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Copulatory behaviour and paternity determined by DNA fingerprinting in kestrels: effects of cyclic food abundance

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Abstract. It has been proposed that mate guarding is the primary paternity insurance in birds, and only those species in which the male is compelled to leave his mate unattended during the fertile period resort to frequent copulation as a means of assuring paternity. A higher frequency of both extra-pair copulation (EPC) and extra-pair paternity (EPP) has been predicted in non-guarding species. These predictions were examined in Eurasian kestrels, *Falco tinnunculus*, breeding in western Finland. During the course of a 3-year vole cycle, the mean within-pair copulation frequency was 0.72 per h and the mean number of extra-pair intrusions 0.07 per h. The proportion of EPCs was only 1% of copulations. The within-pair copulation frequency was higher in a year of increasing food abundance (1991) than in the years of decreasing (1992) and low food abundance (1990), although the kestrel breeding density was lower in 1990 than in 1991–1992. Males spent more time mate guarding in 1991–1992 than in 1990 (overall mean 40%). Single-locus DNA profiling revealed EPP in 7% of 27 broods and 5% of 112 offspring in 1991 but none in 1990 or 1992 (19 and 29 broods, respectively). Kestrel males successfully used both frequent pair copulation and mate guarding as complementary paternity guards. The low frequency of EPP may reflect the importance of male parental care in determining the reproductive success of raptors. Females may jeopardize future aid from their mates by soliciting EPCs from intruders. This strategy might markedly reduce female fitness, because the male is the main provider for female and young from before egg laying until the late nestling period.

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Extra-pair copulations (EPCs) have been documented in a wide range of birds (reviews in Ford 1983; Westneat et al. 1990; Birkhead & Møller 1992). To assess the adaptive value of EPCs in sexual selection and mating systems and their consequences to individual fitness, one has to know to what extent such copulations lead to fertilization. In the last 10 years advances in molecular genetics have provided new methods for the exact determination of genetic parentage (Jeffreys et al. 1985). DNA fingerprinting and related techniques make it possible to detect both extra-pair fertilizations and intraspecific brood parasitism (egg dumping; e.g. Burke & Bruford 1987; Wetton et al. 1987; Weatherhead & Montgomerie 1991). EPCs leading to successful fertilization have been documented in more than 40 studies, the majority of which involve small passerines (Table 12.2 in Birkhead & Møller 1992).

Male birds can adopt two alternative means of paternity assurance: mate guarding, where males closely follow their mates during the fertile period and prevent copulations with another male, or a very high frequency of within-pair copulation, by which males can at least augment their chances of paternity (e.g. Birkhead 1987). Birkhead et al.

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(1987) and Birkhead & Møller (1992) proposed that mate guarding is the primary means of paternity assurance in birds. They suggested that only species in which mate guarding is difficult, such as birds of prey and colonial seabirds, use frequent pair copulation. They have also suggested that, in species without mate guarding, the frequency of EPC and of extra-pair paternity (EPP) should be relatively high compared with guarding species.

In most birds of prey, adoption of a close mate-guarding strategy is difficult owing to the distinct inter-sexual division of duties in the breeding season: females produce and incubate eggs and brood the young, whereas males provide for their families from before egg laying until the nestlings are half-grown (e.g. Newton 1979; Korpimäki 1981; Mikkola 1983). During the female's fertile period, male birds of prey should try to ensure paternity, although at the same time provision their mate to allow her to gain weight and maximize egg production. Thus, males have to compromise between leaving the nest vicinity to collect food for their mate or staying close to her to assure paternity.

The Eurasian kestrel, *Falco tinnunculus* (hereafter kestrel), is a small open-country raptor in which inter-sexual division of breeding duties is similar to that in other birds of prey (see Village 1990; Tolonen & Korpimäki 1994). Kestrels mainly feed on microtine rodents (voles) of the genera *Microtus* and *Clethrionomys* in central and northern Europe (e.g. Korpimäki 1985, 1986, and references therein). In northern Europe, vole densities fluctuate more than 100-fold in 3–5-year cycles (e.g. Hansson & Henttonen 1985; Korpimäki & Norrdahl 1991; Hanski et al. 1993). Such cyclic food conditions dramatically affect the breeding performance of kestrels. In western Finland, annual breeding density ranged from four to 98 nests per 100 km^2 during 1977–1992 (Korpimäki & Norrdahl 1991; Korpimäki 1994), and clutches were larger in good vole years (between-year range in annual mean clutch size 4.5–5.8; Korpimäki 1984, unpublished data).

