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Genetic evidence of multiple parentage in broods of cliff swallows

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Summary. Parental exclusion analyses based on allozyme data were performed on 105 families of cliff swallows (*Hirundo pyrrhonota*) from southwestern Nebraska, USA. The protein products of seven polymorphic loci were resolved from blood, and at least one parental exclusion occurred at six of these loci. One or both putative parents were excluded for 35 nestlings from 22 different families. The mean number of non-kin nestlings in these families was 1.59. Non-kin nestlings were found in families with brood sizes ranging from two to five. A greater percentage of families in an 1100-nest colony had non-kin offspring than in two smaller colonies, although the difference was not statistically significant. Application of genetic models to these data and the observed distribution of parental exclusions suggested that multiple parentage in cliff swallows results more often from intraspecific brood parasitism than from forced extra-pair copulations. Based on the calculated probabilities of detecting non-kin, we estimate that 23.7% of all nestlings in our population are not the offspring of one or both of their putative parents. We estimate that about 43% of all cliff swallow nests in Nebraska contain at least one offspring resulting from intraspecific brood parasitism, and that about 6% of nests might contain offspring resulting from extra-pair copulations.

Introduction

There is increasing evidence that animals sometimes invest parental care in unrelated offspring. Parasitism of parental care can occur in two major

ways. A male may be cuckolded and hence the offspring he cares for may not be his own, or (in egg-laying animals) another individual's egg may be added to a clutch to be cared for by the unwitting and often unrelated "hosts" of either sex. Among birds, there is evidence of both extra-pair copulations (which can lead to cuckoldry) and parasitic egg-laying by conspecifics (e.g., Gladstone 1979; Yom-Tov 1980; Power et al. 1981; Ford 1983; McKinney et al. 1983; Andersson 1984; Brown 1984; Birkhead et al. 1985; Gavin and Bollinger 1985; Emlen and Wrege 1986; Gibbons 1986; Frederick 1987; Moller 1987a; Westneat 1987a; Hoffenberg et al. 1988; Brown and Brown 1989). Some researchers have used observational methods alone to study these phenomena, and this approach has yielded much useful information. However, observational methods are limited in that they usually cannot measure the actual frequency and success of alternative reproductive tactics. For example, Emlen and Wrege (1986) note that forced extra-pair copulation attempts in birds are dramatic, conspicuous events and that this conspicuousness may have led investigators to an inflated impression of these events' genetic importance. In white-fronted bee-eaters (*Merops bullockoides*) in Kenya, extra-pair copulations may represent at most only a 5% probability of fertilization for a given female per nesting attempt (Emlen and Wrege 1986). To evaluate to what extent extra-pair copulations successfully lead to fertilization and result in offspring, one must perform genetic analyses of offspring and putative parents (Sherman 1981; Westneat et al. 1987). Similarly, estimates of the frequency of intraspecific brood parasitism, based solely on observational methods, tend to grossly underestimate parasitism's frequency (Brown and Brown 1989). Electrophoretic analyses of allozyme variation enable us to at least partially

estimate the degree of brood parasitism overlooked by more conventional ornithological methods.

Standard starch-gel electrophoresis of allozymes has proven useful in investigating genetic relationships among individuals in broods or litters in a variety of animals (e.g., McCracken and Bradbury 1977; Foltz and Hoogland 1981; Hanken and Sherman 1981; Gowaty and Karlin 1984; Gavin and Bollinger 1985; Joste et al. 1985; Evarts and Williams 1987; Westneat 1987b; Wrege and Emlen 1987; but see Mumme et al. 1985 and Hoffenberg et al. 1988). Electrophoretically distinct allozymes permit paternity and maternity "exclusion" analyses and reveal which offspring are unrelated to either or both parents. Recent development and refinement of genetic models to estimate the frequency of and probability of detecting non-kin offspring (Westneat et al. 1987; Wrege and Emlen 1987), given known proportions of alleles in the population, should facilitate electrophoretic studies of parentage. These models and the use of DNA fingerprinting techniques to assign precise genetic relationships within families (Jeffreys et al. 1985; Wetton et al. 1987) may soon have a major impact on sociobiology.

