IN THE EUROPEAN STARLING: THE EFFECT OF POLYGYNY<sup>1</sup>

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**Abstract.** We determined the frequency of extra-pair paternity and intraspecific brood parasitism in European Starlings (*Sturnus vulgaris*) by performing multilocus DNA fingerprinting on 22 complete families from a population in southern Sweden. Among 92 nestlings, we did not detect any successful intraspecific brood parasitism. This confirms observational data that successful intraspecific brood parasitism is rare in this population. We detected eight extra-pair young. Except for one case, where all (two) nestlings were fathered by another male than the one attending the nest, no more than one nestling in each nest was fathered by an extra-pair male. Although the degree of extra-pair paternity was higher in nests of secondary females than in those of primary and monogamous females, this difference was not significant. There was no relationship between a male's paternity with his primary and secondary female during the same season. There was a tendency for males to loose paternity in the broods of secondary females when the fertile periods of his females overlapped considerably. We suggest that the relative ease by which female Starlings can be mate-guarded, the fact that mate-attraction and mate-guarding do not have to be exclusive activities and the high within pair copulation frequency in Starlings may keep the degree of extra-pair paternity low.

**Key words:** *Sturnus vulgaris*; DNA fingerprinting; extra-pair paternity; polygyny.

### INTRODUCTION

Among birds, monogamy is the predominant mating system (e.g., Lack 1968, Silver et al. 1985). It has become increasingly clear, however, that apparent social pair bonds do not always reflect the genetic structure of a population. Using various techniques, it has been shown that the putative father or mother often is not the genetical father and mother of the offspring in a nest (Westneat et al. 1990, Birkhead and Møller 1992). DNA fingerprinting and related methods have proved to be especially powerful in revealing the genetic mating pattern in bird populations (Jeffreys et al. 1985, Burke and Bruford 1987, Wetton et al. 1987, Burke 1989, Gibbs et al. 1990).

Extra-pair offspring might have several origins. (1) Females might perform intraspecific brood parasitism (Yom-Tov 1980, Andersson 1984). (2) Females might participate in copulations outside the pairbond (extra-pair copulations, EPC) (Ford 1983, McKinney et al. 1984).

(3) Rapid mate-switching might occur and the replaced male might achieve some paternity in the subsequent brood (McKinney et al. 1984, Møller 1985). This paper is mainly concerned with extra-pair paternity (EPP) due to extra-pair copulations, although egg-dumping and rapid mate-switching will be briefly discussed.

DNA fingerprinting and related methods have shown that the degree of EPP varies widely between species. For example, nearly every offspring was fathered by the putative father in monogamous Dunnocks *Prunella modularis* (Burke et al. 1989), the Willow Warbler *Phylloscopus trochilus* and the Wood Warbler *P. silbatrix* (Gyllenstein et al. 1990) and the Zebra Finch (Birkhead et al. 1990). On the other hand, in the Red-winged Blackbird *Agelaius phoeniceus* (Gibbs et al. 1990, Westneat 1993), the Indigo Bunting *Passerina cyanea* (Westneat 1990), the Shag *Phalacrocorax aristotelis* (Graves et al. 1992) and the Tree Swallow *Tachycineta bicolor* (Liffield et al. 1993) EPP has been found to be high with 25–28%, 35%, 18% and 38% of the offspring being fathered by a male other than the putative father.

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Furthermore, EPP has also been found to vary considerably between different populations of the same species. For a Norwegian population of the Pied Flycatcher *Ficedula hypoleuca*, Lifjeld et al. (1991) found a very low frequency of EPP (4%), whereas Gelter and Tegelström (1992), also using DNA fingerprinting, found it to be 24% for a Swedish population.

There are many hypotheses explaining variation in EPP both within and between populations (reviewed by Birkhead and Møller 1992). EPP may result from forced copulations (e.g., Afton 1985). Since most birds lack intromittent organs, some cooperation from the female is necessary for successful copulation (Fitch and Shugart 1984). However, females may mate with extra-pair males to minimize costs of copulations (Westneat 1992).