We have examined the mating behaviour of kestrels in relation to varying food abundance in western Finland. The most common mating system was monogamy, in which one male and one female formed a seasonal pair bond and reared a brood together (see also Village 1990). Some 5–20% of males were polygynous in years of vole abundance, however, whereas no cases of polygyny were detected in years of vole scarcity (Korpimäki 1988, unpublished data). These kestrels are migrants, and on average, males arrived a few days before females in late March to early May (Palokangas et al. 1992). Pairs were usually formed within 2 days of male arrival.

In this paper we report on annual differences in the copulatory behaviour (pair copulation and EPC frequency) and mate guarding of kestrels in the course of a 3-year vole cycle, which induced marked changes in kestrel breeding density. We used DNA analysis of hypervariable single loci to determine the proportion of EPP and, where possible, assign extra-pair paternity. Finally, we have assessed the prediction that in species that are not able to closely guard their mates, the frequency of EPC and EPP should be relatively high compared with guarding species (Birkhead & Møller 1992).

METHODS

Field Work

The study site was in Kauhava and Lapua, in the province of South Ostrobothnia, western Finland (62°59′–63°10′N, 22°50–23°20′E). The main site (Alajoki, ca 100 km^2) is a level uniform agricultural area broken by small islands of trees, bushes and woods, two peatland bogs, large ditches and two rivers (Korpimäki 1984; Korpimäki & Norrdahl 1991). The rest of the site (ca 200 km^2) consists of smaller agricultural areas (1–10 km^2) surrounded by coniferous forests.

Kestrels bred in nestboxes mounted on the gables of barns or solitary trees. The total number of nestboxes was 200 at Alajoki and 120 in the small agricultural areas during 1990–1992. We checked the boxes regularly to determine the start of egg laying, clutch size, hatching date and number of hatched and fledged young. We removed pellets and prey remains that had accumulated on the bottom of the boxes after the breeding season for the analysis of diet (Korpimäki 1984, 1987b).

The total number of kestrel nests in the boxes was 28 in 1990, 56 in 1991 and 57 in 1992. Within our study area, we chose a sub-area containing 19 nests in 1990, 27 nests in 1991 and 29 nests in 1992, from which we collected blood samples of parent kestrels and their young for DNA analysis. In 1991–1992, with high kestrel density, all the

sampled nests originated from our main study area (Alajoki), but in 1990 we also sampled kestrels in smaller agricultural areas. To reveal the identity of potential extra-pair fathers, we took blood from five males in 1990 and six males in 1992 that bred in the vicinity of sampled nests. We captured parent kestrels when feeding their 10–20-day-old young using a swing-door trap attached to the front of the box (Korpimäki 1988). We measured their body mass and wing, tarsus and tail lengths, and marked them with a metal band and an individual combination of plastic coloured leg bands. The proportion of trapped falcons in the breeding population was 93% in 1990, 82% in 1991 and 79% in 1992. We banded all young when the oldest nestling in the brood was 25 days old.

We collected blood in microcapillary tubes after puncturing the brachial vein. Nestlings were sampled when they were 2–4 weeks old. The blood (60–75 µl) was immediately suspended in a lysis buffer and placed within 6 h in a −18°C freezer for permanent storage (Seutin et al. 1991).