For the last six years we have been studying reproductive strategies in colonially nesting cliff swallows (*Hirundo pyrrhonota*). Intraspecific brood parasitism is common in cliff swallows, occurring when females lay eggs in other individuals' nests (Brown 1984; Brown and Brown 1989) and when birds physically transfer eggs into nearby nests (Brown and Brown 1988). Based on observations of color-marked birds parasitizing nests and by checking for irregularities in egg-laying sequences, we estimated that at least 22% of all nests contain one or more parasitic eggs (Brown and Brown 1989). Extra-pair forced copulation attempts also occur commonly in cliff swallows, both while birds gather mud away from the colony sites and among birds in neighboring nests at the colonies (Butler 1982; Brown 1985). We undertook an analysis of parentage in cliff swallow broods, using allozyme variation observable in blood, (1) to supplement and if necessary revise accordingly our estimates of the frequency of brood parasitism previously based on observational methods alone (Brown and Brown 1989), and (2) to evaluate the potential success of forced extra-pair copulation attempts and the degree to which male cliff swallows may be cuckolded. Knowing how often these reproductive strategies lead to genetic payoffs is crucial in understanding how parasitism of parental care may have evolved in birds.

Cliff swallows are small migratory passerines

that nest in colonies throughout much of western North America. The species' general biology has been well studied (Emlen 1941, 1952, 1954; Mayhew 1958; Samuel 1971; Grant and Quay 1977; Withers 1977; Brown 1985). Cliff swallows build gourd-shaped nests out of mud pellets that are attached underneath overhanging rock ledges on the sides of cliffs and canyons. They feed exclusively on insects caught in flight, and colonies serve as centers in which individuals acquire information from other birds on the location of food sources (Brown 1986, 1988). The birds are highly social in all of their activities, feeding, preening, mud-gathering, and loafing in large groups (Emlen 1952; Brown 1985). Cliff swallows are usually single-brooded, and hematophagous ectoparasites are responsible for much of the observed nestling mortality (Brown and Brown 1986).

Methods

Study site

This study, part of continuing long-term research on the social behavior of cliff swallows, was done in Keith and Garden cos., Nebraska, USA, near the University of Nebraska's Cedar Point Biological Station, from May to August, primarily in 1986–87. Cliff swallows are abundant in Nebraska and have probably occurred there at least since before European settlement of North America (e.g., Nichols, cited in Pearson 1917). We studied colonies that were located on structures such as bridges and highway culverts and on cliff sites along the south shore of Lake McConaughy. Since 1982 there have been 276 cliff swallow colonies totalling 97980 nests in or near the study area (Brown 1985). Colony size ranged from 2 to approximately 3500 nests (\bar{X} = 355 nests, SD = 561), and birds also nested solitary. The most common colony size was about 400 nests.

Capturing birds and sampling blood

Parental cliff swallows were caught inside their nests for the taking of blood samples. Both the male and female of each pair sleep together in the nest beginning as soon as they arrive in the study area and continuing until nestlings approach fledging, as in other swallows (Brown 1980). If we found only one parent present in a nest at night (only a few cases), we excluded that family from the data set. We entered culverts containing cliff swallow nests about two hours after sunset (about 22:00 MDT) and plugged with cotton the narrow entrances of the birds' gourd-shaped nests. We returned to the colony at dawn the next day and removed the adults inside the plugged nests. If we entered the colony quietly and without lights, it was possible to plug nests and exit without any disturbance to the colony. In rare instances inadvertent noises by us flushed one or several adults out of their nests at night, but these birds apparently later returned with no ill effects on their nesting activity. Our selection of nests to plug and hence families to sample was determined largely by nocturnal accessibility. In the two smaller colonies studied, mud and standing water underneath the nests prevented us from reaching at night, quietly, roughly one-half

Table 1. Allele frequencies, total exclusions per locus, and probabilities of detecting an exclusion (for the extra-pair copulation and brood parasitism models) for seven polymorphic loci found in blood of Nebraska cliff swallows

