

Extra-pair fertilizations in a predominantly monogamous bird: genetic evidence

DAVID F. WESTNEAT

Department of Biology, University of North Carolina, Chapel Hill, North Carolina 27514, U.S.A.

Abstract. Parentage in indigo buntings, *Passerina cyanea*, was examined by electrophoresis of soluble enzymes in muscle tissue. There were nine polymorphic loci which segregated independently. Of 257 offspring sampled in the 2 years, 37 (14.4%) had genotypes incompatible with one of the putative parents. Since evidence suggested that intraspecific egg parasitism did not occur, all of these offspring probably came from successful extra-pair copulations. More neighbouring males had genotypes that fitted the genotypes of the excluded young than expected by chance. Most of these males already had nesting females of their own. The probability of cuckoldry depended on the male's age but not on whether or not he was polygynous. Some males probably fathered many offspring through extra-pair copulations while others were cuckolded. These results are examined in light of behavioural observations on this species. Because parental exclusions underestimate the frequency of extra-pair fertilizations, an estimate of the actual rate of extra-pair fertilizations is reported. The consequences of these results for theories about mating systems and sexual selection are discussed.

Extra-pair copulations have now been reported in a large number of avian species (Ford 1983; McKinney et al. 1984). These matings are interesting not only because they have been predicted by theories on the evolution of the male's mating behaviour (Trivers 1972; Maynard Smith 1977), but also because they create differences between the social associations of individuals and gene flow in the population (Gowaty 1985). Such differences might have a profound impact on the study of mating behaviour since, at least for males, the traditional measure of reproductive success is the number of young a male's female(s) raises (Vehrencamp & Bradbury 1984). It is therefore very important to know how many offspring come from extra-pair copulations (EPCs).

To date, most of the studies that have attempted to estimate the success of EPCs have relied on observations of copulations (MacRoberts 1973; Mineau & Cooke 1979; Fujioka & Yamagishi 1981; Werschkul 1982; Røskaft 1983; Fitch & Shugart 1984; Birkhead et al. 1985; Frederick, in press). Observations of copulations might give inaccurate estimates of the rate of fertilizations for several reasons (Westneat 1987; Frederick, in press). First, the observer is rarely able to see all the copulations that occur. Second, EPCs might differ in visibility from within-pair copulations (WPCs) which would bias estimates of their frequency. Third, because

most birds lack an intromittent organ, it is often difficult to determine whether any particular copulation successfully transferred sperm. Fourth, female receptivity to EPCs varies considerably among individuals and species, and it is unknown what effect these differences might have on fertilization rates (Van Tienhoven 1983; Fitch & Shugart 1984; Frederick, in press). Finally, little is known about the effects of sperm competition, sperm precedence, and the timing of fertilization for most birds (Cheng et al. 1983; Smith 1984).

Genetic markers, such as plumage polymorphisms (Burns et al. 1980) or electrophoretically distinct allozymes (Sherman 1981; Mock 1983), can be used to exclude an individual as the genetic parent. Parental exclusion thus provides a more accurate method for measuring the success of EPCs. Several authors have employed genetic markers and have shown that EPCs do fertilize some young (Burns et al. 1980; Gavin & Bollinger 1985; Joste et al. 1985; Mumme et al. 1985). However, these authors have reported anecdotal cases of successful EPCs and have not attempted to estimate the frequency of extra-pair fertilizations.

Observations of EPCs in indigo buntings, *Passerina cyanea*, have been reported by Payne (1983). Most males in this species pair with only one female at a time, but do little to raise the offspring (Verner & Willson 1969; Carey & Nolan 1979; Payne 1982).

Males might be expected to pursue EPCs on a frequent basis (Trivers 1972; Maynard Smith 1977). In this paper I present data on the frequency of parental exclusions from electrophoretic analysis of muscle isozymes in indigo buntings. From these data I will estimate the frequency of extra-pair fertilizations.

METHODS

Details of the population of buntings and of the general field methods used are given in the preceding paper (Westneat 1987).

During May through August 1983–1985, pairs were regularly observed on their territories (Westneat 1987). Individual birds were captured and uniquely banded. I avoided capturing males when their female might have been fertile (after the female arrived and before the last egg was laid). Males were caught in mist-nets within their territories. Those in their first breeding season (young males) could be distinguished from older males by the presence of brown greater primary coverts (Taber & Johnston 1968; Carey & Nolan 1975, 1979; Payne 1982). Females were captured by netting them near the nest when the young were 3–7 days old. Nestlings were banded with unique combinations of two-colour split bands and a FWS aluminium band at 5–7 days of age.