We made time budgets of kestrels during the period of courtship feeding (i.e. after pair formation but before egg laying) in 1990–1992. The total observation time was 6–8 h per pair (11 pairs in 1990, 20 in 1991 and 23 in 1992) on rainless days, and we divided observation periods into two equal parts in the morning and afternoon. We observed focal pairs at a distance of 200–500 m from their future nests with binoculars and telescopes so that their behaviour was undisturbed. We chose sites from which there was good visibility of both the nest and the main hunting areas. We differentiated pair males from extra-pair males on the basis of colour bands or individual differences in plumage coloration. Yearling males have a female-like plumage (Village 1990), and in adult (≥2 year old) males the back colours vary from brown to brick-red, the size of dark back-spots vary from tiny to broad, and tail colours vary from brown to blue-grey (Palokangas et al. 1994). We used focal sampling (Altmann 1974), and made behavioural observations by continuous recording, because this measure of behaviour is more reliable than the time sampling method (Martin & Bateson 1986). We measured the proportion of the time spent mate guarding (i.e. the attendance of the male in the vicinity of the nest with his mate) and in flight hunting, the number of within-pair copulations, extra-pair attempts (i.e. intrusion of an extra male to the vicinity of female and nest) and extra-pair copulations per h, the number of prey delivered by males to their mates per h, and the number of display flights per h. Our definition of mate guarding is somewhat different to the normal one (see Introduction), because it also includes other activities (e.g. resting) of males in the vicinity of the nest with the female. When males are in the vicinity of their mates, however, they are far more able to prevent copulations with other males compared to when they are hunting. Males usually hunt 1–2 km (up to 3 km) from their nests (Korpimäki et al. 1994). Perching, wind-hovering and soaring are the main hunting modes of kestrels (e.g. Village 1983; Tolonen & Korpimäki 1994). Flight hunting, i.e. directional flights between the future nest site and the hunting area, flight bouts during hunting, wind-hovering, soaring and gliding consume more energy but yield more prey per unit time than hunting from a perch (Masman & Klaassen 1987; Masman et al. 1988). Consequently, we used the proportion of the total budget time spent flight hunting (i.e. the hunting effort) as an estimate of reproductive effort in males (see Tolonen & Korpimäki 1994, in press). We excluded one yearling male recorded in 1991 and 1992 from the analysis, because the breeding success of yearling males is lower than that of adult males (Village 1990; Hakkarainen et al. 1993).

Two *Microtus* voles, *M. agrestis* and *M. epiroticus*, and the bank vole, *Clethrionomys glareolus*, are the main prey species of kestrels in our study area (Korpimäki 1985, 1986). We estimated vole densities by snap-trapping in April, June and August both at Alajoki (three 3-km^2 sample plots) and in the small agricultural areas (three 3-km^2 sample plots). We modified a short-line method from the small quadrat method of Myllymäki et al. (1971) to suit the conditions of intensively cultivated fields (Norrdahl & Korpimäki 1993). We randomly placed trap lines in ditches inside each plot and set 10 mouse snap-traps and one rat-snap trap 10 m apart for 2 nights in each selected ditch. We trapped eight ditches per trapping occasion per plot during 1991–1992 (giving a total of 176 trap nights per trapping occasion per plot) and used 10 ditches per trapping occasion per plot 1990 (a total of 220 trap nights).

We performed two-tailed statistical analyses using SYSTAT (Wilkinson 1989).

Laboratory Analyses

We extracted DNA as described by Wetton et al. (1992), restricted it with excess *Mbo* I and determined the concentration by fluorometry. All samples were diluted to 0.15 µg/µl with 2 × loading buffer (4% Ficoll 400, 40 mM EDTA pH 8.0, 0.05% Bromophenol blue, 0.05% xylene cyanol). Then we loaded 1.5 µg of diluted genomic DNA along with 10 ng of an internal marker, consisting of a 4:1 mix of 1-kb DNA ladder (Gibco/BRL) to lambda DNA digested with *Xho* I, in the wells of a 20 × 22-cm 0.8% agarose gel in TAE buffer (40 mM Tris, 40 mM acetic acid, 1 mM EDTA (pH 8.0)). Electrophoresis at 48 V for 44 h resulted in the loss of fragments less than 2 kb from the end of the gel. DNA was depurinated and denatured by soaking for 20 min in 0.2 M HCl and 35 min in 1.5 M NaCl, 0.5 M NaOH and neutralized in two changes of 1.5 M NaCl, 1 M Tris pH 7.0 for 45 min. DNA was transferred by capillary blotting with 20 × SSC onto Zetaprobe GT nylon membrane (Bio-Rad) and fixed by baking at 80°C in vacuo for 120 min.

Inserts of single-locus minisatellite DNA isolated from the peregrine falcon, *F. peregrinus*, charomid clones c*Fpe*MS5, c*Fpe*MS13 and c*Fpe*MS17, and the merlin, *F. columbarius*, clones c*Fco*MS2 and c*Fco*MS19 (J. H. Wetton & C. A. May, unpublished data) were labelled with [α^{32}P]-dCTP by random priming (Feinberg & Vogelstein 1984) and sequentially hybridized along with 10 ng of separately labelled 6.6-kb lambda *Hind* III fragment. The inclusion of the lambda probe aided alignment of the autoradiographs by revealing the 15.0-kb lambda *Xho* I internal marker fragment, which includes the 6.6 kb *Hind* III fragment (Wetton et al. 1995). Pre-hybridization, hybridization, washing and probe stripping followed standard protocols (May et al. 1993), no competitor DNA was required and stringent washes were carried out at 0.1 × SSC, 65°C. The final hybridization to a cocktail of separately labelled kilobase ladder and 6.6-kb lambda *Hind* III fragment revealed the internal markers.

