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EXTRAPAIR FERTILIZATION IN MONOGAMOUS BULL-HEADED SHRIKES REVEALED BY DNA FINGERPRINTING

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ABSTRACT.—We analyzed parentage in a wild population of the Bull-headed Shrike (*Lanius bucephalus*) by DNA fingerprinting. Male Bull-headed Shrikes pair with a single female and defend a large all-purpose territory. Generally, both parents provide substantial parental care. Among 99 nestlings from 24 clutches, 10 (10%) nestlings from 4 (17%) clutches had extrapair paternity, but were offspring of the females. These results show that copulation frequency is not necessarily a good measure of reproductive success even in monogamous birds where no extrapair copulations are observed. The average band-sharing proportion between full siblings was the same as that between parent and offspring, but the variance was greater. This is likely the case because the band-sharing proportion reflects the gene-sharing proportion. *Received 6 May 1991, accepted 9 February 1992.*

OVER 90% of birds are thought to be monogamous (Lack 1968), and males of these species employ mate guarding to avoid being cuckolded (Møller 1985). Nevertheless, recent evidence from field observations and genetic analyses has clearly demonstrated that extrapair copulations (EPCs) and extrapair fertilizations (EPFs) occur in monogamous species. Among colonially breeding species of monogamous birds like Bank Swallows (*Riparia riparia*; Beecher and Beecher 1979) and Cattle Egrets (*Bubulcus ibis*; Fujioka and Yamagishi 1981), EPCs occur frequently. In Purple Martins (*Progne subis*; Morton et al. 1990) and Zebra Finches (*Taeniopygia guttata*; Birkhead et al. 1990), EPCs often result in fertilization, and intraspecific brood parasitism (ISBP) has been documented. In House Sparrows (*Passer domesticus*), which have a loosely colonial breeding system, up to 8% of the offspring were not fathered by their putative male parent (Wetton et al. 1987).

EPCs have been recorded even in species with large all-purpose territories, where individuals are relatively difficult to observe. In Indigo Buntings (*Passerina cyanea*), EPCs often resulted in EPFs (Westneat 1987a, b), while in monogamous Willow Warblers (*Phylloscopus trochilus*), EPCs resulted in few EPFs (Gyllensten et al. 1990). These results show that copulation frequency is not necessarily a good measure of reproductive success. However, there are only a few genetic studies of strongly monogamous and territorial species; all have found low frequencies of EPFs.

The Bull-headed Shrike (*Lanius bucephalus*) has a strongly monogamous breeding system and has a large all-purpose territory (radius ca. 100 m; Yamagishi 1982b). No EPCs have been observed (Yamagishi and Saito 1985). In monogamous birds for which no EPCs have been observed, there are few studies involving the determination of parentage; an exception is an analysis of Dunnocks (*Prunella modularis*) under a monogamous system (Burke et al. 1989). Their study showed that in monogamous pairs no EPFs occurred, so that the reproductive success of a pair was related to the number of fledged young. To test if this was also the case for Bull-headed Shrikes, we looked for evidence of EPFs and ISBP using DNA fingerprinting, which can be a powerful tool for determining parentage (Jeffreys et al. 1985b).

STUDY AREA AND METHODS

Study area and capture techniques.—The study was conducted from March to June 1989 in Oizumi city park, Osaka Prefecture, central Japan (34°34'N, 135°32'E). The park covers an area of about 70 ha, is about 20 m in elevation, and is planted mainly with oaks (*Quercus*). The incubating female and/or the female and the male that fed their nestlings were captured with a mist net, and were regarded as the putative father and mother of the brood. All males that produced extrapair offspring were captured during incubation or nestling period (i.e. when their female mate was not fertilizable, therefore, a capture of the male did not affect our results). Age determination was based on the coloration of the wing coverts (Yamagishi 1982a).

DNA fingerprinting.—DNA was prepared from 100 μl blood samples collected by jugular venipuncture

with a heparinized syringe. Blood samples were obtained from 23 pairs and 99 nestlings (brood size one to six) of 24 families (two broods were obtained from the same pair), and seven males and six females that did not succeed in breeding, for a total of 158 individuals. Blood samples were suspended in 3 ml of SET buffer (0.15 M NaCl, 0.05 M Tris-HCl, 1 mM EDTA; pH 8.0) and stored at −60°C within two weeks. Subsequently, 15 μl proteinase K (10 mg/ml) and 0.3 ml 10% SDS were added to the thawed sample and incubated overnight at 37°C. DNA was extracted three times with phenol/chloroform, once with chloroform alone, and then precipitated with ethanol and dissolved in TE buffer (10 mM Tris-HCl, 1 mM EDTA; pH 7.6). Usually, 400 to 800 μg DNA was obtained. About 20 μg was digested for 15 h with 24 units of *Hin*fI at 37°C, phenol extracted and ethanol precipitated.

DNA fragments were fractionated by electrophoresis in 1% agarose gels (25 cm in length) in Tris-Borate buffer at 1.2 V/cm for 45 h until fragments of 2 kb had migrated about 20 cm. Gels were successively soaked in 0.25 M HCl for 15 min twice, 0.5 M NaOH/1.5 M NaCl for 45 min, and 0.5 M Tris-HCl/1.5 M NaCl with pH 7.5 for 45 min. DNA fragments were transferred with 20× SSC (3 M NaCl, 0.3 M sodium citrate; pH 7.0) onto a nylon membrane (Biodyne A, Pall Biosupport). The membranes were briefly washed in 2× SSC, and then baked at 80°C for 3 h.