I removed small samples of pectoralis muscle tissue for electrophoresis from parents and offspring when they were banded. The biopsy was adapted from a procedure developed by Baker (1981) and is described in detail by Westneat (1986a) and Westneat et al. (1986). After I sutured the wound, I released the bird immediately. The biopsy procedure had no major effects on adult survival or ability to reproduce (Westneat et al. 1986).

I biopsied a total of 449 individuals; 65 males, 40 females and 105 young in 1983, and 39 males, 30 females and 170 young in 1984. Seven additional adults were re-biopsied in 1984, and three offspring biopsied in 1983 returned to breed in 1984 (one was re-biopsied). Muscle samples of complete families were obtained for 98 broods and 257 offspring.

The muscle samples were placed in individual plastic vials with two drops of Tris-EDTA-HCl buffer pH 7.4 (Selander et al. 1971) and frozen immediately on dry ice. The samples were stored on dry ice for the 4–9 months until analysis.

Soluble muscle enzymes were analysed by standard techniques of horizontal starch-gel electrophoresis (Shaw & Prasad 1970; Selander et al. 1971; Harris & Hopkinson 1976; Avise et al. 1980a, b; Zink 1982). From the existing literature on avian isozymes, I picked 19 enzymes likely to be polymorphic (Barrowclough & Corbin 1978; Avise et al. 1980a, b; Zink 1982). I could consistently resolve only 15 of these. Details on the gel and buffer systems used for all polymorphic enzymes are shown in Table I.

When possible, families were run on the same gels. However, the order of the samples on each gel was randomized in an attempt to score each gel without knowledge of the identity of each sample or of the genotypes of its relatives. C. F. Aquadro confirmed my scoring methods. Cases where I had trouble assigning a genotype were not used in any subsequent analyses. The relative mobilities of each allozyme were measured for each gel and all gels were photographed with either colour slide or black-and-white-film after they were scored.

Analysis

In most of the analyses of the data I assumed that each offspring was fertilized independently. While this might not be a completely realistic assumption, it is probably more accurate than the assumption that all offspring are fertilized by one insemination. Statistical analysis by broods is also complicated by variable brood sizes and so broods were not used in most of the analyses.

RESULTS

Genetics

I found eight enzymes polymorphic in 1983 and an additional locus in 1984 (see Table I). The banding patterns of heterozygotes were consistent with those expected from the known subunit structure of each enzyme (Selander et al. 1971; Harris & Hopkinson 1976; Avise et al. 1980a). Genotype frequencies were not significantly different from those expected by Hardy–Weinberg equilibria in either year (G -tests, Sokal & Rohlf 1969) for all but one locus. This locus (PGI) had fewer heterozygotes than expected in 1984 ($G=9.8$, $df=2$, $P<0.05$), but not in 1983 ($G=1.9$, $df=2$, $P>0.05$). One significant result in 18 tests is reasonably likely by chance alone. In addition, no

Table I. Names, allele frequencies, and buffer, gel and running conditions for the nine polymorphic enzymes found in indigo buntings*