Each 1-kb range between consecutive rungs of the ladder was divided into 10 bins of equal width. The lower boundary of the first bin was centred upon the largest marker fragment, creating a continuous range of non-overlapping bins which become progressively broader at each rung due to the logarithmic separation of fragments. The allele sizes for each locus were determined by aligning the autoradiogram with that of the internal marker ladder by means of the baseline 15.0-kb lambda *Xho* I band common to both hybridizations. We compared the bin allocations of the paternal alleles observed in extra-pair young with those of each adult male to identify the extra-pair sires. To avoid false exclusions resulting from slight mis-alignment of the autoradiograms, we considered potential matches if the estimated size of the adults band differed from that of the extra-pair nestling by less than 200 bp (i.e. two bins' width). Exclusions were unlikely to have resulted as a consequence of de novo mutation because of the low observed frequency per allele, which ranged from 0.005 (c*Fpe*MS5) and 0.0016 (c*Fpe*MS13 and c*Fpe*MS17) to none (c*Fco*MS2 and c*Fco*MS19) among 307 legitimate offspring.

Observed heterozygosities (H) at the five loci ranged from 0.94 to 0.96 among 152 unrelated adult kestrels from our study population. As a consequence of these high heterozygosities, the majority of individuals were informative for testing for co-segregation between loci. Sequential LOD score analysis (Morton 1957) of the families of 56 adults with five or more offspring revealed weak linkage between two loci (c*Fco*MS2 and c*Fpe*MS17, $\theta = 0.2$, LOD > 3) but we found no evidence of linkage between any other combination (LOD < − 3 for all $\theta \leq 0.2$). Tight linkage between minisatellites can result in associations between alleles at different loci, thereby reducing the likelihood of detecting extra-pair fertilizations. Linkage between c*Fco*MS2 and c*Fpe*MS17, however, was sufficiently weak that such associations would only be maintained between close relatives, which are unlikely to be encountered in our nomadic population (mean turnover of 75% for breeding males and 92% for females during 1983–1992; Korpimäki 1988, unpublished data). The probability of false paternal inclusion can thus be estimated as $P_{fi} = 2q - q^2$ where $q = 1 - H$ (Bruford et al. 1992). The combined P_{fi} is of the order of 2×10^{-5}, which equates to the probability that a cuckolded attendant male will be wrongly assigned as the biological parent. Taking into account the loss of resolution resulting from binning still results in a P_{fi} less than 2×10^{-3}. Thus, single locus profiling also provides a powerful means of identifying the true biological parents when these are run on another gel.

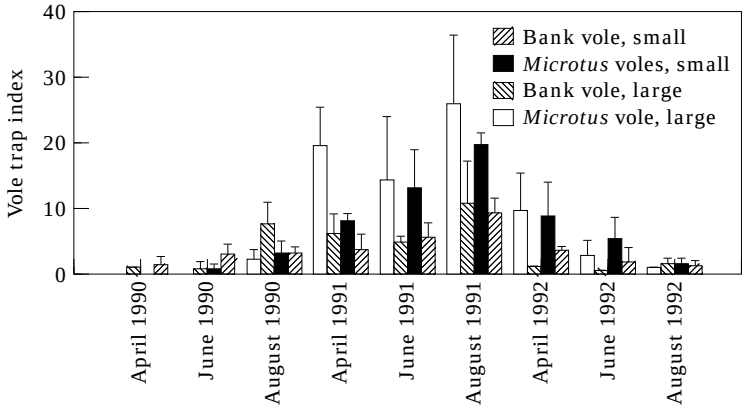

Figure 1. Trap index (trapped individuals per 100 trap nights) of two *Microtus* voles, *M. agrestis* and *M. epiroticus*, and the bank vole in a large agricultural area (Alajoki) and small agricultural areas in April, June and August 1990 to 1992. The bars give the mean (+SD) values for three sample plots (each 3 km^2) in two agricultural area categories.

RESULTS

Food Abundance and Breeding Performance

In 1990, densities of the *Microtus* and bank voles were low in the entire breeding season of kestrels (May–July), but started to recover from late July onwards. In the following year (1991), vole densities were intermediate during the laying period of kestrels (late April to late May), but increased rapidly to peak numbers during the nestling period (June to early July). The 1992 vole abundances were intermediate at the time of egg laying (late April to early May), but crashed to very low numbers in the late nestling and fledging periods (late June to July; Fig. 1).