Membranes were incubated for 12 to 15 h at 37°C in prehybridization solution (50% formamide, 5× SSC, 5× Denhardt, 0.1% SDS, 0.05 M sodium-acetate pH 6.5, 45 μg/ml sheared salmon-sperm DNA), then hybridized in the same solution with the labeled probe (1×10^7 cpm) for two days at 37°C. Membranes were washed three times, for 30 min each, with 2× SSC, 0.1% SDS at 25°C. Autoradiography was performed with *X*-ray film (Fuji RX) for 3 to 10 days at −60°C.

Minisatellite sequences 33.6 and 33.15 (Jeffreys et al. 1985a) were used as hybridization probes. The 0.7-kb and 0.6-kb *Eco*RI/*Pst*I fragments containing 33.6 and 33.15, respectively, were each recloned into plasmid pUC119 and then were designated pIN33.6 and pIN33.15, respectively. Approximately 100 ng of single-stranded pIN33.6 or pIN33.15 DNA were labeled to 1×10^7 cpm by extending its complementary strand as follows. Single-stranded template DNA (20 ng/μl) was annealed with M13 sequencing primers, and an extension reaction was carried out with Klenow enzyme (0.4 units/μl) and ^{32}P-dCTP (4 μCi/μl). Labeled probe DNA was precipitated using ethanol.

Fingerprinting analysis.—We hybridized *Hin*fI digests from the 24 families with probe 33.6, and the banding patterns of offspring were compared with those of the putative parents. All comparisons of banding patterns were made within the same gel, and only fragments longer than 3 kb were analyzed. Bands present in the offspring, but not present in either of the putative parents, were termed mismatched bands. The existence of many mismatched bands in a nestling strongly suggested that at least one of its putative parents was not its genetic parent. However, as one or two mismatched bands in a nestling were likely simply due to mutation, we then changed from probe 33.6 to 33.15 in order to assess which one(s) resulted from mutation. If no mismatched bands were revealed by probe 33.15, we concluded that the nestling was the offspring of its putative parents.

For nestlings that were not genetic offspring of at least one of their putative parents, we calculated the similarity coefficient *D* (Wetton et al. 1987) between the offspring and each putative parent in order to decide whether the offspring was the result of EPF or ISBP. The coefficient is calculated as

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

where N_A and N_B are numbers of fragments in individuals A and B, respectively, and N_{AB} is the number shared by both. The *D*-value varies from zero, when no bands are shared to one when all bands are identical. Distributions of *D*-values were evaluated. In the statistical treatment of *D*, we followed Westneat (1990). Using this method, *D*-values could provide sufficient evidence to analyze parentage.

RESULTS

Breeding behavior.—In the population we studied, Bull-headed Shrikes are not migratory, and females and males have separate territories in winter. At the end of February, nest construction starts upon the arrival of females in males' winter territories. All pairs are monogamous and can breed twice during the breeding season, which extends from February to July (Yamagishi and Saito 1985). Nest construction is performed mainly by females, and incubation is only by females, while males feed their mates during the egg-laying and incubation periods (Yamagishi 1981). Both parents feed their nestlings. Some males with territories are unpaired, and these males intrude into the territories of adjacent pairs more frequently than do paired males (Yamagishi 1982b).

Pair fertilization (PF).—DNA fingerprints obtained with probe 33.6 revealed banding patterns that varied considerably between individuals (Fig. 1, Table 1). Individuals averaged 26.5 bands (range 16–37). *D*-values between presumably unrelated individuals averaged 0.30 ± SD of 0.063. Assuming that all bands are inherited separately, the mean probability of identical fingerprints between two unrelated individuals is estimated to be lower than $0.30^{26.5} = 1.4 \times 10^{-14}$. For 81 of 99 nestlings examined, all bands

corresponded to those of the putative parents, indicating that the young were the genetic offspring of the parents.

Eight nestlings from three families (family V, chicks B, C, D; family J, chicks A, C, D; family W, chicks A, D) had only one or two mismatched bands (Appendix 1). To evaluate whether mutations or EPFs/ISBPs are the cause of such mismatched bands, we made DNA fingerprints of these three families using probe 33.15. No mismatched bands were revealed (Appendix 2). In contrast, a nestling (family J, chick B) that had two mismatched bands in fingerprints with probe 33.15 had no mismatched bands in those with probe 33.6. Therefore, we concluded that one or two bands with 33.6 and two bands with 33.15 were the result of mutations. Thus, these nine offspring were also judged to be genetic offspring of their putative parents. However, chick B of family W had 10 and 6 mismatched bands with 33.6 and 33.15, respectively. Therefore, it is likely that these bands were the result of EPF or ISBP.