Enzyme	Allele	Frequencies		Buffer conditions	Running conditions
		1983	1984		
α -Glycerophosphate dehydrogenase (α -GPD)	100	0.979	0.973	Poulik†	7 h at 200 v
	60	0.017	0.018	Tray pH: 8.2	
	135	0.004	0.009	Gel pH: 8.7	
Mannose 6-phosphate isomerase (MPI)	100	0.963	0.949	Poulik†	7 h at 200 v
	105	0.037	0.038	Tray pH: 8.2	
	94	0.000	0.013	Gel pH: 8.7	
6-Phosphogluconate dehydrogenase (6-PGD)	100	0.936	0.926	<i>N</i> -Aminopropyl‡	8 h at 210 v
	60	0.039	0.035	morphiline	
	148	0.015	0.022	Tray pH: 6.0	
	120	0.005	0.013	Gel pH: 6.1	
	208	0.005	0.004		
Phosphoglucomutase (PGM)	100	0.964	0.979	Tris citrate†	13 h at 180 v
	70	0.012	0.013	Tray pH: 6.3	
	88	0.012	0.000	Gel pH: 6.7	
	144	0.008	0.004		
	217	0.004	0.004		
Isocitrate dehydrogenase (IDH)	100	0.975	0.976	Tris citrate†	13 h at 180 v
	185	0.025	0.024	Tray pH: 6.3 Gel pH: 6.7	
Glutamate oxaloacetate transaminase (GOT)	100	0.899	0.924	Tris HCl†	15 h at 200 v
	27	0.089	0.059	Tray pH: 8.2	
	200	0.012	0.017	Gel pH: 8.5	
Phosphoglucose isomerase (PGI)	100	0.920	0.911	Tris HCl†	15 h at 200 v
	240	0.080	0.089	Tray pH: 8.2 Gel pH: 8.5	
Peptidase B (Pept B)	100	0.912	0.897	Tris maleate†	24 h at 125 v
	79	0.049	0.047	Tray pH: 7.4	
	115	0.033	0.052	Gel pH: 7.4	
	90	0.006	0.004		
Peptidase C (Pept C)	100	0.919	0.931	Tris maleate†	24 h at 125 v
	114	0.065	0.051	Tray pH: 7.4	
	90	0.011	0.009	Gel pH: 7.4	
	74	0.005	0.009		

* All stain recipes found in Selander et al. (1971) except the peptidases (Harris & Hopkinson 1976).

† Selander et al. 1971.

‡ Allendorf et al. 1977.

offspring inherited an allele inconsistent with its mother's genotype. Thus I considered all nine enzymes to be reliable genetic markers for parental exclusion analyses.

The nine loci segregated independently in both years based on all possible pairwise *G*-tests, with the expected numbers of individuals heterozygous for zero, one, or both of the tested loci. If the loci were linked, fewer individuals should have been heterozygous at only one of the paired loci than expected by chance. Out of 72 pairwise tests, one

comparison (PGI \times Pept C; 1984) was significant ($G = 7.99$, $df = 2$, $P < 0.05$). In 1983, this comparison was not significant ($G = 1.72$, $df = 2$, $P > 0.05$). Since one significant result out of 72 tests is likely by chance alone, I concluded that there was no linkage disequilibrium between loci.

Parental Exclusion

Of the 257 young that were biopsied, 37 had genotypes inconsistent with the genotype of one of

their putative parents. Ten of these 37 had genotypes incompatible with the genotype of the resident male. For example, male OGRX was an MM homozygote (at the PGI locus) paired with ORXO, an FF homozygote. All of their offspring should have been MF heterozygotes. One, however, was an FF homozygote, which eliminated OGRX as the father. In the remaining cases the offspring had an allele present in both putative parents and one not present in either of the parents (ambiguous exclusion).

Parental exclusion can occur from intraspecific egg parasitism (or egg dumping; when a female lays an egg in another female's nest) as well as from extra-pair fertilization. Such egg dumping has been found in a variety of birds (Yom-Tov 1980; Brown 1984; Gowaty & Karlin 1984; Frederick 1985). If the parasitic female mated with a male other than the putative father, egg dumping should result in offspring with genotypes that exclude the female as often as the male (Westneat et al., in press). In the buntings the genetic data did not reveal any cases of a conclusive female exclusion, whereas in 10 cases the male was excluded. This result suggests that egg dumping is rare in this population.

Nest checks during egg laying are an additional means of detecting egg dumping. The appearance of two eggs in the nest on the same day would be convincing evidence of egg dumping (but see Welty 1982 for review of exceptions). R.B. Payne (personal communication) never found a case of two eggs being laid in 1 day in his study. Out of 44 instances I never detected the laying of two eggs in 1 day. One female in 1985 laid her second egg 3 days after the first egg appeared in the nest. A neighbouring female might have laid the earlier egg, but it is also possible that the resident female's normal egg laying was interrupted for several days. Although nest checks do not provide strong evidence for the absence of egg dumping, the data from electrophoresis and nest checks together suggest that egg dumping is rare. I therefore assume that all cases of exclusion were the result of extra-pair fertilizations and not intraspecific egg parasitism.