Breeding density of kestrels was highest in the increasing vole year 1991, but slightly lower in the decreasing year 1992, and markedly lower in the low year 1990 (Table I). It closely followed spring densities of voles (Fig. 1) which is also evident in the long-term data from 1977 to 1992 (Korpimäki 1984, 1994). Egg laying started nearly 2 weeks earlier in 1991 and 1992 than in 1990, and the between-year variation in mean laying date was significant (ANOVA: $F_{2,45}=4.58$, $P=0.02$; Table I). Clutches were largest in 1991, followed by 1992 and 1990 (ANOVA: $F_{2,41}=5.03$, $P=0.01$).

Table I. Breeding characteristics of kestrels and number of nests where breeding and behavioral data were collected during 1990–1992

	1990	1991	1992
Breeding density (nests per 100 km^2)	19	51	40
Mean laying date	12 May	30 April	2 May
SD	8	11	11
Mean clutch size	5.0	6.0	5.6
SD	0.9	0.6	0.9
Number of nests	11	20	22

Copulation and Nest Attendance

The mean ± SD pair copulation frequency was 0.718 ± 0.546 per h (range 0.000–2.700; number of pairs 53) during 1990–1992. In addition, two EPCs were recorded, one each in 1991 and 1992 (overall frequency 0.007 per h; only 1% of all copulations). However, extra-pair attempts (i.e. intrusions of extra males to the vicinity of females and nests) were recorded more frequently (overall $\bar{X} \pm \text{SD} = 0.070 \pm 0.139$ per h). In 40% of the attempts, a pair male was present and behaved aggressively towards the intruder, thereby preventing EPC. In 27% of the cases, a pair female was aggressive towards the intruder, and in 33% of these, she resisted attempted copulation.

We found significant between-year variation in the within-pair copulation frequency during 1990–1992 (ANOVA: $F_{2,50}=3.90$, $P=0.027$; Fig. 2). This result was because the falcons copulated significantly more in the increasing vole year (1991) than in the decreasing vole year (1992) (Tukey test: $P=0.04$). Pair copulation frequency

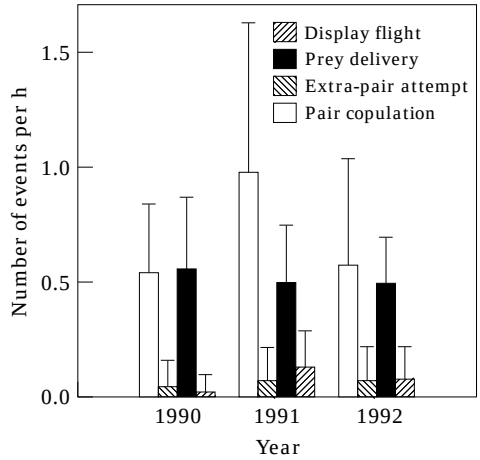

Figure 2. Mean (+SD) number of within-pair copulations, extra-pair copulation attempts, prey deliveries of males and display flights of males per hour in the low (1990, total number of pairs 11), increase (1991, 20) and decrease (1992, 22) vole years.

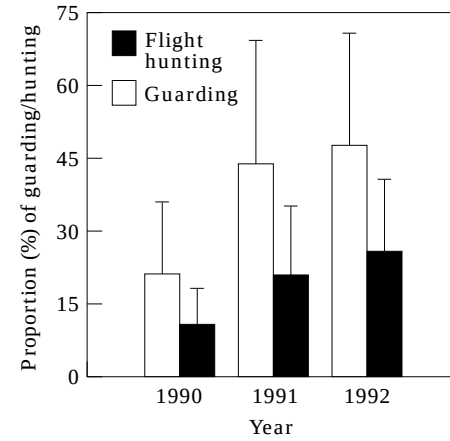

Figure 3. Mean (+SD) proportion (%) of budget time devoted by males to mate guarding and flight hunting in the low (1990), increase (1991) and decrease (1992) vole years. Total number of males as in Fig. 2.

also tended to be higher in 1991 than in 1990, the low vole year (Tukey test: $P=0.08$). The occurrence of extra-pair attempts did not vary between years (Kruskal–Wallis test: $H=1.45$, $df=2,50$, $P=0.48$; Fig. 2). The prey delivery rate averaged 0.514 ($\pm$0.232) per h during 1990–1992 and did not vary between years ($F_{2,50}=0.27$, $P=0.76$; Fig. 2). The frequency of display flights averaged 0.089 ($\pm$0.142) per h during 1990–1992 with no significant between-year variation ($F_{2,50}=2.33$, $P=0.11$; Fig. 2).

