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Condition-dependent control of paternity by female purple martins: implications for coloniality

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Abstract Proposed causal links between extra-pair copulation (EPC) and colony formation in socially monogamous birds hinge on the question of which sex controls fertilizations. We examined in colonial purple martins *Progne subis* (1) whether EPCs were forced or accepted by females, and (2) the degree to which apparently receptive females were able to obtain EPCs against their mates' paternity defenses. Paternity analyses of multilocus DNA fingerprinting confirmed previous findings of a marked relationship between age class and extra-pair fertilizations (EPFs), with young males losing paternity of 43% ($n = 53$) of their putative offspring compared to 4% ($n = 85$) by old males. All assignable extra-pair offspring were sired by old males, with one male obtaining most EPFs each year. Contrary to the hypothesis that EPCs are forced, EPF frequency within age class did not increase with seasonal increases in the number of males per fertile female. Whereas the male control hypothesis predicted that the male age class that mate-guarded more would be cuckolded less, the reverse was true: young males guarded significantly more intensely. The male age class difference in cuckoldry could not be explained by the possibility that young and inexperienced females (which are usually paired to young males) were more vulnerable to forced copulation because EPFs were

unrelated to female age. These findings suggest that females (1) pair with old males and avoid EPCs, or (2) pursue a mixed mating strategy of pairing with young males and accepting EPCs from old males. The receptivity to EPCs by females paired to young males put them in conflict with their mates. Two factors determined the paternity achieved by young males: (1) the relative size of the male to the female, with young males achieving much higher paternity when they were larger than their mates, and (2) the intensity of mate-guarding. Both variables together explained 77% of the variance in paternity and are each aspects of male-female conflict. Given female receptivity to EPCs, mate-guarding can be viewed as male interference with female mating strategies. We conclude that EPCs are rarely or never forced, but the opportunity for females paired to young males to obtain EPCs is relative to the ability of their mates to prevent them from encountering other males. Evidence of mixed mating strategies by females, combined with other features of the martin mating system, is consistent with the female-driven "hidden lek hypothesis" of colony formation which predicts that males are drawn to colonies when females seek extra-pair copulations.

Key words Coloniality · Purple martin · *Progne subis* · Cuckoldry · Lek

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Introduction

The recent discovery that extra-pair copulation (EPC) is prevalent among socially monogamous birds (Westneat et al. 1990; Birkhead and Møller 1992) has produced interest in how fertilizations are influenced by male and female behavior. Whereas most earlier studies assumed that EPCs were forced by males, in many species females solicit or permit EPCs (Smith 1988; Wagner 1991a, 1994; Kempenaers et al. 1992;

Lifjeld and Robertson 1992; Graves et al. 1993; Stutchbury et al. 1994). When females pursue EPCs, the potential exists for high rates of extra-pair fertilizations (EPFs) to occur. Female preferences for certain males and variation in male competitive ability may produce skews in male EPC success that can substantially increase the intensity of sexual selection in socially monogamous species (Møller 1988, 1992; Wagner 1992a; Hill et al. 1994). The pursuit of EPCs by female birds therefore, has ramifications for many facets of avian evolution.

Recently it has been proposed that extra-pair copulation can promote colony formation, either through benefits to males (Morton et al. 1990) or females (Wagner 1993). The female-driven hidden lek hypothesis predicts that when females pursue extra-pair copulations, the same mechanisms that produce leks operate (Wagner 1993, 1996). The hypothesis proposes that the pre-egg laying clustering for EPCs of males and females sets the stage for colony formation. After clustering for EPCs occurs, other hypothetical post-laying advantages of coloniality, such as enhanced food-finding and reduced predation (Wittenberger and Hunt 1985), can accrue. Although a skew in male EPC success would allow only a minority of males to benefit from EPCs, many males would be forced to breed near males who might cuckold them if that is where females prefer to breed.

In colonial purple martins (*Progne subis*) there is a strong relationship between male age class and extra-pair fertilization frequency (Morton et al. 1990). Male purple martins exhibit delayed plumage maturation: males age two years or more attain the definitive purple whereas yearlings retain a mainly brown and white female-like juvenile plumage, despite undergoing a complete molt (Stutchbury 1991a). Morton et al. (1990) observed that older males could increase their fitness by recruiting young males and cuckolding them, and suggested that the benefits old males accrued from EPCs select for coloniality. This idea was supported by the different breeding schedules of the two age classes, with older martins arriving and breeding earlier (Morton and Derrickson 1990). When the mates of the old males complete egg-laying, old males perform a loud pre-dawn song which appears to attract migrating young males and females to the colony. At this time, old males are emancipated from nest-building and mate-guarding and can pursue the mates of the young males for EPCs, which appear to be forced (Morton 1987). However, in order for the hidden lek hypothesis to explain martin colony formation it is necessary that females willingly accept EPCs, despite appearing to resist male attempts. Although young males and their mates should avoid breeding near old males if EPCs are forced, it is possible that females prefer to pair with old males; if none are available then females might pursue a mixed mating strategy of pairing with a young male and accepting EPCs from an old male. While this

would be disadvantageous to young males, they could achieve greater fitness by breeding in a colony and sharing paternity with old males than by foregoing reproduction that year.

Our principal objective was to test whether males or females control extra-pair fertilizations, because the proposed casual links between EPC and coloniality hinge on this question. Another goal was to both exclude and assign paternity of extra-pair offspring in order to evaluate the intensity of sexual selection produced by extra-pair fertilizations. During the course of the research an additional goal emerged when we discovered a significant relationship between paternity and body size of young males and their mates. This led us to use morphological data to examine the relevance of body size to the question of which sex controls fertilizations.