Females might also gain from EPCs. Different patterns are expected depending on what a female may gain by seeking EPCs. Here we concentrate on genotypic advantages, since it seems unlikely that female birds gain phenotypic advantages (like nutrients or male help) by participating in EPCs (Westneat et al. 1990).

According to the *mate acquisition hypothesis* (Colwell and Oring 1989), extra-pair copulations is a way for females to test prospective mates. This hypothesis predicts extra-pair copulations to occur during mate selection.

The female might take part in EPCs to insure herself against the mate being infertile (the *infertility insurance hypothesis*) (Gibson and Jewel 1982). Since females of most or all bird species are able to store sperm (Birkhead and Møller 1992), one or a few EPCs should be enough to insure against male infertility. We would then expect EPP to occur at a low frequency, but occasionally find whole families where the putative father had no paternity (if he is infertile) or low paternity (if his sperm is of poor quality) (Wetton and Parkin 1991). If a male has low paternity in one brood, he also should have low paternity in all his other broods.

Females might increase the genetic variance among their offspring by participating in EPCs, thereby reducing the variance in offspring survival (the *genetic variability hypothesis*) (but see Williams 1975). According to this hypothesis, we expect EPP to be evenly dispersed among families (Westneat et al. 1990).

According to the *sexual selection hypothesis*, females might obtain "good genes" for their off-

spring by mating with high-quality or ornamented males (Mineau and Cooke 1979, Møller 1988, Smith 1988). According to this hypothesis, we would expect low quality males to have low paternity but high quality males to father the whole brood. Hence, EPP will occur in a small proportion of broods, but in these account for a high proportion of offspring (Westneat et al. 1990). Furthermore, we would expect some males to consistently show low paternity and others high paternity in their broods. In polygynous species, males with more than one female might be the more attractive mates and therefore achieve a higher paternity in their broods than monogamous males (Westneat et al. 1990, Kempenaers et al. 1992).

Variation in EPP might also depend on the males' abilities to defend exclusive mating access. Defense of paternity might be achieved by either copulating frequently within pairs (McKinney et al. 1984, Birkhead et al. 1987) or through mate-guarding (Beecher and Beecher 1979, Birkhead 1979). According to the *mate-guarding hypothesis*, variation in paternity can be explained by variations in the ability of males to guard their mate(s). A male might trade mate-guarding against other activities such as attracting additional mates or guarding the nest (Westneat et al. 1990, Bollinger and Gavin 1991, Hasselquist and Bensch 1991). Mate-guarding of the primary female might be traded against attraction of a secondary female, resulting in a higher degree of extra-pair paternity for the offspring of the primary, than of the secondary, female. Furthermore, in polygynous species we might expect males with more than one female to be unable to guard all females efficiently if the females' fertile periods overlap. Hence, we expect paternity to be lower for polygynous males than for monogamous ones. For polygynous males we expect paternity to be lower the greater the degree of overlap between female fertile periods. Whether a male invests most in guarding the primary or secondary female may depend on the relative value of the broods, which might change over the season (Perrins 1965).

The purpose of the present study was to estimate the degree of extra-pair paternity and maternity in the European Starling *Sturnus vulgaris*. The European Starling is a facultatively polygynous passerine that breeds either solitarily or in colonies (Pinxten and Eens 1990; Smith et al., in press). The male defends a small territory in-

cluding the nest-site, but no food resources (Feare 1984). In south Sweden, Starlings arrive at the colonies in early spring (mid March), but do not lay their eggs until late April or early May. Breeding is highly synchronous, with most females laying within a week of each other (Karlsson 1983). During the female's fertile period, she is intensively guarded by her mate (Power et al. 1981, Pinxten et al. 1987). Egg-dumping (Yom-Tov et al. 1974, Lombardo et al. 1989, Romagnano et al. 1990, Pinxten et al. 1991), extra-pair copulations (Hoffenberger et al. 1988, Eens and Pinxten 1990) and rapid mate-switching (Pinxten et al. 1993) have all been reported for the European Starling.

## METHODS

### STUDY AREA

The study was performed in 1991 in the Revinge area in southern Sweden. The area consists mainly of permanent pastures grazed by cattle. The study included three colonies with 15 nestboxes each and one colony with 40 nestboxes.