Mutations occurred in 10 of 2,283 bands (i.e. the rate of mutations was 4.4×10^{-3} per band in fingerprints with 33.6). This rate is near the general rate of about 4×10^{-3} (Jeffreys et al. 1988, Burke and Bruford 1987). Because the average number of bands per individual was 26.5, the mean probability of a nestling having one mutated band was

$$ {}_{26.5}C_1 \times (4.4 \times 10^{-3})^1 \times (1 - 4.4 \times 10^{-3})^{26.5-1} = 0.10. $$

The probability of two mutated bands was 5.8×10^{-3}, and that of three or more mismatched bands was 2.2×10^{-4}. Thus, the occurrence of three or more mismatched bands is unlikely to be due to mutation. The observed rate of offspring having one mutated band was $6/89 = 6.7 \times 10^{-2}$, and that of two mismatched bands was $2/89 = 2.2 \times 10^{-2}$. The observed rate of offspring having two mismatched bands was not significantly higher than the expected rate (binomial test, $P = 0.10$). With probe 33.15, the average number of bands per individual was 19.7. Assuming that the rate of mutations with 33.15 is the same rate as that for 33.6, the mean probabilities of finding one and two mutated bands with both probes are 0.17 and 1.7×10^{-2}, respectively. These probabilities are higher than that with only probe 33.6. Considering these calculations, it is appropriate to regard all of the

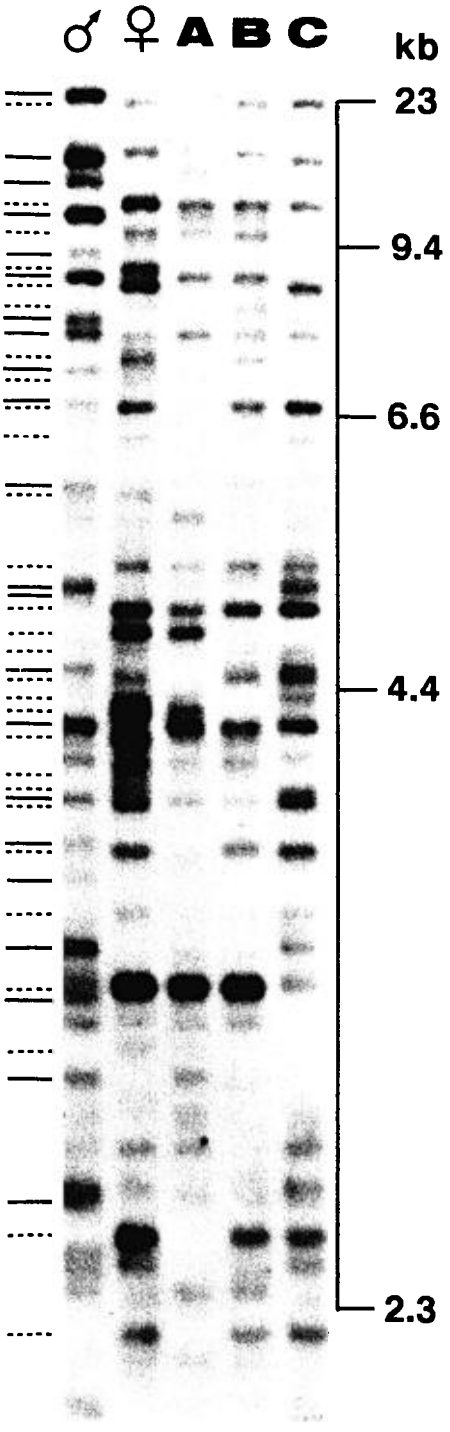

Fig. 1. Fingerprints obtained with probe 33.6 for a pair and their three nestlings (family C). Solid lines indicate paternal-specific bands and broken lines maternal-specific bands. ♂, putative father; ♀, putative mother; A, B, C, nestlings.

TABLE 1. Analysis of fingerprints of family C.

Individual	Number of bands				D with		Conclusion[a]
	Total	Mismatched	Shared with male	Shared with female	Male	Female	
Male	26	—	—	5	—	0.18	—
Female	31	—	5	—	0.18	—	—
Chick A	24	0	13	16	0.52	0.58	PF
Chick B	25	0	13	17	0.51	0.61	PF
Chick C	29	0	14	19	0.51	0.63	PF

[a]PF = pair fertilization.

cases with one or two mismatched bands as being due to mutation.

Extrapair fertilization (EPF).—*D*-values between parents and their offspring with few or no mismatched bands averaged 0.62 (n = 178), while *D*-values between adults sampled at random from this population averaged 0.30 (n = 40; Table 2). These two distributions are distinct at the 0.99% confidence limit (Fig. 2A). Ten nestlings from four families had 4 to 12 mismatched bands (Fig. 3, Appendix 3). All *D*-values between these nestlings and their putative mothers were relatively high, averaging 0.62, and were within the parent-offspring distribution. *D*-values between these nestlings and their putative fathers were low, averaging 0.35, and these fell in the unrelated distribution (Fig. 2B). One value of *D* was relatively high, 0.46, and fell within neither the 99% confidence limit of parent-offspring nor that of unrelated adults; the nestling had four mismatched bands. Therefore, we concluded that the nestling resulted from an EPF. The *D*-value between the putative father and mother was relatively high, 0.41, and this may have contributed to the high value of *D* between the nestling and the unrelated putative father. Our conclusion is that all of these 10 nestlings were genetic offspring of the putative mothers, but were not genetic offspring of the putative fathers.