In pursuing our primary objective, testing for male versus female control, we reasoned as follows. When unescorted females alight on the ground to gather nesting material they are subjected to aggressive-appearing EPC attempts by males (Brown 1978; Morton 1987). Females resist, but their resistance occurs in a wide range of forms. Some fly away before males can approach near enough to attempt mountings, while others continue foraging for material until the male is able to mount, at which point females emit an alarm call and struggle to escape (Morton 1987). This range in responses could be caused by variation in female experience in avoiding EPCs or variation in female receptivity to EPCs. In the latter case, receptive females may subtly permit mountings while also resisting as a ploy to test males (Westneat et al. 1990; Wagner 1991b). Because such behaviors are extremely difficult to interpret, we searched for objective methods to determine whether extra-pair copulations are actually forced.

The male control hypothesis predicts that the probability of a female being subjected to forced EPCs is determined by the number of males available to pursue her (Morton et al. 1990). Since the operational sex ratio (i.e. the number of males per fertile female on a given day; Emlen and Oring 1977) increases with date, with more males becoming available to chase later-laying females (Morton et al. 1990), the male control hypothesis predicts a negative correlation between laying date and paternity, regardless of male age. Alternatively, the age-related pattern of EPFs could be explained by females controlling fertilization and accepting EPCs when paired to young males and refusing EPCs when paired to old males. The female control hypothesis predicts that male age alone determines paternity.

Another test of male versus female control uses contrasting assumptions about the purpose of mate-guarding. Whereas mate-guarding is usually considered a male tactic to keep other males from their mates, when females seek EPCs, mate-guarding is also a method to keep the female from other males (Birkhead

et al. 1990; Gowaty and Bridges 1991; Kempnaers et al. 1992; Wagner 1992b). If EPCs are forced, then males should mate-guard to keep other males from their mates. In this case, mate-guarding intensity is predicted to increase with the operational sex ratio. Alternatively, if males guard to prevent their mates from accepting EPCs, then mate-guarding intensity should be independent of the operational sex ratio.

An additional opportunity to test which sex controls fertilizations arose in one season when there was an exceptionally wide spread of laying dates and few young males breeding. The male control hypothesis predicts that early breeding old males should cuckold late old males similarly to how they hypothetically force the mates of late young males in other years. It also predicts that late old males will mate-guard more intensely than early old males. The female control hypothesis predicts that EPF frequencies for late old males should be low, and not different from early old males, and that mate-guarding intensity will not increase for late breeders.

Methods

We studied purple martins in Severna Park, Maryland from April to July in 1991, 1992, and 1993. The study colony consists of three 24-compartment aluminum martin houses which can be lowered and raised on 4.3-m poles (see Morton 1987; Morton et al. 1990). We recorded the arrival dates of color-banded individuals to the nearest 3 days. Mate-guarding intensity was scored by recording whether or not the male escorted his mate to the ground to gather nest material (Morton 1987). Nest boxes were checked at least twice weekly; because one egg is laid per day and the modal clutch size is four or five, we were able to record the date of clutch initiation within 1 day.

We trapped adults in a single night each year at the time when most eggs had hatched but few chicks had fledged, which was 3, 8, and 3, July respectively. We used custom-made traps which simultaneously drop over the entrances of all the cavities (Morton and Paterson 1983). We weighed each adult to the nearest 0.5 g using a pesola scale and measured the flattened wing chord to the nearest 1 mm using a stop-ended wing ruler.

DNA fingerprinting

We produced DNA fingerprints for 22 of 27 families in 1992 and 22 of 24 in 1993, which included 69 of 88 hatchlings in 1992 and 73 of 95 in 1993. None of the fingerprinted pairs bred together in both years. We calculated the percentage of the brood sired by the putative father for males for which at least two offspring were fingerprinted, which was for all but two males in 1992 and one in 1993.

We sampled blood by puncturing the brachial or tibio-tarsus vein and collecting 50–100 μ l in microcapillary tubes. The blood was immediately dispensed into a lysis buffer (0.01 M Tris, 0.01 M NaCl, 0.002 M EDTA, 1% SDS) and stored at 4°C. DNA was extracted by phenol/chloroform extraction and 6 μ g was digested with *HaeIII*. Restriction fragments were separated by electrophoresis for 60 h at 25 V through a 0.8%, 1 X TBE agarose gel. Southern blotting was performed according to Piper and Parker (1992). Probes were labeled with alpha 32 P dCTP by random priming, and hybridization and washes followed Piper and Parker (1992).

Filters were exposed to X-ray film (Amersham Hyperfilm MP) with one intensifying screen for 1–6 days.

We ran gels with the putative parents and chicks in adjacent lanes. Scoring was done without prior knowledge of male or female age. We scored bands as similar if they were within 0.5 mm and of similar intensities. Bands that were similar, but brighter in one lane than the other, were scored as two bands in the lane with the brighter band. In all cases, bands were scored in a conservative manner; fragments were only considered different if the difference was clear on the autoradiograph.

Paternity exclusions

Probe 33.6 was used for all paternity exclusions. In cases where light banding patterns yielded less than average numbers of bands we also used probe 33.15 to confirm or reject the interpretation of the banding patterns from probe 33.6. Probe 33.6 produced a mean of 17.0 (SD = 3.4) bands per individual and probe 33.15 produced 17.7 (SD = 3.6). The average band-sharing coefficient among presumably unrelated adults was 0.122 (SD = 0.005, $n = 187$).