Males spent on average 40.3% ($\pm$28.9%) of their time in mate guarding and 20.7% ($\pm$14.1%) of their time in flight hunting. The proportion of both activities showed significant between-year variation ($H=9.76$, $df=2,50$, $P=0.008$ for mate guarding, and $H=9.15$, $df=2,50$, $P=0.01$ for flight hunting; Fig. 3). This result was attributable to males guarding their mates less in 1990 (the low vole year) than in 1991–1992. In addition, males tended to spend less time in flight hunting in 1990 than in 1991–1992.

Genetic Parentage

In all the nests analysed for parentage during 1990–1992, the female that was trapped in the nest when the young were 2–3 weeks old was the genetic mother of the young. Of 63 young tested in 1990 and 144 young tested in 1992, all were consistent with the attendant male (Table II). The males of sampled nests were all adults ($\geq$2 years old) in 1990, but single yearling males were sampled in both 1991 and 1992. In 1991, six (5%) of 112 nestlings were incompatible with their attendant male at several loci (mean=4.2, minimum=3). Because the probability of three or more mutations in the same individual was less than 10^{-6}, we inferred that these nestlings had been sired by a male other than the attendant. In the first brood with extra-pair offspring, all four nestlings mismatched with the attendant male. The distribution of the two paternal alleles observed at each locus between the four nestlings implied that they were all the offspring of a single sire. This falcon might have been an unsampled yearling male that once unsuccessfully attempted

Table II. Extra-pair paternity determined by DNA fingerprinting in kestrels breeding in western Finland during 1990–1992

Year	Extra-pair offspring		Extra-pair broods		No. of offspring	No. of broods
	N	%	*N*	%		
1990	0	0	0	0	63	19
1991	6	5.4	2	7.4	112	27
1992	0	0	0	0	144	29
All years	6	1.9	2	2.7	319	75

Table III. Clutch size, brood size and time interval at the start of egg laying in primary and secondary nests of three bigynous males DNA profiled in 1991–1992

Year	Primary nest		Secondary nest		Time interval (days)	Distance* (km)
	Clutch size	Brood size	Clutch size	Brood size		
1991	6	3	6	5	15	1.6
1992	6	6	6	6	18	0.8
1992	5	3	5	5	14	1.1

*The linear distance between primary and secondary nests.

EPC during our 8-h observations. The cuckolded adult male was presumably a poor food provider, because he did not deliver prey to his mate during our 8-h observations, although he used 31% of the time in flight hunting. Also, the within-pair copulation frequency (0.33 per h) was low compared to the yearly mean (0.97 per h). In this case, however, we cannot rule out a rapid change of mate, because the attendant male was identified after the fertile period of his mate. The other two extra-pair nestlings were identified in a brood of four, the remainder of which matched perfectly with the attendant pair. The attendant male, who was the only yearling sampled in 1991, was excluded as the father of the mismatching young at all five loci, but an adult male breeding 0.7 km away possessed all of the paternal alleles. The paternity of this male was confirmed by both single and multilocus fingerprinting. This male sired all four young in his own nest.

The proportion of EPP broods did not show significant between-year variation (chi-squared test: $\chi^2 = 3.65$, $df = 2$, $P = 0.16$), but the proportion of EPP young did so ($\chi^2 = 11.30$, $df = 2$, $P = 0.004$). The latter test assumes that fertilizations within a brood are independent.

Broods of three bigynous adult males were sampled for DNA analysis (Table III). The time interval of laying dates between primary and secondary nests (i.e. between first and second mates of the male; e.g. Korpimäki 1988, 1991) averaged 16 days and the linear distance averaged 1.2 km. These bigynous males sired all of the young in their two nests.

DISCUSSION

This study examined the effect of largely varying breeding density upon copulation rates, mate guarding and genetic parentage in a bird species that uses frequent pair copulation for paternity assurance (see also Dunn et al. 1994). In this kestrel population, breeding density was closely related to prey abundance and seems to influence these variables. First, the pair copulation frequency was higher in a year of increasing food supply than in years of decreasing and poor food supply (Fig. 2), although the kestrel breeding density was lower in a year of vole scarcity than in increasing or decreasing years of vole abundance (Table I). Second, males invested more time in mate guarding in increasing and decreasing food years than in a year of food scarcity (Fig. 3). Third, DNA profiling revealed EPP only in a year of increasing food abundance (Table II).