| Locus | No. of alleles | Allele frequencies ($n = 1200$) | Total exclusions at locus | Total probability of detecting an exclusion at locus | |
|--|----------------|-----------------------------------|---------------------------|--|------------|
| | | | | (EPC model) | (BP model) |
| Glucosephosphate isomerase (gpi) | 4 | 0.959, 0.015, 0.013, 0.013 | 1 | 0.0400 | 0.0760 |
| Mannosephosphate isomerase (mpi) | 5 | 0.955, 0.018, 0.011, 0.011, 0.005 | 7 | 0.0438 | 0.0829 |
| Phosphogluconate dehydrogenase (pgd) | 3 | 0.984, 0.008, 0.008 | 0 | 0.0159 | 0.0310 |
| Malate dehydrogenase-1 (mdh-1) | 2 | 0.955, 0.005 | 1 | 0.00495 | 0.00973 |
| Malate dehydrogenase-2 (mdh-2) | 3 | 0.991, 0.007, 0.002 | 1 | 0.00892 | 0.0175 |
| Isocitrate dehydrogenase (idh) | 3 | 0.972, 0.022, 0.006 | 8 | 0.0272 | 0.0527 |
| Peptidase with glycyl-leucine (pep-gl) | 5 | 0.833, 0.117, 0.031, 0.012, 0.007 | 19 | 0.146 | 0.235 |

of each colony. In these cases all nests plugged were from the half of the colony located over relatively dry land. In the third colony all nests were located over flowing water, and we selected nests as randomly as possible in the dark from all parts of the colony.

Nocturnal capture of parents occurred in June and did not commence until birds had full clutches or until after eggs had hatched. Cliff swallows deserted their nests if captured at night prior to completion of egg-laying, but after that time desertions were very rare. Parents were removed from nests by placing a mesh bag attached to a metal ring around the outside of each nest's entrance. Birds would usually fly out into the bag as soon as the cotton was removed from the entrance. If individuals were reluctant to emerge, small portions of the nest's neck were chipped off and the adults removed by hand. These minor alterations to the nests had no effect (Brown and Brown 1986, 1987), especially since we rebuilt most of the damaged nests with mud. The maximum number of nocturnal visits necessary to capture parents at any one colony was three, and fewer visits were needed at two of the colonies. Colonies sampled and their total sizes were ones of 140 active nests in 1986 and ones of 340 and 1100 active nests in 1987. The former two colonies were at the same site located in Garden Co., and the latter colony was located about 45 km away in Keith Co. All cliff swallow families for which data are reported here inhabited nests that were periodically fumigated to remove the effects of ectoparasites (Brown and Brown 1986). This led to the nestlings in the sampled nests probably being in above average physical condition and may have reduced potential nestling mortality that might have occurred after hatching and before nestling blood samples were taken. After sampling blood from both parents at a nest, we later returned to that nest (during daylight) when the nestlings were between 12 and 21 days of age. At that time nestlings were temporarily removed for blood-sampling. Nestling cliff swallows at these ages appeared fully homeothermic. Status of all nests was monitored regularly throughout the season using a dental mirror and flashlight inserted through each nest's neck (see Brown and Brown 1986).

An initial group of 40 adult cliff swallows was captured at a 750-nest colony in July 1986 (the same Keith Co. site occupied by the 1100-nest colony sampled in 1987) for a preliminary survey of allozyme variability. These individuals were captured in mist nets strung across the culvert entrances. The birds in this initial sample were residents in the colony but were an apparently random sample of the study population in all other respects.

Parental cliff swallows were sexed by cloacal protuberance, and all adults and nestlings were weighed and banded with United States Fish and Wildlife Service bands. Blood samples were taken by brachial vein puncture using a lancet and heparinized capillary tubes. We attempted to collect about 0.1 ml of blood from each individual. Birds were held until the wound was fully sealed, and clotting was enhanced in some cases by applying an ice cube to the wound area. There was no evidence of any mortality resulting from blood collection from either adults or nestlings. All nestlings sampled either fledged or fell victim to unrelated events such as nests' falling from the substrate. Many adults were captured again a day to a month or more after sampling, and in virtually all cases there was no visible evidence of a wound. In only one case was there any indication that blood collection caused a parent to desert its nest. Capillary tubes containing blood were placed immediately after collection into an ice cooler and transferred to liquid nitrogen within six hours. In 1986 we centrifuged all samples and separated red cells and plasma prior to freezing. Since no polymorphic loci were found in plasma, in 1987 we did not centrifuge samples prior to freezing. Samples were eventually transferred directly to an ultralow (-70°C) freezer at Cornell University for storage.