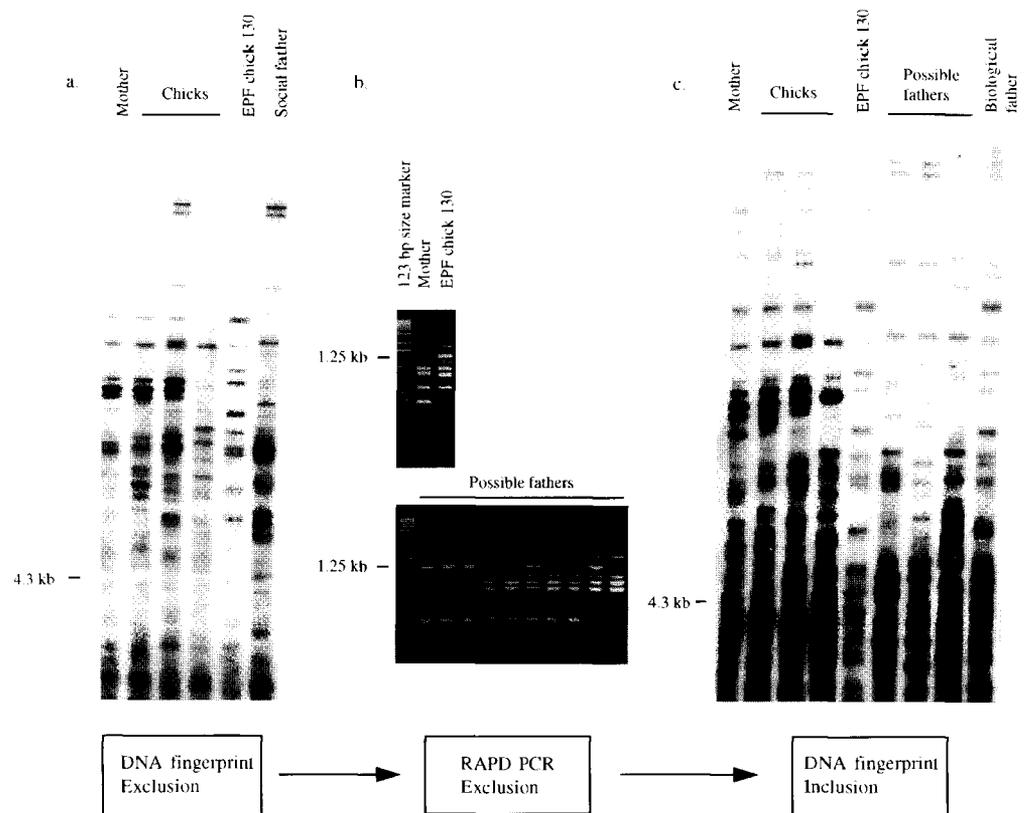
We calculated the average rate of mutation by assuming that individuals with only one and two novel fragments were the biological offspring of the social parents and received the novel fragments by mutation (Westneat 1993). The proportion of chicks with at least one novel fragment was 0.30, and the proportion with two was 0.16. This corresponds to an average mutation rate to a novel fragment of 0.23 per individual. Thus, the expected probability of observing three novel fragments from mutation alone is $0.23^3 = 1.2 \times 10^{-2}$, and four novel fragments is $0.23^4 = 2.8 \times 10^{-3}$. Because no chicks were observed with three novel fragments, we concluded that chicks with four or more novel fragments were from EPFs or brood parasitism. We also examined the relationship between band sharing and number of novel fragments. Average band sharing coefficients for chicks with zero, one, or two novel fragments ranged from 0.364 to 0.722 for putative fathers and 0.310 to 0.727 for mothers. For those chicks with more than three novel fragments, band sharing ranged from 0.057 to 0.277 for putative fathers. Thus, the criterion of four or more novel fragments is robust.

There were eight individuals that met only one of these criteria and were thus ambiguous. In six of these cases, no novel fragments were observed, but low band sharing coefficients were present; DNA patterns were lighter for the chick samples than the paternal samples, yielding fewer bands in the chick lane and skewing the band sharing coefficients towards low values. Because no novel fragments were present, we were conservative and did not exclude the social parents. These light DNA patterns also yielded skewed band sharing coefficients for two cases of chicks and social mothers, and in both cases the mother was not excluded. In two cases these decisions were confirmed after running subsequent gels. In others, insufficient DNA was available.

Paternity assignments

In order to streamline the process of assigning paternity for 1992, we devised a new application of randomly amplified polymorphic DNA (RAPD) polymerase chain reaction (PCR). We screened 42 RAPD primers from the OPF, OPM, and OPG kits (Operon Technologies, Inc.) to identify DNA markers that exclude a portion of the potential males before final paternity assignment using Southern blots hybridized with Jeffrey's probes using PCR and electrophoresis conditions as described by Levitan and Grosberg (1993), except using a Perkin Elmer Cetus base model thermal cycler, and 30 ng of template. The criterion for using RAPD markers was that the DNA fragment was bright, clearly separated from other bands on the gel, and repeatable using

Fig. 1a–c Example paternity analysis for chick 130 from the 1992 colony. **a** Paternity exclusion by DNA fingerprinting with Jeffrey's probe 33.6 rejected the social father from being the genetic father. **b** A polymorphic fragment approximately 1.24 kb in length was produced by RAPD PCR with primer OPM-15 that was present in the offspring but not in the mother. Five of ten possible fathers shown were excluded. **c** Paternity inclusion by DNA fingerprinting with Jeffrey's probe identified one of four males as the genetic father of chick 130



similar PCR conditions with the same DNA. Polymorphic markers were included in the analysis if they were present in the offspring but not in the mother.