### CAPTURING OF ADULTS

Starlings arrived to the colonies in the beginning of March onwards. Adult Starlings were captured both before breeding (10 March–14 April) and during the incubation and nestling feeding periods. For each bird captured, we determined wing length to the nearest 1 mm with a ruler, tarsus length to the nearest 0.05 mm with calipers, and mass to the nearest 0.1 g using a Pesola spring balance. All birds were measured by the same person. Sex and age (second-year bird vs. older bird) were determined according to Svensson (1984). Each bird was equipped with a unique combination of three color bands and with one numbered aluminium band. We collected 100  $\mu$ l of blood from each captured bird by jugular venipuncture. Collected blood was immediately added to 1 ml of SET-buffer (0.15 M NaCl, 0.05 M TRIS, 0.001 M EDTA), stored temporarily on ice, and frozen to  $-50^{\circ}\text{C}$  later the same day.

### BREEDING DATA

During the egg-laying period, nestboxes were visited daily after 10:00 (Swedish winter time). All eggs were individually marked with a permanent marker. We defined as parasitic, all single eggs laid more than one day before the laying of the clutch started. We also defined as parasitic, those

eggs laid more than one day after the last egg of the clutch. In addition, we assumed that a parasite was involved when more than one egg was laid per day (Lombardo et al. 1989).

Nestlings were banded fourteen days after hatching. At the same time we took a blood sample from each nestling as described above.

### DETERMINATION OF MATING STATUS

Most males and females assigned to nests were seen incubating or carrying food to nestlings. However, since in some cases the male did not participate in the rearing of the secondary female's clutch/brood, for the remainder of the cases we used the criteria that the male had defended the nestbox, built the nest and/or visited the nestbox just before the laying of the first egg (Smith et al., in press). We defined a female as monogamous, primary or secondary depending on the number of other females mated to her mate and the laying date of her first egg relative to those females. Hence, we assume that the first female mated to a male also laid her clutch before later arriving females (see Smith et al. [in press] for a support of this assumption). Since nearly all breeding males were captured and banded it was possible to assign mating status to nearly all females (Smith et al., in press). In two cases both females mated to the same male initiated laying on the same day. In one case we knew which female mated first with the male. In the second case we assumed that the older of the two females was the primary female, since in 13 out of 14 cases when the ages of the primary and the secondary female differed, the primary female was the older one.

For this study we selected eight polygynous families (one male, two females mated with him and nestlings from two broods) and six monogamous families. We only used birds breeding during the early synchronous period (cf. Pinxten et al. 1990), defined here as clutches initiated within 14 days after the initiation of the first clutch in the study population. Only 6% of females, including renestings, initiated clutches later than this. For a few families, we lacked blood samples from all family members since we either did not capture one of the parents or since all nestlings died before blood samples were taken. We selected families randomly among those where we had blood samples from both parents and all nestlings.

## DNA FINGERPRINTING

DNA was extracted by adding 10  $\mu$ l of SDS (20%) and 13  $\mu$ l of Proteinase K (10 mg/ml) to 500  $\mu$ l of thawed SET-buffer with blood samples and incubating it over night at 55°C. DNA was purified by extractions with phenol, phenol/chloroform-isoamylalcohol and chloroform-isoamylalcohol. The dissolved DNA was precipitated with 0.1 vols 3 M sodium acetate and two vols absolute ethanol, washed with 75% ethanol and vacuum dried. The DNA was solved and stored in 50–150  $\mu$ l 1  $\times$  TE and the DNA concentrations measured on a spectrophotometer. About 10  $\mu$ g of total cellular DNA was digested with 18–20 units of the restriction enzyme *Alu* I for three hours in 37°C and subsequently electrophoresed through a 25 cm long 0.8% horizontal agarose gel in 0.5  $\times$  TBE buffer. The gels were run at 26 V for about 70 hours until fragments smaller than 2 kb had migrated off the gel. The DNA was transferred to nylon filters (Hybond N+) by a vacuum blot while the gels were soaked in 0.4 M NaOH. Filters were hybridized over night at 64°C in 5  $\times$  SSPE, 5  $\times$  Denhardt's solution, 0.5% SDS and the radioactively labelled probe 33.15 (Jeffreys et al. 1985). Filters were washed at 64°C in 2  $\times$  SSPE and 0.1% SDS for 30 min and 2  $\times$  30 min in 1  $\times$  SSPE and 0.1% SDS. The filters were autoradiographed for 1–7 days at –70°C.