Allozyme analysis

Electrophoresis was done at Cornell's Laboratory for Ecological and Evolutionary Genetics under the direction of B. P. May. Blood samples were analyzed and interpreted following standard horizontal starch-gel electrophoretic procedures given in May et al. (1979) and May (1980). Using our initial 40-bird sample, the following loci were tested and found for cliff swallows to be monomorphic, to exhibit poor resolution, or to otherwise be unusable: *ada*, *ak-1*, *ak-2*, *aat*, *ck*, *dia*, *est-A*, *est-B*, *gdh-nad*, *gpt*, *gr*, *gapdh*, *g2dh*, *g3p*, *gda*, *ldh-1*, *me*, α -*man*, *mup*, *np*, *pgm*, *pgk*, *pro-1*, *pro-3*, *sod*, and *tpi*. Seven loci were found to be polymorphic (Table 1), and these loci were scored for all 105 cliff swallow families for which we had complete samples. Electrophoretic genotypes were determined for a total 600 individuals. At each of the seven loci parental and offspring genotypic frequencies corresponded to that expected under Hardy-Weinberg equilibrium (χ^2 test, $P > 0.10$ for each locus). There was no indication that these loci were linked in any way. In all cases where initial scoring of gels revealed an offspring with a genotype in any way inconsistent with its putative par-

ents', that offspring and the parents were re-analyzed and re-scored with reference to a standard. In only one case was the initially inconsistent score reversed upon re-examination.

Theoretical models

Westneat et al. (1987) developed genetic models to calculate the probabilities of detecting offspring unrelated to their putative parents ("non-kin" offspring), based on observed allele frequencies. These models allow one to partition detection probabilities into specific exclusion categories, assuming either extra-pair copulations or intraspecific brood parasitism as the sole sources of non-kin. This leads to a predicted distribution of exclusions in different categories for each model. For the extra-pair copulation model, Westneat et al. (1987) present general equations for n alleles. For the brood parasitism model, however, Westneat et al.'s equations apply only to two-allele systems. Most of our polymorphic loci had more than two alleles (Table 1). Thus, for brood parasitism we used equations for three alleles developed and supplied to us by D.F. Westneat (personal communication). The three-allele equations for brood parasitism were contained in a Basic computer program, and this program furnished by Westneat was used for all calculations for both the extra-pair copulation and brood parasitism exclusion models. For our loci with more than three alleles, we lumped the rarest ones together in order to use Westneat's equations (see Westneat et al. 1987).

Results

Parental exclusions

Genotypes were determined for 349 nestling cliff swallows; 35 (10.0%) exhibited genotypes that excluded as real parents one or both of their putative parents. Genotypes of these nestlings and their putative parents are listed in the Appendix. In all but three cases, the exclusion was ambiguous; either the putative mother or father was excluded but it was not possible to know which (see Appendix). These 35 nestlings were distributed among 22 families; 21.0% of all families sampled ($n = 105$) had one or more non-kin offspring. The mean number of non-kin offspring among these 22 families was 1.59 (SD = 0.73). There were 11 families with more than one non-kin offspring; in two of these (containing two and four nestlings, respectively) *all* nestlings were of non-kin genotypes. Five three-nestling broods and four four-nestling broods each had two non-kin nestlings represented. At least one exclusion of a parent occurred at six of the seven polymorphic loci, with *idh* and *pep-gl* having the majority of exclusions (Table 1). Thirty-three exclusions were based on an inconsistency at one locus; the remaining two exclusions were based on inconsistencies at two loci each.

Exclusions occurred in families with brood sizes ranging from two to five. The number of families with exclusions and the total number of fami-

lies of that size sampled were: 0 of 6 for broods of one nestling; 2 of 13 for broods of two nestlings; 9 of 32 for broods of three nestlings; 10 of 49 for broods of four nestlings; and 1 of 5 for broods of five nestlings. Brood size did not affect the likelihood of a family having a detectable non-kin offspring ($\chi^2 = 2.84$, $df = 4$, $P = 0.59$). In the 140-nest colony, four families (of 27 sampled; 14.8%) had at least one non-kin offspring; in the 340-nest colony, four families (of 26 sampled; 15.4%) had at least one non-kin offspring; and in the 1100-nest colony, 14 families (of 52 sampled; 26.9%) had at least one non-kin offspring. Among these families, the mean number of non-kin offspring per family was 1.50 (SD = 0.58) in the 140-nest colony; 1.75 (SD = 0.50) in the 340-nest colony; and 1.57 (SD = 0.85) in the 1100-nest colony. Although there was a trend for the percentage of families with non-kin offspring to increase with colony size for these three colonies, the trend was not significant ($\chi^2 = 2.21$, $df = 2$, $P = 0.33$).