Screening of primers was completed in two stages. First, 13 (31%) of the primers were excluded from further analysis because they produced fewer than five bands, smeared banding patterns, or non-repeatable patterns between two or more PCR reactions. Of the remaining 29 markers, five exhibited polymorphisms that appeared as a distinct band in at least one extra-pair offspring, but not the social mother (Fig. 1b). Because RAPD markers are inherited in a Mendelian pattern (Williams et al. 1990), bands present in the chicks but not in the social mother must be inherited from the genetic father. Thus these primers were used to screen all males for the presence or absence of the band. Males not exhibiting the marker were excluded as potential fathers (Fig. 1b).

We found at least one RAPD marker present in the chick but not in its social mother for 12 of 17 EPF chicks. This excluded an average of 25.6 ($SD = 3.7$) males as genetic fathers. We used probes 33.15 and 33.6 to compare the EPF chicks with remaining potential fathers for which DNA was available, according to the criterion described for exclusions in 10 cases (e.g., Fig. 1c). We could not assign the genetic father to two EPF offspring for which RAPD markers were identified, and to the remaining five offspring where no RAPD markers were available. For these chicks, only 60% (168 of 228) of possible male-offspring dyads were analysed by DNA fingerprinting and RAPD PCR because of insufficient DNA or failure to identify an acceptable polymorphic RAPD marker, or both. For all six extra-pair chicks from 1993, the genetic father was identified by DNA fingerprinting with probe 33.15 according to the original criteria for paternity exclusions.

Analyses

Data in the form of percentages were arcsine transformed for their use in parametric tests. To combine data on laying dates between

years we used the percentile ranks of laying dates. The statistics of Mann-Whitney *U*-tests and Spearman rank tests are reported for ties.

Results

Paternity and male age class

The DNA fingerprinting results confirm the relationship between male age class and extra-pair paternity reported by Morton et al. (1990). In both years combined, 43% of chicks of young males were sired through extra-pair copulations compared to 4% of old males, and while 47% of young males were cuckolded, only 8% of old males lost any paternity (Table 1). The ten cuckolded males achieved paternity of a mean of 28% of their putative offspring; five males achieved 0% and the other five averaged 55% (range 33–75%). Paternity of young males was bimodally distributed, with young males either achieving 100% or 0–50% paternity of their broods.

Of 26 extra-pair offspring, we were able to assign paternity to 8 of 20 in 1992 and 6 of 6 in 1993, of which all were sired by old males ($\chi^2 = 9.6$, $df = 1$, $P = 0.002$). In five of six broods in which more than one extra-pair offspring was assigned paternity, the EPFs had been obtained by one male. Paternity assignments were skewed, with one old male obtaining most of the EPFs in each year. In 1992, an old male in house A sired all

Table 1 Comparison of extra-pair fertilization frequencies in the nests of young and old males. The percentage of males cuckolded was calculated for males which had a minimum of 2 chicks fingerprinted. In addition to these there were three males (one young

and one old male in 1992, and one old in 1993) with one chick that was fingerprinted and not excluded as the offspring of the putative father. Young males were significantly more likely to be cuckolded than old males (Fisher's exact test, $P = 0.008$)

Year	Young males		Old males	
	No. cuckolded/no. males	no. EPF/no. offspring	no. cuckolded/no. males	no. EPF/no. offspring
1992	7/13 (54%)	20/40 ^a (50%)	0/7 (0%)	0/26 (0%)
1993	1/4 (25%)	3/13 (23%)	2/17 (12%)	3/59 (5%)
Total	8/17 (47%)	23/53 (43%)	2/24 (8%)	3/85 (4%)

^a The one nestling produced by intra-specific brood parasitism is not included

seven offspring in the nests of two young males in house B. In 1993, an old male in A sired two of three chicks of another old male in A, and three of three of a young male in B. A second old male in A obtained paternity of one of four chicks of an old male in B. In short, one old male obtained seven of eight assignable EPFs in 1992, and another obtained five of six EPFs in 1993.

We found one apparent case of intra-specific brood parasitism in which both the putative mother and father mismatched one of three offspring. Both the male and female of this pair were of the young age class and bred in house B in 1992. The genetic father of the offspring was a young male who was the nearest neighbor. We did not identify the mother, but could not exclude the female paired to the genetic father.

To examine the effect of extra-pair paternity on male fertilization success we compared the variance in actual fertilization success with that in male brood size (i.e., no. of chicks fingerprinted). The variance in fertilization success was 400% higher ($F = 2.82/0.71 = 3.97$; $n = 41$, $P < 0.001$), indicating that EPFs produced significant potential for sexual selection to operate among males.

Which sex controls extra-pair fertilizations?

The male control hypothesis predicts that the age class difference in paternity is accounted for by the fact that old males breed early, allowing them to mate-guard until egg-laying and then pursue the later-laying mates of young males for EPCs. The female control hypothesis predicts that the earlier breeding dates of old males does not affect paternity.

The mates of old males laid a mean of 10 days earlier than the mates of young males in 1992 (11 June, $SD = 5.7$, $n = 17$, versus 21 June, $SD = 5.9$, $n = 20$, Mann-Whitney U -test: $z = -4.71$, $P = 0.0001$; Fig. 2a). However, despite overlap between the male age classes in their mates' laying dates, all old males achieved 100% paternity versus only 50% for young males (Fig. 2b). Statistically, the paternity that males achieved in their broods was unrelated to their mates' laying dates (Spearman rank test: $r_s = -0.36$, $n = 20$,

$P = 0.12$). The negative sign of the correlation is due to the high EPF frequencies and later breeding dates of young males. Whereas the male control hypothesis predicts that the correlation should be negative and significant within age class, the correlation for young males is positive and non-significant ($r_s = 0.15$, $n = 13$, $P = 0.60$).