Three broods contained two or more illegitimate offspring. To test whether these broods were sired by more than one extrapair male, *D*-values between nest mates were calculated. The *D* between known full siblings averaged 0.62 (n = 159) and that between known half siblings averaged 0.40 (n = 5; Table 2). The two distributions were distinct at the 0.95% confidence limit, but were not distinct at the 0.99% limit (Fig. 2C). There are two reasons why these distributions are not as different as those of *D* between unrelated adults, and between parents and offspring. First, the average *D* between half siblings was significantly higher than that between unrelated adults (Table 2; t = 3.9, P < 0.001); second, the variance of *D* between full siblings was significantly wider than that between parent and offspring (Table 2; F = 1.7, P < 0.01).

D-values were high (0.52 and 0.67, respectively; Table 3) between nest mates of two broods (family L and B′) whose paternities were unidentified, and were within the full-sibling distribution (Fig. 2D). In each of these two broods, one male seems to have sired both nestlings. Two values of *D* between nest mates of family A′ (between chicks A and C, and between C and D) are outside the full-sibling distribution and within the half-sibling distribution at the 95% confidence limit, but the other values contrast (Fig. 2D, Table 3). We are unable to determine whether the father of chicks A, C, or D might have been different from that of chicks B and E.

In this population of Bull-headed Shrikes, EPFs involved 10.1% (10/99) of nestlings and 16.7% (4/24) of broods. EPFs occurred throughout the breeding season from early April to late May. The frequency of EPFs before early April, which was the relatively synchronous breeding period, is lower than that after late April (1/14 = 7.1% and 3/10 = 30%, respectively; Fisher's exact probability test; P = 0.18). Only one (male A′) of four males that took care of unrelated offspring was a yearling; all others were adults. All four females that were fertilized by an extrapair male were adults (>1 year). The frequency of cuckolded males and cuckolding females was higher in adults than in yearlings, but differences were not statistically significant (Fisher's exact probability test; P = 0.59 and P = 0.14, respectively).

Extrapair copulation (EPC).—In families A′ and

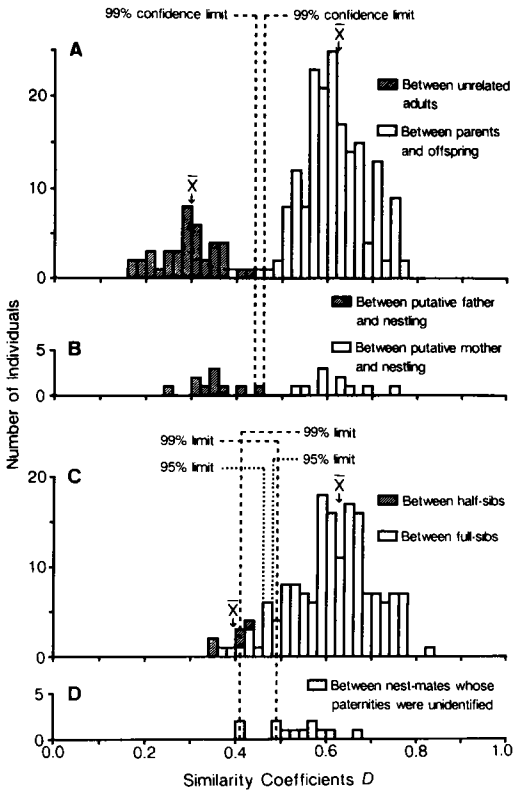

Fig. 2. Distribution of similarity coefficients *D*. (A) Comparison of *D*-values between presumed unrelated adults (slashed bars) and between each parent and their offspring (those offspring with few or no mismatched bands; open bars). (B) *D*-values between nestlings with many mismatched bands and their putative parents (slashed bars, putative father; open bars, putative mother). (C) Comparison of *D*-values between full siblings (open bars) and between half siblings (slashed bars). Full siblings were nest mates with few or no mismatched bands. *D*-values between half siblings calculated by comparing nestlings that had few or no mismatched bands with their nest mates, which had many mismatched bands. (D) *D*-values between nest mates whose paternities were unidentified (open bars).

B', resident males did not sire any of the nestlings. Therefore, these could be cases of mate switching after egg laying. Evidence against this possibility comes from the observation that when mate change involving the male occurs, the male does not feed the offspring of the previous male (Yamagishi 1981:65). The fact that all resident males fed their young in this study strongly suggests that mate switching did not occur. EPFs could have arisen through EPCs or