With this assumption, a total of 37 of 257 (14.4%) of all young in the two seasons were fathered by a male other than the resident. Of the 98 broods for which the genotypes of both parents were known, 24 (24.5%) had at least one young that was a result of an EPC. In two broods, the genotypes of the offspring implicated two males as the partial fathers of the brood. Each brood as a set

contained three different alleles. In one case both putative parents were homozygous for the same allele (CC). One offspring was also homozygous for that allele, but the other two were heterozygous for that allele and two rare alleles (CA and CB). Thus this brood was fathered by two males, presumably the resident and an intruder of genotype AB. In the other case of multiple paternity, the putative father was heterozygous for two rare alleles (AD), the female was homozygous for the common allele (BB) and the three offspring were AB, BD and BB.

In three other broods all of the young had genotypes excluding the resident male. In the remaining broods I assumed that the resident was the actual father if the genotypes of the offspring were consistent with the genotype of the resident male.

Actual Fathers

Normally, electrophoretic data can be used only for parental exclusion and not for the assignment of paternity or maternity. However, in some of the broods with at least one excluded offspring a neighbouring male (territory within 200 m) had a genotype that fitted the genotype of the excluded offspring. Most of these neighbours carried a rare allele; conversely most (18 of 21) of the excluded males had the most common genotype. Since the frequency of the most common allele averaged 0.94 over all loci, then the frequency of males carrying any rare allele at a given locus ($2pq + q^2$) must have averaged 0.12. Excluded males had an average of three neighbours with known genotypes, so that the probability that at least one neighbour carried a rare allele by chance was 0.32. If the true fathers were a random selection from the population, we would expect only 0.32 times 18 (the number of cuckolded males with common genotypes) or 5.8 males to have neighbours with genotypes consistent with the excluded young. Eleven of the 18 males cuckolded (i.e. victims of extra-pair fertilization) had one or more neighbours whose genotypes matched the young, a significant difference (binomial probability, $P < 0.01$; Hays 1981). Of the remaining seven males, five had some neighbours with unknown genotypes. The other two were apparently cuckolded by males that were not close neighbours. These two males had no neighbours within 200 m on one side of their territories and their more distant neighbours on that side had unknown genotypes. Breeding males in this popu-

lation on occasion have travelled over 400 m from their territories.

Breeding Status of Likely Actual Fathers

Most males apparently achieving successful EPCs already had a female breeding on their territories. Of the 48 territorial neighbours (of cuckolded males) with known genotypes, 24 had genotypes that suggested a successful EPC. I knew the breeding status of 18 of these males at the estimated time of EPC; 15 already had females with active nests; 10 of these females were incubating eggs and five were caring for young.

Age and EPCs

Males that were 1 year of age were significantly more likely to be victims of extra-pair fertilizations than were older males (28.3% to 10.8% of young; $\chi^2=9.01$, $df=1$, $P<0.01$). The same comparison by broods revealed that 36.8% of broods had at least one offspring that excluded a young male, whereas 21.5% excluded an old male. However, this difference is not significant ($\chi^2=1.14$, $df=1$, $P>0.05$).

Whether or not older males were more successful at EPCs than males in their first breeding season is not clear. Of the 48 neighbours (of cuckolded males) with known genotypes, six were young males. Two of these had genotypes that matched the genotypes of the young of excluded males, but in both cases an older neighbour also had a matching genotype. In no case did a young male have the only possible genotype that fitted the young, whereas nine older males did have the only genotype. However, this trend is not significant (Fisher exact tests $P=0.345$; Siegel 1956).

Polygyny and EPCs

Some male indigo buntings pair with more than one female simultaneously (Carey & Nolan 1979; Payne 1982). During the years of my study, 11.6% of all fledged young came from nests of second or third females on an individual male's territory (R.B. Payne, unpublished data).

There was no difference between a monogamous and a polygynous male's likelihood of being cuckolded (15.4% to 10.7% of young; $\chi^2=0.81$, $df=1$, $P>0.05$). Secondary females were not more or less

likely to be inseminated by non-mate males than primary or monogamous females (3.8% to 15.6% of young; $\chi^2=2.55$, $df=1$, $P>0.05$) or than primary females alone (Fisher exact probability test with Tocher's modification; $P>0.05$; Siegel 1956).

Polygynous males apparently gained as many EPCs as monogamous males. Of the 48 neighbours (of cuckolded males) with known genotypes, seven were polygynous at some point during the season. Two of these seven had genotypes suggesting a successful EPC; a result not significantly different from that expected by chance ($\chi^2=0.07$, $df=1$, $P>0.05$). One of these males had two females incubating eggs at the time of the EPC. The other had been polygynous earlier in the season, but at the time of the EPC he had only one female nesting on his territory.