A hypothetical experiment for male control is to widen the range of breeding dates of old males to test whether earlier old males cuckold later old males. Although there may be no practical method to

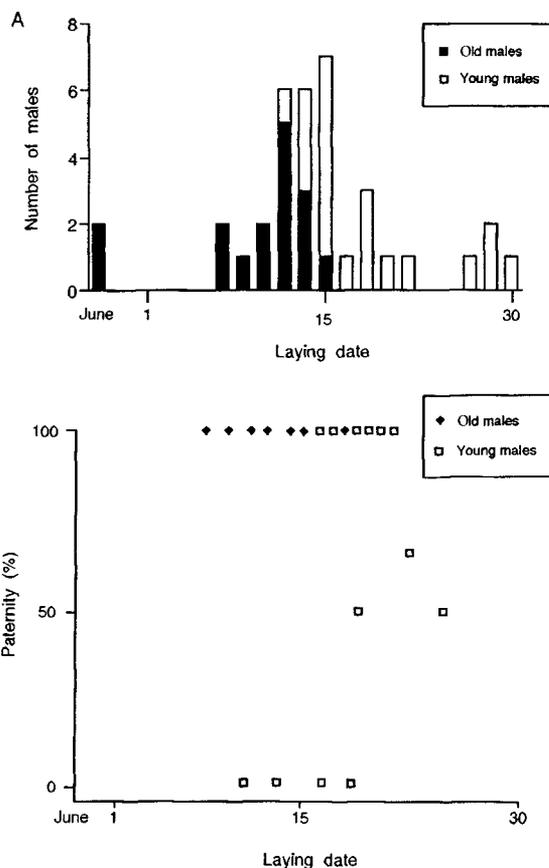


Fig. 2 a The distribution of egg-laying dates of the mates of old and young males in 1992, and **b** the percentage paternity achieved by old and young males plotted against laying date

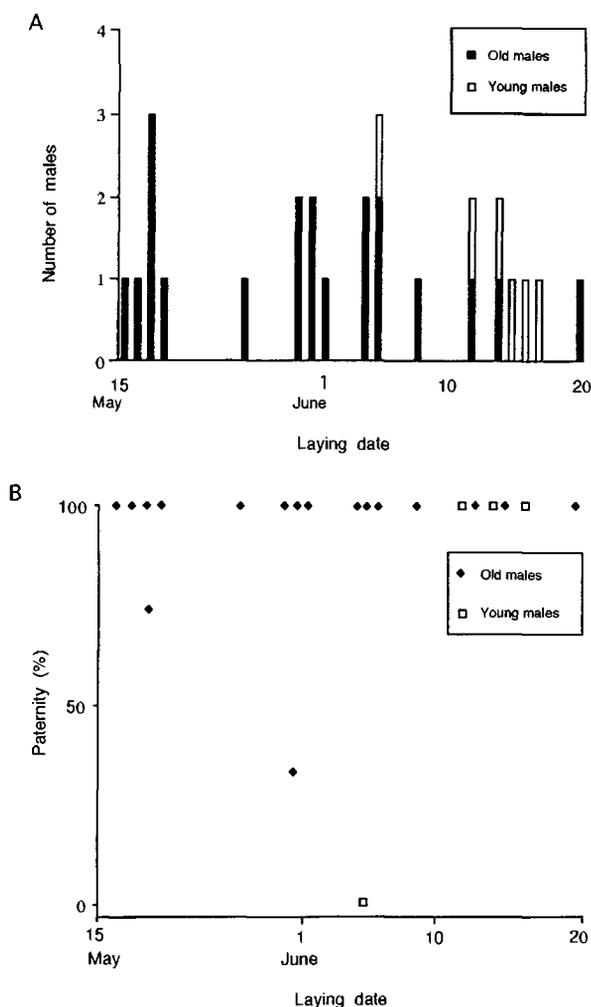


Fig. 3 **a** The distribution of egg-laying dates of the mates of old and young males in 1993, and **b** the percentage paternity achieved by old and young males plotted against laying date

manipulate laying schedules, natural variation provided this test in 1993 when there was an unusual spread of laying dates compared to other years. Whereas in 1991 and 1992, the mates of old males laid within a range of 13 and 20 days, in 1993 the range was 34 days (Fig. 3a). The standard deviation of breeding date for old males in 1993 was 10.4, higher than for any of the 14 years of records, and nearly twice the mean of 5.8 (E. Morton, unpublished work).

If the increased operational sex ratio faced by later breeders increases the risk of cuckoldry due to male control, then late breeding males were predicted to respond by increasing their mate-guarding intensity. However, the correlations of mate-guarding intensity and laying date percentile for the three years combined were not significant for young males ($r_s = 0.07$, $n = 22$, $P = 0.76$), and even suggested a negative trend for old males ($r_s = -0.35$, $n = 24$, $P = 0.097$). (The sample sizes of some age and year classes were insufficient to perform each correlation separately).

Although these results fail to support male control, they could be caused by old males guarding more intensely than young males. However, during the 15 days prior to egg-laying, old males guarded significantly *less* intensely, escorting their mates during 52.9% (SD = 24.8, $n = 33$) of female departures versus 71.6% by young males (SD = 20.2, $n = 25$, $z = 2.85$, $P = 0.004$).