## STATISTICS

Analyses were performed with SYSTAT (Wilkinson 1990). All proportions were arcsine square-root transformed. One-tailed tests were used only when explicit hypotheses were tested. This is indicated in the text.

## RESULTS

### SCORING OF FINGERPRINTS

The *Alu* I combined with Jeffreys' 33.15 produced a highly variable multiband pattern (see also Pinxten et al. 1993). When scoring bands, we considered bands with similar electrophoretic mobility (less than 0.5 mm apart) identical (cf. Birkhead et al. 1990). When a band present in one individual might have been obscured by stronger bands in the individual with which it was compared, it was excluded from the analysis (cf. Birkhead et al. 1990). Families were always run on the same gel with parents on either side

of the nestlings in their broods. Band-sharing coefficients (Wetton et al. 1987) for unrelated individuals were calculated by comparing parents.

When analyzing DNA fingerprints, it is important that bands segregate independently of each other. Pinxten et al. (1993) found for the European Starling, using Jeffreys 33.15 and *Alu* I, that bands segregated independently.

It was possible to score bands between 3 kb and 23 kb. We scored on average 16.0 (SD 5.0) bands in nestlings and 16.3 (SD 5.5) bands in parents. The bandsharing coefficient between parents was 0.167 (SD 0.113,  $n = 22$ ). We scored a minimum of six bands in a nestling, giving a probability of false inclusion of a pair of  $8.2 \times 10^{-4}$  (Burke et al. 1989). The number of paternal bands scored in each offspring was 6.7 (SD 2.8). However, the minimum number of scored paternal bands was three, giving a probability of false inclusion of an unrelated individual as high as 0.005 (Jeffreys et al. 1985, Davies et al. 1992). However, we do not regard this as a serious problem since we did not compare multiple males with the offspring.

We found up to seven unique bands in one nestling. We assume that at least two unique bands might be the result of mutation (cf. Westneat 1990). Figure 1 shows the relationship between band-sharing coefficients with the putative mother and the putative father and the number of unique bands in a nestling. The offspring with less than three unique bands had high band-sharing coefficients with both the putative father ( $\bar{x} = 0.54$ ,  $SD = 0.10$ ) and the putative mother ( $0.56 \pm 0.10$ ), whereas those offspring with more than two unique bands had high band-sharing coefficients with the putative mother ( $0.58 \pm 0.09$ ), but not with the putative father ( $0.11 \pm 0.10$ ). In fact, there was no overlap between the band-sharing coefficients between the nestling and the putative father for those that had two or fewer unique bands and those that had more than two unique bands. Hence, we conclude that those nestlings with more than two unique bands were fathered by a male other than the one attending the nest.

### FREQUENCY OF EXTRA-PAIR MATERNITY

We found no genetic evidence of egg-dumping among the selected nests. Observational data in-