Male Reproductive Success

Compared to measurements of reproductive success based on social associations of males and females, males gained or lost considerable reproductive success through EPCs. For example, in 1984, male WGGX produced three young with a female on his territory. Two of his neighbours, XWWS and GXRY, were cuckolded. XWWS had one brood of two young, one of which had a genotype excluding XWWS as the father. GXRY had two broods of three young each; in the first brood all three young were not his, in the second brood two young had genotypes that were inconsistent with GXRY's genotype. All of these excluded young in the three broods had rare alleles at two loci. At the 6-PGD locus the actual father(s) had to have been -A, -B, or AB, and at the PGI locus the actual father(s) had to have been -F or FF. WGGX male had the genotype AB at 6-PGD and FF at PGI, so with all likelihood he was the actual father. This means that in 1984, WGGX fathered a total of nine offspring (not just the three on his own territory) and XWWS and GXRY each fathered only one offspring (instead of the two and six in nests on their territories).

In 1983, WGGX was cuckolded and apparently fathered only one offspring instead of two. Because he had a rare genotype, several of the neighbouring males had genotypes that fitted the genotype of the offspring. One of these neighbours was XWWS, but since there were other neighbours that had unknown genotypes the chances of his being the actual father were slim. In no case in the 2 years did

a male that was cuckolded achieve a successful extra-pair fertilization himself in the same year. In 1983, one young male was cuckolded and had a genotype that fitted the genotype of a neighbour's offspring. However, two other old males also fitted the genotype of the same neighbour's offspring. In all, the 24 males that had genotypes suggesting they achieved a successful EPC had 45 offspring of their own. We would expect that if these males were as likely to be cuckolded as the average male, then 5.7 (10.3% of 1983 young, 16.9% of 1984 young) of their offspring should have had genotypes that excluded the putative father. Only two did so, but this difference is not significant (normal approximation of binomial with correction for small sample size; $z = 1.4$, $P > 0.05$; Hays 1981).

DISCUSSION

In indigo buntings, the social consortships between males and females do not reflect exclusive mating relationships. Extra-pair copulations were responsible for fertilizing at least 14.4% of all offspring. These data confirm the results from observations of the mating behaviour of this species (Westneat 1987) that EPCs are an important feature of the mating system of indigo buntings. Furthermore, these data show that EPCs can, and often do, lead to extra-pair fertilizations.

Electrophoresis does not detect all cases of successful EPCs (Gowaty & Karlin 1984; Westneat et al., in press). For example, if an intruding male had had the same genotype as the resident, then I would not have detected a successful EPC. Because of this, the observed frequency of exclusions of 14.4% must be an underestimate of the actual rate. Methods for calculating the probability of detecting an extra-pair fertilization if it has occurred have been developed (Neel & Schull 1954; Chakraborty et al. 1974; Westneat et al., in press). Briefly, for a two-allele locus there are 27 possible arrangements of genotypes between a putative father, the mother and the actual father. Each arrangement of genotypes has a probability of occurring (x_i) that depends on the allele frequencies. Each mating arrangement can be detected with some probability (e_i). For example, e_i equals 0.5 if the genotype of the putative father is AA, the mother is AA and the actual father is AB. The probability of detection (d) for a locus is then the sum, over all arrangements of genotypes, of the probability of each arrangement

times the probability of detection for that arrangement, $x_i e_i$. For a two-allele locus this reduces to $pq(1 - pq)$ where p is the frequency of one allele, and q is the frequency of the other allele (Neel & Schull 1954). The probability of detection for each of several independent loci (d_i) can be combined to give an overall probability of detection, which is one minus the product of $(1 - d_i)$ (Chakraborty et al. 1974).

The overall probability of detection for the eight independent loci in 1983 and the nine independent loci in 1984 was 0.378 and 0.401 in 1983 and 1984 respectively (Westneat et al., in press). This implies that the observed rate of extra-pair fertilizations of 14.4% (10.3% in 1983, 16.9% in 1984) is only about 40% of the actual rate. By dividing the observed rate for each year by the probability of detection for each year, an estimated 27.2% of all offspring in 1983 and 42.1% of all offspring in 1984 were the result of extra-pair fertilizations.