The age classes of males and females can also be used to test for male versus female control. The female control hypothesis predicts that a female's receptivity to EPCs depends upon the age of her mate and not her own age. Male control predicts that inexperienced females are less able to escape forced EPCs than old females, and therefore young males are cuckolded more because they are more often paired to young females (Morton and Derrickson 1990). However, when our findings are combined with those of Morton et al. (1990), female age is shown not to be a factor. Whereas 56% (9/16) of young females had EPFs when paired with young males, 0% (0/6) did when paired with old males; likewise, only 12% (3/25) of old females had EPFs when paired with old males, but 71% (5/7) did when paired to young males ($\chi^2 = 23.1$, $df = 3$, $P = 0.0001$). We also excluded the possibility that young females were guarded more intensely, because females were guarded according to the age of the male and not the female (Table 2). The predominant effect of male age, rather than female age or laying date, on mate-guarding intensity was demonstrated in a multivariate test (Table 3).

Male age was partially controlled in 1993, allowing for another test of male versus female control. Although young males might be easier to cuckold, there

Table 2 Mate-guarding intensities of old males and young males paired with females of the same and different age classes. There was no effect of female age on mate-guarding intensity (two-way ANOVA on arcsine transformed data. $F = 0.0$, $P = 0.95$). Mate-guarding intensities were calculated for pairs in which a minimum of 10 observations were made during the 15 days prior to the laying of the first egg

Pair age class		Mean	SD	n
Male	Female			
Old	Old	50.1	28.0	23
Old	Young	56.1	15.4	10
Young	Old	71.2	22.8	9
Young	Young	72.4	19.9	15

Table 3 The effects of male age class, female age class and laying date on mate-guarding intensity (ANCOVA, $df = 55, 3$)

Variables	Std. coeff.	t -value	P	r^2	F	P
Male age class	0.423	2.50	0.02	0.17	3.6	0.02
Female age class	0.054	0.39	0.70			
Laying date	-0.042	0.24	0.39			

was an unusual shortage of young males breeding in 1993 – the percentage of breeding males comprising the young age class was only 22% (6 of 27), which was the lowest for the 14 years and only half the mean percentage of 46% (SD = 13) (Morton and Derrickson 1990; E. Morton and R. Wagner, unpublished work). Contrary to the prediction of the male control hypothesis, late-breeding old males were not cuckolded (see Fig. 3b). The mean laying date for 1993 was 3 June (SD = 10.4, range 16 May – 20 June). Of the two old males that were cuckolded, one was tied for third earliest, breeding on 18 May and the other bred on 31 May, 3 days prior to the mean laying date. The cuckolded young male bred on 4 June. Also contrary to male control, mate-guarding intensity by old males did not increase with laying date ($r_s = 0.07$, $n = 16$, $P = 0.77$).

Can males prevent their mates from obtaining EPCs?

If young males guard more than old males and yet are cuckolded more, the question is raised whether mate-guarding actually reduces cuckoldry. Figure 4 shows that paternity of young males significantly increased with mate-guarding intensity. Of the two old males that were cuckolded, one guarded less than average (37%) and the other more than average (71%).

Relative body size played a significant role in predicting the paternity achieved by young males. Whereas paternity was positively correlated with male wing-length and negatively correlated with female wing-length, the strongest morphological relationship was between paternity and the size of males relative to their mates (Table 4). The difference in wing-length between mates (male minus female wing) explained about half the variance in paternity (Fig. 5; Table 4). This suggests either that females are less receptive to EPCs when paired with large (and hypothetically high

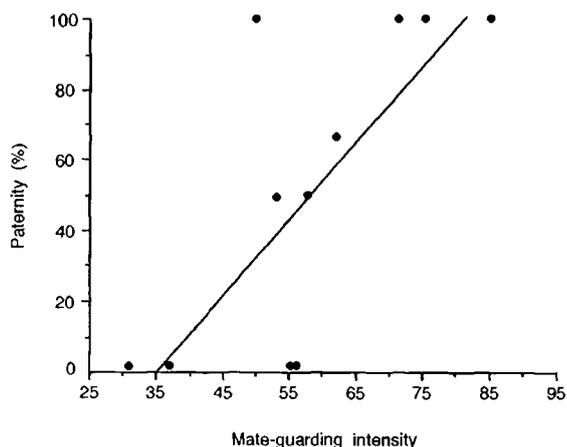


Fig. 4 The relationship between mate-guarding intensity and the paternity achieved by young males in their broods ($r_s = 0.66$, $n = 11$, $P = 0.037$)

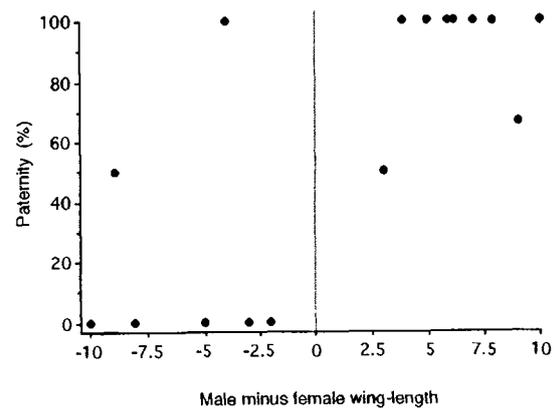


Fig. 5 The relationship between the relative size of young males to their mates (male minus female wing-length) and the paternity achieved by young males in their broods ($r_s = 0.69$, $n = 16$, $P = 0.007$). Wing difference can also be treated as a categorical variable. Young males achieved 87% (SD = 20.0, $n = 9$) paternity when they were larger than their mates, versus only 21.4% (SD = 39.3, $n = 7$; Mann-Whitney U -test, $z = 2.8$, $P = 0.005$) when they were smaller than their mates