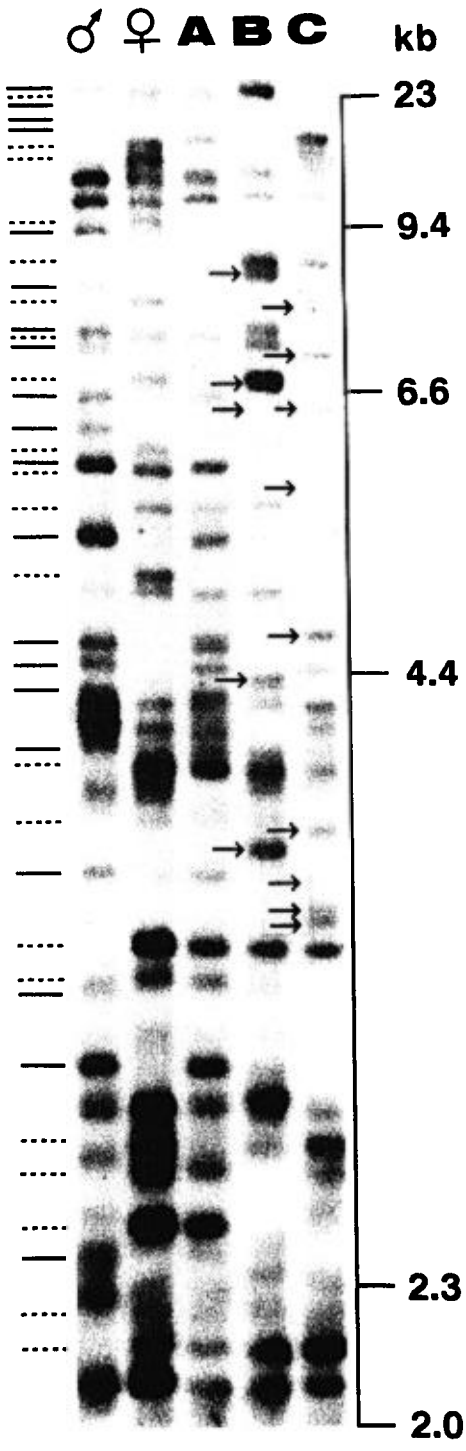

Fig. 3. Fingerprints obtained with probe 33.6 for a pair and their three nestlings (family L). Many mismatched bands present in two nestlings (B, C) as indicated by arrows (see Appendix 3).

TABLE 2. Similarity coefficients D between: presumed unrelated adults; genetic parents and offspring; full siblings; half siblings; and mates. Offspring consisted only of those having few or no mismatched bands. Full siblings were nest mates with no evidence of EPF. D-values between half siblings calculated by comparing nestlings that had no evidence of EPFs with their nest mates, which had evidence of EPFs.

Relation	n	$\bar{x} \pm$ SD (range)
Unrelated adults	40	0.30 ± 0.063 (0.17–0.43)
Parents and offspring	178	0.62 ± 0.069 (0.40–0.77)
Full siblings	159	0.62 ± 0.089 (0.37–0.84)
Half siblings	5	0.40 ± 0.039 (0.35–0.44)
Mates	23	0.35 ± 0.093 (0.16–0.53)

through rapid mate switchings (i.e. when a female pairs with and is inseminated by one male, but then switches males prior to egg laying; Møller 1985). Intensive field observations were not carried out every day. Thus, in three of the four families that had EPFs, it was not clear whether rapid mate switching had occurred. However, in family L, detailed observations clearly indicated no rapid mate switching occurred. The EPFs arose at the time of the second breeding by the pair after their first nestlings were preyed on. The EPFs in four families likely occurred through EPCs.

We attempted to assign paternity of illegitimate offspring. During the copulation stage, the territory of the male of family L was adjacent to three territories of paired males and to three territories of unpaired males; there was no floater. One of the three unpaired males often intruded into the territory of family L during the egg-laying stage, but we did not obtain his blood. To assess whether any of the other five adjacent males was the genetic father, we made DNA fingerprints of these individuals. However, five to nine mismatched bands were observed with each male, indicating that none of the three paired and two unpaired adjacent males examined was the genetic father. Paternity for the other families was not assignable either.

D and relatedness.—To examine how precisely D-values reflect relatedness, the relative values of D (based on probe 33.6) between parents and offspring in relation to D between mates are shown in Figure 4. The plots show considerable scatter. This proves that the dispersion of D between parents and offspring is due not only to a difference of D between mates, but also to the increased margin of error resulting from a limited number of bands. Therefore, we are unable to determine the exact degree of relatedness using the D-values in this study.

However, there was a correlation between parent-offspring D and mates D ($P < 0.001$, $r = 0.48$, $n = 178$), and a simple linear regression,

$$Y = 0.491 + 0.375X \quad (2)$$

was found (Y-intercept SE of 0.054, slope SE of 0.153). This was compared with the theoretical curve, which was derived as follows. Then we assumed that all bands were the same frequency in the population, all bands were inherited separately, and numbers of observed bands were unlimited.

The frequency of the band of a particular molecular weight in the population is represented by $p(0 < p < 1)$, and between two particular related individuals, α represents the probability that both individuals have the band, β repre-

TABLE 3. Similarity coefficients D between siblings calculated from DNA fingerprints of families that included EPF.

	Family A′				Family W			Family L		Family B′
Chick	A	B	C	D	A	B	C	A	B	A
B	0.49[a]				0.41[b]			0.36[b]		0.67[a]
C	0.41[a]	0.55[a]			0.76[c]	0.42[b]		0.35[b]	0.52[a]	
D	0.49[a]	0.58[a]	0.41[a]		0.67[c]	0.44[b]	0.49[c]			
E	0.53[a]	0.58[a]	0.59[a]	0.61[a]						

[a] D-values between siblings whose paternities were unidentified.
[b] D-values between half siblings.
[c] D-values between full siblings.