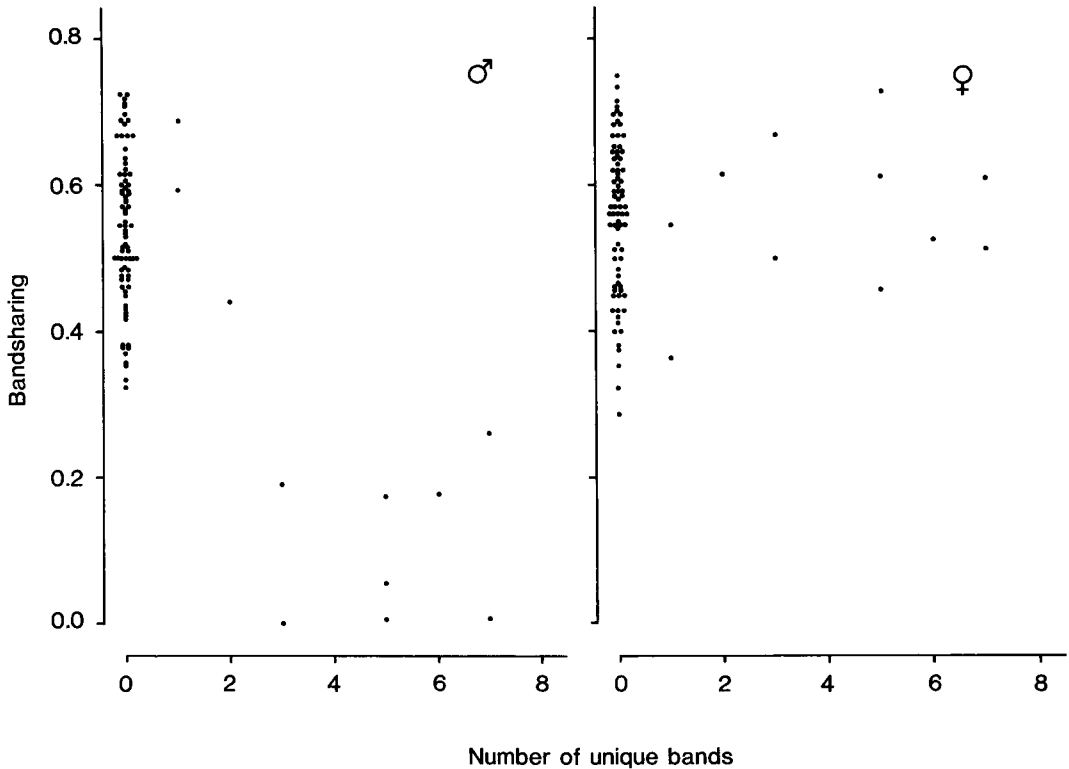


FIGURE 1. The relationship between bandsharing coefficients between an offspring and its putative father or its putative mother and the number of unique bands detected in an offspring.

dicated that four out of 117 eggs were parasite eggs. Of these, however, three were laid before the first egg in the clutch and were thrown out (presumably by the female, Stouffer et al. 1987) and one was laid several days after the clutch was completed and never hatched.

#### FREQUENCY OF EXTRA-PAIR PATERNITY

In our 22 nests, we found evidence of extra-pair paternity in seven (31.8%). However, with one exception, there was only one extra-pair offspring in each nest. Hence, only eight out of 92 nestlings (8.7%) were fathered by another male than the one attending the nest. In one nest, only two out of four nestlings survived until blood-sampling and they had another father than the one attending the nest. This male was observed incubating, but since he was not banded until incubation we cannot exclude rapid mate-switching. The expected number of nests with no extra-pair paternity if extra-pair young was randomly dis-

tributed across broods (Lifjeld et al. 1993) was 15.15 and did not differ from that observed (15).

There was no significant difference in the degree of extra-pair paternity between nests of different mating status (monogamous, primary, secondary) (Fig. 2; Kruskal-Wallis one-way analysis of variance,  $H = 0.54$ ,  $P = 0.76$ ). The apparent tendency for extra-pair paternity to be higher in secondary nests was partly due to the single case where all nestlings in one nest had another father than the attending male. Furthermore, the average paternity per nest was not different for monogamous and polygynous males (Mann-Whitney  $U = 20.5$ ,  $P = 0.62$ ). Paternity was lower for a male's secondary than for his primary brood, but not significantly so (Wilcoxon matched-pairs signed-rank test,  $Z = 1.07$ ,  $P = 0.29$ ). There was no relationship between how much paternity a polygynous male achieved with his primary and his secondary female (Fig. 3; Spearman rank correlation,  $r_{s6} = -0.05$ ,  $P > 0.5$ ). Polygynous males fathered a higher number of own offspring in the