Table 4 Body size correlates of paternity achieved by young males

Variable	n	r_s	P
Male wing	16	0.55	0.033
Female wing	17	-0.55	0.028
Male minus female wing	16	0.69	0.007
Male mass	16	0.39	0.13
Female mass	17	-0.18	0.47
Male minus female mass	16	0.49	0.058

quality) males, or males that are larger than their mates are better able to deter the female from obtaining EPCs. The latter explanation is suggested by the analyses. In a multiple regression using 16 young males, wing difference correlated with paternity but male wing did not (wing difference $t_p = 2.51$, $P = 0.026$; male wing: $t_p = 0.14$, $P = 0.89$; model $r^2 = 0.53$, $F = 7.3$, $P = 0.007$). Wing difference was also much more strongly associated with paternity than female wing (wing difference $t_p = 1.92$, $P = 0.077$; female wing $t_p = 0.14$, $P = 0.89$). A similar but weaker relationship was found using body mass. Whereas male and female body mass did not separately correlate with paternity, the difference in mass between mates correlated with paternity almost significantly (Table 4). Given that mass and wing were not correlated for young males ($r = 0.35$, $n = 16$, $P = 0.18$), the nearly significant correlation between mass difference and paternity supports the interpretation that relative body size, rather than absolute body size, is an important predictor of paternity.

There was no relationship between mate-guarding and wing difference for young males ($r_s = 0.33$, $n = 18$, $P = 0.17$) or mate-guarding and mass difference ($r_s = 0.09$, $n = 18$, $P = 0.93$). Given that mate-guarding and wing difference each explained a significant

portion of the variance in paternity while not correlating with each other, we placed both these variables in a multiple regression with paternity. Together they explained 74% of the variance in paternity of ten young males, and were largely independent of each other (mate-guarding intensity: $t_p = 2.12$, $P = 0.072$; wing difference: $t_p = 2.55$, $P = 0.038$; $F = 10.0$, $P = 0.009$). When mass difference was added to the model, only 3% more of the variance in paternity was explained.

Discussion

Evidence that EPCs are not forced

The marked relationship between male age class and paternity can be explained either by old males forcibly fertilizing the mates of young males, or by the mates of young males permitting EPCs by old males. Contrary to the male control hypothesis, the increase in the operational sex ratio later in the laying period did not result in more cuckoldry within age class then. No old males were cuckolded in 1992, despite most of those sampled breeding within several days of young males, who achieved only 50% paternity. Although this finding would not contradict male control if old males guarded more intensely, the reverse was true, with young males guarding much more intensely. While it is possible that old males are more capable than young males at mate-guarding when they escort the female, they cannot deter other males when they are apart, which is during most of their mates' visits to the ground. Although it is plausible that young females (who are more often paired to young males) are less able to avoid forced EPCs than old females, cuckoldry was unrelated to female age. Old and young females were equally likely to have EPFs when paired to young males, and equally unlikely when paired to old males. The prediction of the male control hypothesis that mate-guarding intensity increases with laying date was not met for either male age class. In another test, in 1993 when laying dates were exceptionally spread and when few young males bred, contrary to the male control hypothesis, late breeding old males were not cuckolded. We therefore conclude that extra-pair copulations are rarely or never forced, and that females paired with young males pursue a mixed mating strategy whereas females paired to old males avoid EPCs.

Mate-guarding and female receptivity to EPCs

When females seek extra-pair copulations, mate-guarding may be unrelated to paternity because females may be able to circumvent their mates' paternity defenses (Wagner 1992c). Mate-guarding intensity may even correlate inversely rather than directly with

paternity if males increase mate-guarding in response to their mates' receptivity to EPCs (Gowaty and Bridges 1991; Kempenaers et al. 1992). Between male age classes, mate-guarding was inversely correlated with paternity: old males achieved high paternity while guarding in relatively low intensities and young males achieved low paternity while guarding in higher intensities. Yet within the young male age class, paternity significantly increased with mate-guarding. This suggests that the higher mate-guarding intensities of young males were a reaction to their mates' greater receptivity to EPCs. It appears that females paired to old males were much less receptive to EPCs than those paired to young males and, consequently, old males guarded less intensely. This leads to the question of why old males guard at all. One reason is that old males are occasionally cuckolded. Another reason may be to prevent the female from being harassed during nest-building (Morton 1987).

Conflict between mates

Morton and Derrickson (1990) described what appeared to be forced copulation attempts in flight between young, but not old, males and their mates. Brown (1978) reported old males chasing their mates but did not observe aggression in flight during nest building that would suggest intra-pair chases were sexual. In contrast to both accounts, we observed many instances of old males attacking their own mates in flight. These attacks resembled EPC chases (see Brown 1978) and could easily be interpreted as such without the benefit of two observers to identify each pair member. Although it is apparent that old males were often aggressive to their mates, the reasons are unclear. One possibility is that males attempt to steer their mates from other males as a paternity guard. This may be true in some cases, however the rareness of cuckoldry for old males, combined with their relatively low mate-guarding intensities, suggest their mates were largely unreceptive to EPCs. Another possibility is that by preventing access between other males and their mates, males guard the pair bond (Wagner 1992d).