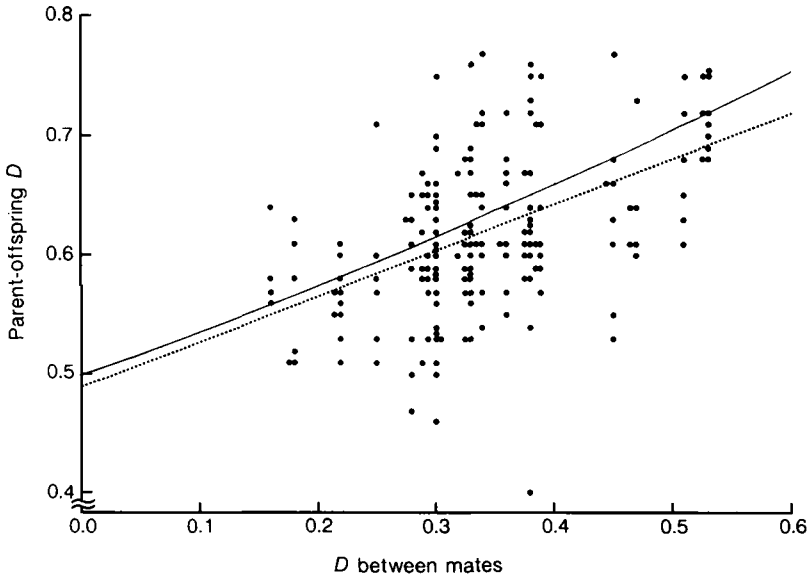

Fig. 4. Relative values of D between parents and offspring in relation to those between mates. Offspring consisted only of those with few or no mismatched bands. Broken line indicates simple linear regression, $Y = 0.491 + 0.375X$. Solid line indicates theoretical curve, $Y = [X + (1 - X)^{0.5}]/[1 + (1 - X)^{0.5}]$.

sents the probability that only one individual has it, γ represents the probability that neither has it ($\alpha + 2\beta + \gamma = 1$). Between parent and offspring,

$$\alpha = p(1 + p - p^2), \quad (3)$$

$$\beta = p(1 - p)^2, \quad (4)$$

and

$$\gamma = (1 - p)^3, \quad (5)$$

so, D between parents and offspring is

$$2\alpha/(2\alpha + 2\beta) = (1 + p - p^2)/(2 - p). \quad (6)$$

Between unrelated individuals,

$$\alpha = p^2(4 - 4p + p^2), \quad (7)$$

$$\beta = p(1 - p)^2(2 - p), \quad (8)$$

and

$$\gamma = (1 - p)^4, \quad (9)$$

so, D between unrelated individuals is

$$2\alpha/(2\alpha + 2\beta) = p(2 - p). \quad (10)$$

Suppose that all bands are the same frequency, p. These equations indicate that in the mates between which the D-value is $p(2 - p)$, D-values between parents and offspring become $(1 + p - p^2)/(2 - p)$. Then X represents D-value between mates, and Y represents D-value between parents and offspring, so,

$$X = p(2 - p), \quad (11)$$

and

$$Y = (1 + p - p^2)/(2 - p). \quad (12)$$

From both equations, X and Y are related to

$$Y = [X + (1 - X)^{0.5}]/[1 + (1 - X)^{0.5}], \quad (0 < X < 1). \quad (13)$$

The simple linear regression was not significantly different from the theoretical curve ($t = 0.37$, $P > 0.5$). This suggests that a more extensive analysis of bands (e.g. by using additional probes) would make it possible to determine the exact degree of relatedness.

Discussion

EPC and EPF.—Even though no EPCs have been observed, 10% of Bull-headed Shrike offspring resulted from EPCs. This result contrasts with the study of predominantly monogamous Willow Warblers, in which 13% EPCs were observed and no EPFs were found (Gyllensten et al. 1990). Percents of EPCs and EPFs in the Indigo Bunting were 13% and 36% (Westneat 1987a, b), in Pied Flycatchers (*Ficedula hypoleu-*

ca) 29% and 24% (Alatalo et al. 1987), in Barn Swallows (*Hirundo rustica*) 7.3% and 26% (Møller 1987), in Zebra Finches 4.5% and 2.4% (Birkhead et al. 1990), and in Dunnocks both less than 1% (Burke et al. 1989), respectively. In birds, the percents of EPCs and EPFs are unlikely to be related to the mating system (i.e. polygynous or monogamous, territorial or colonial). Also, observed copulation frequency is unlikely always to be a good measure of reproductive success. This may be because of incomplete observations or differences of reproductive functions for EPCs among species.

There can be two reasons why observations are incomplete. First, extrapair males may approach females by stealth in order not to be attacked by the resident male. Second, EPCs may occur at specific times of day (e.g. very early in morning). In Bull-headed Shrikes, 30 pair copulations, 13 pair copulations that probably were completed, and no EPFs were seen during day-long observations for 27 days (Yamagishi and Saito 1985). Because our observations were made in territories, the whole of which could be kept in view during day-long watches, the second reason is unlikely to be the cause, unless EPCs occur at night.