The Paternity Assurance Hypothesis

The paternity assurance hypothesis states that very high pair copulation frequency may be a means by which males try to secure their genetic paternity (e.g. Birkhead et al. 1987; Birkhead & Møller 1992), because the probability of fertilization by a male is closely proportional to the relative amount of sperm delivered by him to a particular female. In addition, the last copulation before fertilization is usually more successful than the earlier ones, and a male copulating frequently with his mate will have a high probability of being the last before fertilization (Birkhead & Møller 1992).

Interspecifically, the paternity assurance hypothesis predicts higher copulation frequencies among colonially nesting raptors than among solitary raptors (Birkhead et al. 1987; Møller 1987; Simmons 1990). In a Spanish colony of lesser kestrels, *F. naumanni* (a close relative of Eurasian kestrels), the mean copulation frequency was 0.6 per h during the last week before the start

of egg laying (Negro et al. 1992), which is no more than in our kestrels (0.72 per h) and Dutch kestrels (0.6 per h, estimated from Fig. 1 in chapter 4 of Meijer 1988). Therefore, these data do not give obvious support for the interspecific prediction of the paternity assurance hypothesis.

Intraspecifically, the paternity assurance hypothesis predicts that copulation frequency should also increase with breeding density, because at high breeding densities the occurrence of extra-pair intrusions should also increase (see also Westneat et al. 1990). In accordance with this prediction, there was an almost significant increase in pair copulation frequency in 1991 with high kestrel breeding density (51 nests per 100 km^2) compared to that in 1990 with low kestrel breeding density (19 nests). Kestrel pairs copulated significantly more in 1991 than in 1992, however, although there was no obvious difference in their breeding density (51 versus 40 nests per 100 km^2). This between-year difference in pair copulation frequency might occur because the density of non-breeding (mainly yearling) males (and thus the occurrence of intruders) may have been higher in 1991 than in 1992, because DNA analysis only revealed EPP in 1991. However, we did not record a between-year difference in the EPC attempts. Alternatively, kestrels might invest more in mating under increasing than decreasing food conditions (Fig. 1). In the later phase of the vole cycle, declining food abundance reduces the future survival of nestlings and independent offspring, whereas in the increase phase, offspring survival is better (e.g. Korpimäki 1987a; Korpimäki & Lagerström 1988). As a consequence, the reproductive value of offspring is greater in the increasing than in the decreasing phase of the vole cycle, and it might be adaptive if reproductive effort is high in years of increasing food abundance (see also Hakkarainen & Korpimäki 1994a, b for reproductive effort of vole-eating Tengmalm's owls, *Aegolius funereus*).

Paternity Assurance and Cuckoldry

The sperm storage duration of American kestrels, *F. sparverius* (morphologically close to Eurasian kestrels) is 8.1 days (Table 3.3 in Birkhead & Møller 1992). Using this period and a laying interval of 48 h (Piechocki 1982) for our kestrels means that the fertile period of females will start about 10 days before the first egg is laid and end 8 (five-egg clutch) to 10 days (six-egg clutch) after the first egg is laid (calculated according to the formula in Birkhead & Møller 1992, page 66). The mean copulation rate of our kestrels was 0.72 per h prior to egg laying. Assuming a fertile period of 18 (five-egg clutch) to 20 days (six-egg clutch) with a daily activity time of 16 h (Tolonen 1992) gives 207–230 copulations per clutch. This copulation rate is higher than that recorded in merlins and African marsh harriers, *Circus ranivorus*, but is about the same as in ospreys, prairie falcons, *F. mexicanus*, and lesser kestrels (Table IV). Even higher copulation rates have been recorded for goshawks, *Accipiter gentilis*, and American kestrels (Table IV). Eighty-two per cent of 57 bird species copulated less than or within 40 times per a clutch (data from Figure 8.1. in Birkhead & Møller 1992).