Extra-pair copulations or brood parasitism

In most of the cases in which a putative parent was excluded (32 of 35), the exclusion was ambiguous with respect to either the mother or father. This meant that definite exclusion of either paternity and/or maternity was not possible for most of the families with non-kin offspring. We therefore applied Westneat et al. (1987)'s models and their extension to three-allele systems (Westneat personal communication), to estimate the probability of detecting a non-kin offspring given our allele frequencies and to predict the kind of parental exclusions we should observe. The probability of detecting a non-kin individual at each polymorphic locus is given in Table 1, assuming either that all non-kin result solely from extra-pair copulations (cuckoldry) or that all non-kin offspring result solely from intraspecific brood parasitism. Combining the probabilities for all loci and for all specific types of parental exclusions (using the equation in Westneat et al. 1987), the overall probabilities of detecting non-kin offspring in our sample of birds are 0.260 if non-kin result solely from extra-pair copulations and 0.421 if non-kin result solely from brood parasitism.

We partitioned the probabilities of detection into specific exclusion categories for the extra-pair copulation and brood parasitism models (see Westneat et al. 1987), leading to the total detection probability (combined from all loci) for each category of each model (Table 2). The proportions of exclusion types follow from the total detection

Table 2. Partitioning of the detection probabilities into probabilities for each type of exclusion (based on the data in Table 1) and observed versus expected distributions of exclusion types

| | Male only | Female only | Both male and female | Ambiguous exclusion |
|--------------------------------------|-----------|-------------|----------------------|---------------------|
| <i>Extra-pair copulation model</i> | | | | |
| Probability of detection | 0.0508 | – | – | 0.216 |
| Proportion of exclusions | 0.189 | 0.0 | 0.0 | 0.811 |
| Expected no. exclusions ($n = 35$) | 6.6 | 0.0 | 0.0 | 28.4 |
| Observed no. exclusions | 1 | 0 | 2 | 32 |
| <i>Brood parasitism model</i> | | | | |
| Probability of detection | 0.0304 | 0.0304 | 0.0202 | 0.361 |
| Proportion of exclusions | 0.0687 | 0.0687 | 0.0458 | 0.817 |
| Expected no. exclusions ($n = 35$) | 2.4 | 2.4 | 1.6 | 28.6 |
| Observed no. exclusions | 1 | 0 | 2 | 32 |

probabilities for each category (Table 2). Given that we observed 35 exclusions, we can generate an expected distribution of exclusion types if exclusions result solely from either extra-pair copulations or brood parasitism (Table 2; also see Westneat et al. 1987).

The observed distribution of parental exclusions (Table 2) differed significantly from that expected if exclusions resulted solely from extra-pair copulations ($\chi^2 = 5.21$, $df = 1$, $P = 0.022$). The observed distribution of parental exclusions did not differ significantly from that expected if exclusions resulted solely from brood parasitism ($\chi^2 = 2.21$, $df = 1$, $P = 0.137$; three of the categories were lumped to achieve an expected frequency > 5). Although the number of unambiguous exclusions detected was quite small, evidence suggests that non-kin offspring among cliff swallow broods may result from intraspecific brood parasitism more often than from extra-pair copulations.

Frequency of multiple parentage

Allozyme analyses indicated that 35 nestlings from 22 nests were unrelated to either the putative mother or father. If we assume that these non-kin offspring result from intraspecific brood parasitism (see above), the overall probability of detecting non-kin is 0.421. A genetic estimate of the actual frequency of non-kin offspring can be obtained by dividing the observed frequency (0.10 of all offspring) by the probability of detection (Westneat et al. 1987). This leads to an estimate that 23.7% of all nestling cliff swallows in our population are unrelated to one or both of their putative parents. If non-kin nestlings are distributed among families in the observed ratio (35:22), we can estimate that 52 families in our sample of 105 actually contained at least one non-kin offspring. Thus, an estimated

49.5% of cliff swallow nests in Nebraska may contain at least one offspring unrelated to one or both of its putative parents.