In some species (e.g. Bull-headed Shrikes, Indigo Buntings, Barn Swallows), EPCs seem to be more efficient than PCs and, in others (e.g. Willow Warbler, Zebra Finches), EPCs seem to be less efficient. In Bull-headed Shrikes the proportions of EPFs within four broods were high (i.e. 5/5, 1/4, 2/3, 2/2), suggesting that EPCs are effective in gaining paternity. This may be because the number of pair copulations is relatively small (i.e. only about 20 during one breeding cycle, which was obtained by day-long observations during the nest-building and egg-laying stages; Yamagishi and Saito 1985) and because efficiencies of copulations are different (e.g. cuckolders may obtain final insemination before egg laying, perhaps gaining last-male sperm precedence, as in Zebra Finches; Birkhead et al. 1988). However, these factors may not explain the situation where an extrapair male fertilized the entire brood. One possibility is that the resident male might be infertile.

The behavior of females will strongly affect whether males succeed in EPFs. Females have not been observed to leave their territories during the copulation period. Male intruders are attacked not only by the resident male, but often by both the resident male and female. It is probable that females passively accept EPCs when the resident male does not notice the other male intruding in the territory, because it is highly unlikely that the resident male will inflict costs on the female since, for example, this would decrease parental care for his offspring. Also, in this way females could avoid being injured in a fight against the intruder. It also is conceivable that females gain by pairing with good fathers and by intentionally copulating with other males that possess "good" genes (Smith 1988).

Distributions of D*-values.*—The average of D between full siblings was the same as that between parent and offspring, but the variance was significantly greater (Table 2; $F = 1.7$, $P < 0.01$). This may reflect the fact that offspring and a parent share exactly one-half of the genes, but full siblings share on average one-half, with greater variance. In DNA fingerprints, the measured D-value varies between father-offspring and mother-offspring because of the presence of null alleles (fragments that are smaller than 3 kb). A more extensive analysis of bands can suppress the variance. In addition, a more extensive analysis of bands might make it possible to distinguish "high-shared" full siblings from "low-shared" full siblings. The distinction may be important for behavioral ecology because high-shared siblings might be more cooperative than low-shared siblings.

The average of D between mates is significantly higher than that between unrelated adults in the same population (Table 2; $t = 2.2$, $P < 0.05$). Moreover, the variance of the former is significantly greater than that of the latter (Table 2; $F = 2.2$, $P < 0.05$), suggesting that some Bull-headed Shrikes in this population paired with related mates, whereas others were not genetically related to their mates.

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LITERATURE CITED

ALATALO, R. V., K. GOTTLANDER, AND A. LUNDBERG. 1987. Extra-pair copulations and mate guarding

in the polyterritorial Pied Flycatcher, *Ficedula hypoleuca*. Behaviour 101:139–154.

BEECHER, M. D., AND I. M. BEECHER. 1979. Sociobiology of Bank Swallows: Reproductive strategy of the male. Science 205:1282–1285.

BIRKHEAD, T. R., T. BURKE, R. ZANN, F. M. HUNTER, AND A. P. KRUPA. 1990. Extra-pair paternity and intraspecific brood parasitism in wild Zebra Finches *Taeniopygia guttata*, revealed by DNA fingerprinting. Behav. Ecol. Sociobiol. 27:315–324.

BIRKHEAD, T. R., J. E. PELLATT, AND F. M. HUNTER. 1988. Extra-pair copulation and sperm competiion in the Zebra Finch. Nature (Lond.) 334:60–62.

BURKE, T., AND M. W. BRUFORD. 1987. DNA fingerprinting in birds. Nature (Lond.) 327:149–152.

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 (Lond.) 338:249–251.

FUJIOKA, M., AND S. YAMAGISHI. 1981. Extramarital and pair copulations in the Cattle Egret. Auk 98: 134–144.

GYLLENSTEN, U. B., S. JAKOBSSON, AND H. TEMRIN. 1990. No evidence for illegitimate young in monogamous and polygynous Warblers. Nature (Lond.) 343:168–170.

JEFFREYS, A. J., N. J. ROYLE, V. WILSON, AND Z. WONG. 1988. Spontaneous mutation rates to new length alleles at tandem-repetitive hypervariable loci in human DNA. Nature (Lond.) 322:278–281.

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

JEFFREYS, A. J., V. WILSON, AND S. L. THEIN. 1985b. Individual specific 'fingerprints' of human DNA. Nature (Lond.) 316:76–79.

LACK, D. 1968. Ecological adaptations for breeding in birds. Methuen, London.

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. 1987. Behavioural aspects of sperm competition in Swallows (*Hirundo rustica*). Behaviour 100:82–104.

MORTON, E. S., L. FORMAN, AND M. BRAUN. 1990. Extra-pair fertilizations and the evolution of colonial breeding in Purple Martins. Auk 107:275–283.

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

WESTNEAT, D. F. 1987a. Extra-pair copulations in a predominantly monogamous bird: Genetic evidence. Anim. Behav. 35:865–876.

WESTNEAT, D. F. 1987b. Extra-pair fertilizations in a predominantly monogamous bird: Observations of behavior. Anim. Behav. 35:877–886.