Confidence limits on these estimates can be calculated (Westneat et al., in press). Briefly, the error in the estimate comes from two sources. First, there is sampling error in the probability of detection which arises from sampling error on the allele frequencies. The 95% confidence limits on the probabilities of detection were 0.321–0.431 in 1983 and 0.343–0.454 in 1984. Second, the observed number of cases also has some sampling error. A combination of these two gives 95% confidence limit on the estimate of the rate of extra-pair fertilizations of 10.2–50.5% in 1983, and 24.7–65.9% in 1984 (Westneat et al., in press).

There was a considerable difference between years in the estimates of extra-pair fertilizations. This difference could be due to chance, but testing the difference statistically is difficult because the variance on the estimate is not known precisely. This difference could also have been a result of a difference between years in the number of biopsied offspring from young males. In fact, the fraction of offspring of young males was lower in 1984 (18%) than in 1983 (25%). Another possible explanation for the difference between years is that there were more offspring in 1983 with some loci unscored. Since I assumed that all the loci were scored for each individual when I calculated the probability of detection, unscored loci would have decreased the probability of detection. The rate for 1983 therefore is more of an underestimate than the rate for 1984.

Comparison of the estimates of the number

young produced by EPCs based on the genetic data with similar estimates based on observations of behaviour highlight the difficulties of estimating reproduction by observations of copulations. Field observations of mating behaviour in buntings (Westneat 1987) indicated that only about 13% of all copulations during a female's fertile period were EPCs. Adjustments for biases in observation techniques, visibility and chance of success change that figure somewhat, but it is not clear in which direction. Estimates of the number of extra-pair fertilizations based on observations (Birkhead et al. 1985; Westneat 1987; Frederick, in press) may be unreliable and researchers should strive to measure reproductive success directly by genetic analysis.

The results of this study show that extra-pair fertilizations and multiple paternity are not synonymous. Multiple paternity is defined as the fathering of young in a brood by at least two males (Gavin & Bollinger 1985; Gowaty 1985). The genetic data from buntings revealed only two broods with confirmed multiple paternity, but in three broods all the offspring were fathered by a male other than the resident. Thus extra-pair copulations (or egg dumping) can result in multiple parentage but will not always do so.

The genetic data on exclusions also confirm observations (Westneat 1987) that resident males on neighbouring territories are the individuals that gain reproductive success through EPCs. Often females are already nesting on these males' territories. The pursuit of EPCs is thus an example of a successful mixed mating strategy (Trivers 1972).

This strategy depends on age. Yearling males were significantly more likely to be cuckolded than older males. Although the evidence is not as strong, older males appear more successful at gaining EPCs as well. Since young males have more difficulty gaining a territory and attracting a mate, they also have more difficulty reproducing (Payne 1982). Such age-dependent reproduction is known in a number of species (e.g. Selander 1972; Wiley 1974; Nolan 1978). If breeding affects survival, the susceptibility of young males to cuckoldry might decrease the benefits of breeding sufficiently for selection to act on males to delay breeding (Wiley 1974).

Unless buntings are highly unusual, EPCs in many species might have a greater impact on mating and parenting behaviour than previously thought. First, in this population of buntings EPCs account for more reproduction than polygyny,

which suggests that opportunities for polygyny are rarer than for EPCs. Male buntings might follow a complex mixed strategy of monogamy first and polygyny and EPCs when the opportunity arises. However, measurement of the payoffs of these three options is still difficult because electrophoresis cannot detect every case of an extra-pair fertilization.

Second, some male buntings invested in young that were not their own. Trivers (1972) hypothesized that cuckoldry should affect the evolution of male parental care since selection should act to produce males that avoid caring for young that are not their own. On the other hand, Maynard Smith (1978) and Wittenberger (1981) argued that cuckoldry cannot affect the evolution of paternal care since a male's chances of cuckoldry are likely to be the same from one nesting to another. However, in buntings, males could differ in the likelihood of cuckoldry from one nesting to another. Young males are more likely to be cuckolded their first season than later. A male's likelihood of cuckoldry could also depend on how old his neighbours are, how many neighbours he has, and at what stage his neighbours' females are in the nesting cycle.

Werren et al. (1980) argue that if promiscuous matings are sacrificed for parental care then cuckoldry will affect the evolution of parental care. Since males probably do not provide paternal care for the offspring from their own promiscuous matings, whether or not they are cuckolded would affect the payoffs of seeking additional matings. Most male buntings provide minimal, but variable, parental care (Verner & Willson 1969; Carey & Nolan 1979). Some males feed nestlings and fledglings, others feed only fledglings, while others do very little except nest defence (Westneat 1986b). Males might be assessing their likelihood of paternity and caring for the young less while spending more effort pursuing EPCs.