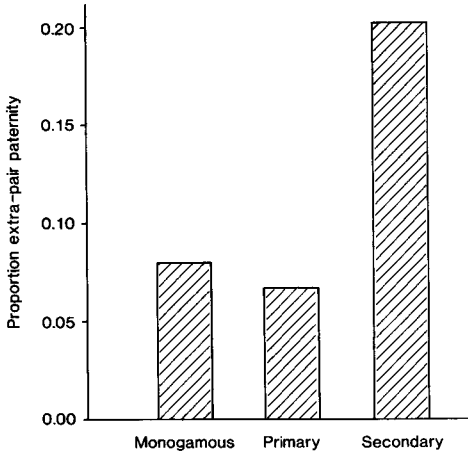


FIGURE 2. The relationship between the proportion of a brood fathered by an extra-pair male and the mating status of the mother.

nests of their females (mean 7.38, SD 0.60) than monogamous males in their single nest ( $4.17 \pm 0.69$ ;  $U = 3$ ,  $P = 0.005$ ).

Nothing is known about the length of the fertile period in the Starling. Following a number of other studies (see Birkhead and Møller 1992), we defined the fertile period as beginning five days before the laying of the first egg and lasting until the laying of the penultimate egg. This period agrees well with the mate-guarding period in Starlings (Power et al. 1981, Pinxten et al. 1987). There was a tendency for the proportion extra-pair paternity to be higher when the female's fertile period was overlapped by the fertile period of another female mated to the same male ( $r_{s14} = 0.38$ ,  $P = 0.08$ , one-tailed; Fig. 4). This was due to an effect on the secondary females' broods ( $r_{s6} = 0.55$ ,  $P = 0.09$ , one-tailed) but not on primary females' broods ( $r_{s6} = 0.12$ ,  $P > 0.25$ , one-tailed).

Among females, the incidence of extra-pair paternity was higher for first-year breeders (16.7%,  $n = 9$ ) than among older breeders (5.9%,  $n = 11$ ), but not significantly so ( $U = 54$ ,  $P = 0.67$ ). There was no relationship between the proportion of extra-pair paternity and female tarsus length ( $r_{s20} = 0.36$ ,  $P > 0.1$ ), or wing-length ( $r_{s20} = 0.10$ ,  $P > 0.5$ ).

When analyzing the effect of morphology for males, we excluded secondary nests to include the same male only once. Since only one male was a first-year breeder we could not analyze the

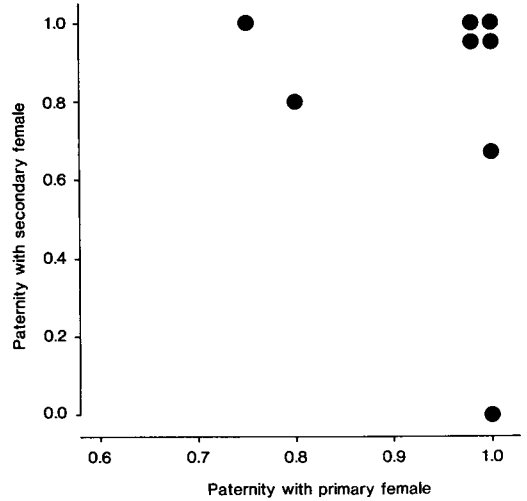


FIGURE 3. The relationship between the proportion of nestlings fathered by polygynous males in the nests of their primary and secondary females.

effect of male age. There was no relationship between the degree of extra-pair paternity and male hackle-feather length ( $r_{s11} = -0.10$ ,  $P > 0.5$ ), male wing length ( $r_{s12} = -0.22$ ,  $P > 0.2$ ) or male tarsus length ( $r_{s12} = -0.24$ ,  $P > 0.2$ ).