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

WETTON, J. H., R. E. CARTER, D. T. PARKIN, AND D. WALTERS. 1987. Demographic study of a wild House Sparrow population by DNA "fingerprinting." Nature (Lond.) 327:147–149.

YAMAGISHI, S. 1981. [The bride of Bull-headed Shrike.] Dainippon-tosho, Tokyo (in Japanese).

YAMAGISHI, S. 1982a. Age determination in the Bull-headed Shrike *Lanius bucephalus* based on buff-tips of greater primary coverts. J. Yamashina Inst. Ornithol. 14:96–102.

YAMAGISHI, S. 1982b. [Territory of the Bull-headed Shrike.] Kagaku 52:392–398 (in Japanese).

YAMAGISHI, S., AND M. SAITO. 1985. Function of courtship feeding in the Bull-headed Shrike, *Lanius bucephalus*. J. Ethol. 3:113–121.

APPENDIX 1. Analysis of DNA fingerprints obtained with probe 33.6 of families whose offspring had one or two mismatched bands.

		Number of bands				*D* with		
Family	Individual	Total	Mismatched	Shared with male	Shared with female	Male	Female	Conclusion[a]
J	Male	31	—	—	14	—	0.45	—
	Female	31	—	14	—	0.45	—	—
	Chick A	26	2	15	18	0.53	0.63	PF
	Chick B	25	0	17	19	0.61	0.68	PF
	Chick C	33	2	21	21	0.66	0.66	PF
	Chick D	31	1	17	24	0.55	0.77	PF
V	Male	18	—	—	5	—	0.28	—
	Female	18	—	5	—	0.28	—	—
	Chick A	16	0	11	10	0.65	0.59	PF
	Chick B	18	1	9	11	0.50	0.61	PF
	Chick C	20	1	12	10	0.63	0.53	PF
	Chick D	20	1	9	12	0.47	0.63	PF
W	Male	28	—	—	14	—	0.51	—
	Female	27	—	14	—	0.51	—	—
	Chick A	28	1	21	18	0.75	0.65	PF
	Chick B	31	10	11	20	0.37	0.69	EPF
	Chick C	22	0	18	15	0.72	0.61	PF
	Chick D	23	1	16	17	0.63	0.68	PF

[a] PF = pair fertilization. EPF = extrapair fertilization.

APPENDIX 2. Analysis of DNA fingerprints obtained with probe 33.15.

		Number of bands				*D* with		
Family	Individual	Total	Mismatched	Shared with male	Shared with female	Male	Female	Conclusion[a]
J	Male	21	—	—	8	—	0.34	—
	Female	26	—	8	—	0.34	—	—
	Chick A	19	0	11	15	0.55	0.67	PF
	Chick B	20	2	10	15	0.49	0.65	PF
	Chick C	23	0	12	18	0.55	0.73	PF
	Chick D	21	0	11	15	0.52	0.64	PF
V	Male	21	—	—	7	—	0.39	—
	Female	15	—	7	—	0.39	—	—
	Chick A	18	0	14	10	0.72	0.61	PF
	Chick B	18	0	12	11	0.62	0.67	PF
	Chick C	21	0	17	11	0.81	0.61	PF
	Chick D	16	0	13	9	0.70	0.58	PF
W	Male	16	—	—	5	—	0.29	—
	Female	18	—	5	—	0.29	—	—
	Chick A	18	0	12	11	0.71	0.61	PF
	Chick B	21	6	9	11	0.49	0.56	EPF
	Chick C	24	0	14	15	0.70	0.71	PF
	Chick D	19	0	11	13	0.63	0.70	PF

[a] PF = pair fertilization. EPF = extrapair fertilization.

APPENDIX 3. Analysis of DNA fingerprints obtained with probe 33.6 of families whose offspring had many mismatched bands.

Family	Individual	Number of bands				*D* with		Conclusion[a]
		Total	Mismatched	Shared with male	Shared with female	Male	Female	
A′	Male	28	—	—	11	—	0.39	—
	Female	29	—	11	—	0.39	—	—
	Chick A	25	6	9	16	0.34	0.59	EPF
	Chick B	34	12	10	20	0.32	0.63	EPF
	Chick C	25	9	7	15	0.26	0.56	EPF
	Chick D	29	9	9	17	0.32	0.59	EPF
	Chick E	34	12	11	17	0.35	0.54	EPF
W	Male	28	—	—	14	—	0.51	—
	Female	27	—	14	—	0.51	—	—
	Chick A	28	1	21	18	0.75	0.65	PF
	Chick B	31	10	11	20	0.37	0.69	EPF
	Chick C	22	0	18	15	0.72	0.61	PF
	Chick D	23	1	16	17	0.63	0.68	PF
L	Male	27	—	—	9	—	0.32	—
	Female	30	—	9	—	0.32	—	—
	Chick A	27	0	18	17	0.67	0.60	PF
	Chick B	28	5	10	19	0.36	0.66	EPF
	Chick C	30	9	10	18	0.35	0.60	EPF
B′	Male	33	—	—	12	—	0.41	—
	Female	25	—	12	—	0.41	—	—
	Chick A	28	4	14	20	0.46	0.75	EPF
	Chick B	26	5	12	16	0.41	0.63	EPF

[a] PF = pair fertilization. EPF = extrapair fertilization.