Table IV. Estimated number of within-pair copulations per clutch and the proportion (%) of extra-pair copulations in birds of prey

Species	Number	%	Reference
Eurasian kestrel	207–230	1	1
Lesser kestrel	326	7	2
American kestrel	690		3
Merlin	42	7	4
Prairie falcon	194	0	5
African marsh harrier	50–220	2	6
Goshawk	502		7
Osprey	160	0.3	8

References: (1) this paper, (2) Negro et al. (1992), (3) Balgooyen (1976), (4) Sodhi (1991), (5) Holthuijzen (1992), (6) Simmons (1990), (7) Møller (1987), (8) Birkhead & Lessells (1988).

Because the mean time interval between the start of egg laying in primary and secondary nests was 16 days, the fertile periods of two females of bigynous males somewhat overlapped. After pair formation, males must trade off between pursuing EPCs, attracting additional mates and investing in paternity assurance and courtship feeding at the primary nest. After successful attraction of a secondary female, a bigynous male has to copulate frequently with her and guard and feed two mates not less than 0.8 km apart. Despite this constraint, our results indicate that bigynous males did not lose genetic paternity of their two broods. Polygynous males may thus be high-quality hunters and have a territory with abundant food supply so that they can simultaneously

assure their paternity and feed their two mates prior to and during egg laying.

Male kestrels invested more time in both mate guarding and flight hunting in the increasing and decreasing years of food abundance (1991–1992) than in the year of food scarcity (1990). Therefore, mate guarding and flight hunting were not necessarily alternative behaviour patterns. This may be because kestrel breeding density was higher in 1991–1992 than in 1990, and males were able to use energy-consuming but profitable flight hunting to maximize time used for nest attendance. During the fertile period of their mates, male kestrels spent on average 40% of their time in mate guarding, which is less than in the goshawk (60–70%; Møller 1987) and osprey (60–70%; Birkhead & Lessells 1988), but more than in the lesser kestrel (20–30%; Negro et al. 1992).

Møller & Birkhead (1992a) and Birkhead & Møller (1992) predicted that in species that are not able to guard their mates closely, the EPC and EPP rates should be relatively high compared with guarding species. They supported this prediction with examples from non-raptorial species: the EPC rate of non-guarding species was higher than that of mate-guarding species (Møller & Birkhead 1993), and populations with many close neighbours had more EPCs than populations with few neighbours (Møller & Birkhead 1992b). However, detailed studies of copulatory behaviour and paternity of raptors appeared to fit their prediction poorly. First, the EPC rate of raptors remained low in all studies (0–7%: Table IV) compared to guarding species (Table 12.2 in Birkhead & Møller 1992). Second, the frequency of EPCs and EPC attempts was not markedly higher at high kestrel breeding densities than at low ones. Third, the frequency of extra-pair offspring (2%) was low in our kestrels, as it was in other raptors: 5% for the sparrowhawk, *A. nisus* (Carter and Parkin in Birkhead & Møller 1992), and 0% for the Eleonora's falcon, *F. eleonorae* (Swatschek et al. 1993), the merlin (Warkentin et al. 1994) and the black vulture, *Coragyps atratus* (Decker et al. 1993), contrasting markedly with species in Table 12.2 of Birkhead & Møller (1992).

Because cuckoldry was unusual in our kestrel population, we conclude that frequent within-pair copulation and mate guarding are used successfully by males as complementary, rather than alternative, paternity assurance means. Also, female kestrels did not normally appear to solicit EPCs from intruding males (see also Negro et al. 1992). The reason may be that in open-country raptors, pair males may detect extra-pair events even at a distance, and as a consequence of reduced confidence of paternity, decrease their parental care (e.g. Whittingham et al. 1992; Dixon et al. 1994). The latter, manifest as food provisioning for the whole family, largely determines the reproductive success of birds of prey. Therefore, by soliciting copulations from extra-pair intruders, females mated with high-quality males would risk the parental care of their mates later in the season, as the courtship feeding rate is a good estimate of male food provision during the nestling period (Palokangas et al. 1992, 1994). This risk taking may markedly reduce female fitness. We suggest that only females mated with poor food providers would solicit EPCs, possibly gaining help with parental care later in the season and increasing the genetic quality of their offspring (see Westneat et al. 1990; Birkhead & Møller 1992; Lifjeld et al. 1994, for general costs and benefits of EPCs for females). Studies on passerines have shown that females appear to pursue EPCs with high-quality males (e.g. Møller 1988; Smith 1988; Kempenaers et al. 1992).

Finally, we emphasize that DNA analysis did not reveal any egg dumping and EPP was unusual. Therefore, the number of fledglings produced per nest is a good estimate of reproductive success for both male and female kestrels.

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