We found in total eight unhatched eggs in the

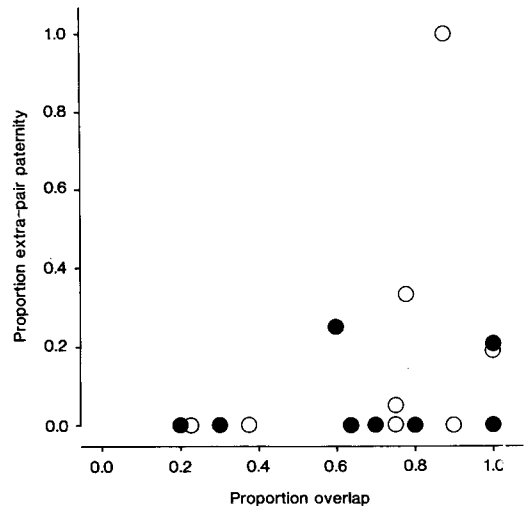


FIGURE 4. The relationship between the degree of extra-pair paternity and the degree to which a particular female's fertile period overlapped with the fertile period of another female mated to the same male. ● indicates primary females and ○ secondary females.

studied nests (excluding one parasitic egg that did not hatch) of which we could examine the content of five. One of these contained a fully developed dead chick and the rest showed no trace of embryos. There was no relationship between the proportion of unhatched eggs and the proportion of extra-pair offspring in broods ( $r_{s20} = -0.09, P > 0.5$ ).

## DISCUSSION

### EGG-DUMPING

We found no genetic evidence of egg-dumping among the studied nests although at least four parasite eggs were laid in those boxes. However, we knew that none of the known parasite eggs hatched. This indicates that our method of finding parasite eggs by using irregularities during laying is rather efficient. It also indicates that egg-dumping only contributes to a minor part of the gene-pool the next generation. The main reason for this is the low success of parasite eggs laid before or after laying of the clutch (e.g., Stouffer et al. 1987) and the problems for parasite females of obtaining access to the nest during the laying period due to nest-guarding (cf. Emlen and Wrege 1986; H.G. Smith, unpubl.).

### EXTRA-PAIR PATERNITY

The frequency of extra-pair paternity among the selected nestlings was 8.7% (31.8% of broods). This figure is much higher than the estimate for starlings by Hoffenberg et al. (1988), 2.1–8.4% of broods, using protein electrophoresis. Their method, however, underestimates the actual frequency of EPP because of the low resolution of the method (Wrege and Emlen 1987). Pinxten et al. (1993), using DNA fingerprinting, estimated the frequency of EPP in starlings to be 9.7% of offspring (28.6% of broods). About half of this could be attributed to rapid mate switching. In total these figures show that the frequency of extra-pair paternity is lower in Starlings than has been found for most other colonially breeding passerine birds (reviewed by Birkhead and Møller 1992).

It seems unlikely that forced copulations explain the pattern of extra-pair paternity. In an extensive study of copulation behavior of European Starlings, Eens and Pinxten (1990) did not observe any forced copulations. Rather, males tried to stimulate already mated females to mate with them by singing close to them.

According to the mate acquisition hypotheses, extra-pair copulations were used to test prospective mates. However, since the starling in south Sweden is single-brooded and females that reneest after breeding failure normally change colonies (H. G. Smith, pers observ.), this hypotheses seems unlikely to apply for the starling.

According to the infertility insurance hypothesis, females could insure against their mate having inviable sperm or sperm with poor fertilizing efficiency by mating with other males (Gibson and Jewel 1982). Since the proportion of extra-pair copulations in starlings is low (Eens and Pinxten 1990; Smith, unpubl.), we would expect males to lose much paternity only when they have sperm of poor quality. We had one male who did not father any young in his brood, but he was clearly not infertile since he fathered all the young of his primary female. There was also no relationship between the frequency of unhatched eggs and the incidence of extra-pair offspring. Hence, our data contrast with those for the house sparrow *Passer domesticus*, for which the infertility insurance hypotheses has been suggested to apply (Wetton and Parkin 1991). Birkhead and Møller (1992) also suggested that the relationship was due to female, rather than male, infertility.

Our data fit the genetic variability hypothesis since EPP was distributed evenly among broods. However, the hypotheses has an inherent weakness. In a sexually reproducing species, offspring will be genetically variable even if a female only copulates with one male (Williams 1975).