Do high levels of successful EPCs mean that male buntings are more promiscuous than polygynous? Definitions of mating systems have been surrounded by more than the usual level of controversy (Selander 1972; Wiley 1974; Emlen & Oring 1977; Wittenberger 1979; Wickler & Seibt 1983; Gowaty 1985; Trail 1985). One outcome of this controversy is that researchers are beginning to distinguish between patterns of associations of males and females and patterns of gene transfer (Gowaty 1985). For example, buntings usually associate with only one member of the opposite sex,

but both males and females often mate with more than one partner. Buntings thus appear to form monogamous pair-bonds, but the gene flow between individuals is very different. One measure of the genetic association between individuals is the breeding sex ratio (Wiley 1974) or gametic contribution ratio (Gowaty 1981, 1985). Both measures are defined as the ratio of the number of males breeding (contributing zygotes to gametes) to the number of females breeding within a season. Alternatively, this measure could be estimated by the ratio of average female reproductive success to average male reproductive success. If the breeding sex ratio is less than one, by definition the species is polygynous.

What is the effect of EPCs on the breeding sex ratio? In buntings, data from both electrophoresis and observations of behaviour (Westneat 1987) indicate that neighbouring, breeding males gain the most EPCs. If this is so, then EPCs do not change the breeding sex ratio. Only if apparently non-breeding individuals gain extra-pair fertilizations, or if many apparently breeding males do not reproduce because they are completely cuckolded, will EPCs change the breeding sex ratio. In buntings this ratio is probably just under one, representing a slightly polygynous mating system.

Trail (1985) has proposed that an index of the intensity of selection be used as a quantitative measure of the mating system. How do EPCs affect the intensity of selection, especially on males? The index of selection is defined as the variance in reproduction over the squared mean (Payne 1979, 1984; Wade 1979; Wade & Arnold 1980; Trail 1985). In buntings, males successful at extra-pair fertilizations and at gaining additional mates are less (but not significantly) likely to be cuckolded. These results are weak evidence that EPCs in indigo buntings might increase the variance in male reproductive success without substantially changing the mean.

It is difficult to infer much about the index of selection on male buntings. First, young males are cuckolded more than older males. Measures of selection need to take age into account (Payne (1984) if there are differences in reproductive success between age classes. Extra-pair copulations in buntings might affect a measure of the intensity of selection not only by altering the variance in reproduction, but also by changing the mean within age classes. On average, young males might reproduce less if it is the older males that are gaining those fertilizations.

It is also important to remember that electrophoresis of bunting muscle detects only approximately 40% of the successful EPCs; 60% remain undetected (Westneat et al., in press). In most cases I also could not assign paternity with much certainty. Precise conclusions about the effect of EPCs on the variance in male reproductive success are dangerous because I could not measure the reproductive success of individuals even with the data from electrophoresis.

The results of this study do suggest that previous estimates of the variance in male reproductive success in many species are likely to be in error. To date, most studies on birds have either tallied the number of offspring a male or female have in their nests (Payne 1979, 1984) or counted the number of mates a male attracts to his territory (Trail 1985). Others have tallied the numbers of copulations gained by males (Payne & Payne 1977; Payne 1984; Birkhead et al. 1985; Trail 1985; Frederick, in press) but as I have mentioned, observations of copulations are likely to be unreliable measures of reproductive success (see also Duvall et al. 1976; Curie-Cohen et al. 1983). If EPCs in any species are successful at a rate similar to (or even less than) that found in indigo buntings, then the intensity of selection as measured by observations or social associations might be very biased. Electrophoresis of muscle tissue can help uncover examples of who is fathering whom, but will not allow assignment of parentage in most cases. Accurate measures of the variance in male reproductive success will not be possible until techniques are developed that give sufficient genetic variation to detect all cases of EPCs and assign parentage with a fair degree of certainty (Gowaty 1985).

Extra-pair copulations are an important reproductive tactic in indigo buntings. It remains to be seen how the strategies of male parental care, polygyny, and the pursuit of EPCs interact to produce the mating system of indigo buntings. Comparable information on the level of extra-pair fertilizations in other species will increase our understanding of the ecological conditions that foster the evolution of these behaviours.

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