Whereas aggression by old males toward their mates may be largely directed at guarding the pair-bond, the high EPF frequencies of young males suggest that female receptivity to EPC is the main cause of within-pair aggression for them. This could explain the interesting correlation between relative body size and paternity of young males. If absolute body size were the stronger correlate of paternity, it could have suggested that females preferred larger males. If this were so, then the age-related pattern of cuckoldry could be due more to size than age, because old males in our sample were larger than their mates more often than were young males. However, if larger

body size was attractive to females such that they sought EPCs when paired to small males, then the females' own size should not have correlated with paternity. Yet, while male wing was positively correlated with paternity, female wing was also correlated with paternity, but negatively. In a multiple regression wing difference, rather than male or female wing, explained a high percentage of the variance in paternity. Most of the remaining variance in paternity was explained by mate-guarding, which was not correlated with wing difference.

The question is then raised whether old males achieve nearly complete paternity because their mates are unreceptive to EPCs, or because they are better able to control their mates through their greater size. The latter is unlikely because, as noted, old males did not escort their mates during most of their departures. In order for the size difference to explain the rarity of EPFs, it would be necessary for old males to somehow intimidate their mates from accepting EPCs, even in their absence. It is more likely that females paired to old males were much less receptive to EPCs than females paired to young males. However, the occurrence of within-pair aggression and the existence of EPFs suggests either that females paired to old males sometimes also pursue a mixed mating strategy, or the ability of females to resist aggressive EPC attempts is not absolute.

Female control and sex ratio

It is interesting how often females paired to young males obtain extra-pair fertilizations despite their mates' attempts to prevent them. Although young males might avoid cuckoldry by breeding away from colonies, Morton et al. (1990) observed that young males were unable to attract females in the absence of other males. A factor that could provide females with the ability to draw young males into colonies is a surplus of young males. In our colony, there are several young males each year that defend nesting cavities but fail to acquire mates, whereas virtually all females and old males find mates. In 14 years, only 6 of 167 (3.6%) old males trapped were unmated versus 23 of 179 (12.9%) of young males and 2 of 302 females (E. Morton and R. Wagner, unpublished work). In 1992, a year of detailed records, 20 young males bred but at least 7 more occupied nesting cavities and failed to find a mate. As the number of females choosing mates increases, so does the skew in the sex ratio, such that the last five females, for example, could choose among as many as 12 males, a sex ratio of 2.4:1. Thus, the mixed mating strategies of females could contribute to colony formation if females exploit this imbalance to force young males to breed near old males and share paternity. Given male-biased skews in the tertiary sex ratios of many species (reviewed by Breitwisch 1989),

the ability of females to force males to breed near preferred males may be widespread. The hidden lek hypothesis predicts that this can contribute to colony formation in species that do not defend feeding territories, as well as cause the clumping of all-purpose territories (Wagner 1993, 1996).

How do females benefit from EPCs?

Given that many female martins accept EPCs, it is important to determine how females may increase their fitness by pursuing a mixed mating strategy. Three hypothetical benefits that have received attention are "good genes", genetic diversity, and insurance against male infertility (reviewed by Westneat et al. 1990; Birkhead and Møller, 1992). Whereas the insurance hypothesis predicts a high percentage of females engaging in EPCs but a low percentage actually being fertilized by them, in martins a high, rather than a low percentage of females were fertilized by EPCs. Furthermore, we would expect insurance to be pursued equally by all females, yet mainly females paired with young males accepted EPCs. It is possible that young males suffer a higher incidence of sterility. However this is unlikely because old and young males paired to old females had similar hatching success (E. Morton and R. Wagner, unpublished data). Also arguing against the insurance hypothesis is that half of the cuckolded males achieved partial paternity, indicating that they were not sterile.

If females sought genetic diversity we would not expect only females paired to young males to accept EPCs; the only rule for females is to copulate with additional males of no particular phenotype. Also arguing against genetic diversity is that unlike in tree swallows *Tachycineta bicolor* (Dunn et al. 1994), EPFs were usually achieved by only one male per nest.

"Good genes" is often proposed as a benefit of EPCs to females (Møller 1990; Kempnaers et al. 1992; Houtman 1992; Graves et al. 1993). The behavior of female martins suggests they may have sought good genes because older males have proven their quality by surviving longer. Older males also possess much brighter plumage, which could advertise for parasite resistance (Hamilton and Zuk 1982). Blood parasites are prevalent in purple martins, and the lower frequencies of infection of young birds may reflect higher mortality because of their immunological naiveté (Davidar and Morton 1992). Females paired to young males therefore, may prefer to be fertilized by old males because they have been tested for genetic resistance to blood parasites.

Conclusions

Our main conclusions are: (1) EPCs are not forced but require the cooperation of the female, (2) females accept

EPCs when paired with young but not old males, and (3) the ability of females to obtain EPCs is relative to their mates' ability to prevent them from encountering other males, which is determined by mate-guarding intensity and body size differences.

With the evidence that females pursue a mixed mating strategy, the conditions required by the hidden lek hypothesis to explain colony formation exist in the purple martin colony. The hypothesis predicts that males cluster in response to females seeking extra-pair copulations. The basis for this prediction is the observation that when females of monogamous species seek EPCs, the same mechanisms that produce leks operate. The skew in extra-pair fertilization success among old males matches the skew in male mating success typical of leks. Old males cluster in the colony and apparently recruit young males and females to breed near them. Old males then obtain EPCs from females paired to young males. The male-biased sex ratio may give females leverage with young males, forcing them to join colonies to find mates.

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