A number of studies have supported the sexual selection hypothesis. Several studies have shown that females actively seek extra-pair copulations with high quality males (e.g., Smith 1988; Møller 1988, 1990; Burley and Price 1991; Kempenaers et al. 1992). Møller (1988) also demonstrated experimentally that female Barn Swallows (*Hirundo rustica*) used the same trait (tail length) when selecting social and genetic mates. As noted by Møller (1992), females should seek EPCs with genetically superior males whenever there is a constraint on female mate choice. In the starling, song seems to be used both for mate-attraction (Cuthill and Hindmarsh 1985, Eens et al. 1990, Mountjoy and Lemon 1991) and when pursuing extra-pair copulations (Eens and Pinxten 1990). At the same time female mate choice is constrained by the fact that she has to choose a male

with an available nest-hole. Hence we expect the sexual selection hypothesis to apply for starlings.

Why then do we not find EPP to be highly clumped among nests? The reason might simply be that mate-guarding is efficient in the starling (Hoffenberg et al. 1988). First, during the female's fertile period male starlings guard their females intensively (Power et al. 1981, Pinxten et al. 1987). Furthermore, copulation frequencies are high. For example, in our population females, during their fertile period, copulated on average 1.8/hr during the morning hours of their fertile period (H. G. Smith, unpubl.). This makes the relative frequency of EPCs low in starlings (Eens and Pinxten 1990). The reason we do not find the patterns expected from the sexual selection hypothesis might simply be that with such low probability of EPP the stochasticity will be high.

If mate-guarding is important in starlings, then we would expect polygynous males, who have to simultaneously guard two females, to show lower paternity in their broods than monogamous ones. We found no significant difference between the average degree of extra-pair paternity for polygynous and monogamous males. This might be due to several reasons. First, since polygynous starlings normally defend adjacent nestboxes (Smith, unpubl.), males may be able to mate-guard and attract additional females simultaneously while their first female is in the colony (Pinxten et al. 1987). Since primary females guard their nests, they spend a considerable amount of time in the colonies and also forage nearby (H. G. Smith, pers. observ.). Second, males might guard their primary females efficiently so that any effect of polygyny will mainly be found for secondary females. It seems that males value the offspring of primary females higher than that of secondary females, since they invest much more paternal care in the former (Smith et al., in press). The degree of extra-pair paternity was higher in secondary females broods than in those of primary females, but not significantly so. However, the power of this test is low due to the small sample size. Third, there might only be a conflict between guarding the primary and the secondary female when the overlap between their fertile periods is high. We found a tendency for paternity to be lower when the fertile periods of females mated to the same male overlapped extensively. Interestingly, this trend was apparent for secondary females, but not for primary ones,

supporting the suggestion that males primarily guard their primary females. Fourth, polygynous males might be the most attractive ones, making females mated to them less prone to participate in EPCs.

Other studies of polygynous species have yielded variable results. Lifjeld et al. (1991) found that polygynous Pied Flycatchers had similar levels of extra-pair paternity in their broods as monogamous ones, although polygynous males may leave their primary female unguarded during her fertile period. For the Red-winged Blackbird, Gibbs et al. (1990) found that EPP eliminated any relationship between apparent and realized reproductive success, whereas Westneat (1993) found no relationship between harem size and the proportion of EPP. Furthermore, Westneat (1993) found no difference in the degree of EPP for females whose fertile periods overlapped compared to those that were fertilizable alone on territories of polygynous males. In the Tree Swallow, extra-pair paternity was much higher for polygynous males than for monogamous ones (Dunn and Robertson 1993). In the Tree Swallow, males do not guard their females (Leffelaar and Robertson 1984), hence this relationship might be explained by betrayed males attracting additional mates (Dunn and Robertson 1993).

An additional reason that the degree of extra-pair paternity might be higher in secondary females' broods is that females might have copulated with other males before mating with a male. For most primary and monogamous females this does not pose any threat to paternity, since they are paired with males well before their fertile period (H. G. Smith, unpubl.). However, secondary females often pair with males soon before egg-laying and therefore may have mated with other males during their fertile period (Smith et al., in press; cf. Pinxten et al. 1993). It is important for secondary females to lay as early as possible to achieve help from the male (Smith et al., in press), while it may benefit the male to delay laying to insure paternity. This may produce a conflict between the sexes over laying time.

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