A second way to estimate the actual frequency of brood parasitism is to combine independently derived empirical frequencies based on nest check data, observations of color-marked birds parasitizing nests, and allozyme data. We previously estimated that approximately 22% of cliff swallow nests contain at least one parasitic egg, based solely on sequences of egg-laying as determined by nest checks and on actual sightings of birds laying parasitic eggs (Brown and Brown 1989). Of the 22 families with electrophoretic parental exclusions, 19 had no evidence of a parasitism in their nest based on nest checks or on observations of egg-laying. Of the 105 total families which were electrophoretically analyzed, 91 had no evidence of a parasitism in their nest based on nest checks or on observations of egg-laying. Thus, 19 of 91 nests (20.9%) had allozymic evidence of parasitism which otherwise would have gone undetected. Combining this estimate and the one derived from observational and nest check data – 22% (Brown and Brown 1989) – we conclude that approximately 43% of cliff swallow nests in our population may contain at least one offspring unrelated to either or both of its putative parents.

We tend to believe that this latter estimate of the percentage of cliff swallow nests with at least one parasitic egg (43%) may be more accurate because it is based on four different methods of assessing parasitism (see Brown and Brown 1989). A potential estimate of the number of nests with at least one offspring resulting from extra-pair copulations, therefore, is the difference between the 49.5% estimate of parasitism (which is based on the probably incorrect assumption that *all* non-kin offspring result from parasitism) and the 43% fig-

ure (the more accurate estimate of parasitism). This leads to an estimated 6.5% of all nests with one or more offspring resulting from extra-pair copulations.

Discussion

Our allozyme analyses indicate that multiple parentage in cliff swallow broods is common. Nearly a quarter of all nestlings distributed among almost half of all nests are genetically unrelated to one or both of the adults tending the nest. Comparison of observed types of exclusions with those expected from the extra-pair copulation model versus the intraspecific brood parasitism model suggests that non-kin offspring result more from brood parasitism than from cuckoldry. Because of the relatively small numbers in the observed and expected categories of these models (other than "ambiguous exclusions"; Table 2), this comparison *per se* would be unconvincing in discriminating between potential sources of non-kin. But given the previously documented occurrence of intraspecific brood parasitism in Nebraska cliff swallows (Brown 1984; Brown and Brown 1988, 1989), the agreement between the observed distribution of exclusions and that expected from brood parasitism is not surprising and suggests that the electrophoretic exclusions probably reflect mostly brood parasitism.

The observed distribution of exclusions (Table 2) provides little evidence that extra-pair copulations in cliff swallows regularly lead to fertilizations and "bastard" offspring, although because of the small number of unambiguous exclusions we cannot rule out extra-pair copulations entirely. We emphasize that our estimated 6.5% of nests with non-kin offspring resulting from extra-pair copulations is tentative and based on little data. Forced extra-pair copulations seem to occur relatively often in cliff swallows (Butler 1982; Brown 1985), but they may represent only a tiny fraction of the total copulations a female receives. Male cliff swallows copulate repeatedly with their mates during nest-building and egg-laying (Brown and Brown personal observation). Although we have not yet quantified the percentage of total copulations that occur between a female cliff swallow and her mate, we agree with Emlen and Wrege (1986) that investigators have probably overemphasized the genetic importance of forced extra-pair copulations. Even studies that have used allozymic exclusion analyses or other experimental techniques (e.g., vasectomies) to conclude that cuckoldry is important, did not thoroughly rule out intraspecific brood parasitism as a source of the observed

non-kin offspring (Bray et al. 1975; Gavin and Bollinger 1985; Evarts and Williams 1987). Only Westneat (1987b) has shown clearly and with adequate sample sizes that extra-pair copulations regularly lead to fertilizations and bastard offspring in the absence of brood parasitism.

There is no evidence whatsoever that male cliff swallows ever guard their mates away from the nest (Brown 1985, personal observation). Males instead devote their time to guarding of their nests, probably in response to the threat of brood parasitism. If, as our data may suggest, cuckoldry in fact occurs relatively rarely in cliff swallows – a species that does not mate-guard – guarding of mates as a principal strategy to insure avian paternity may also be generally overemphasized. At the very least, our results suggest that genetic exclusion analyses are essential before any conclusions can be made about mate-guarding and its relationship to cuckoldry. Observational studies of extra-pair copulations and mate-guarding (e.g. Moller 1985, 1987b, 1987c) must be viewed as inconclusive until the necessary exclusion analyses are undertaken and the effect of intraspecific brood parasitism on any observed parental exclusions is evaluated.

A potential cost of colonial breeding is an increased risk of intraspecific brood parasitism and, for males, cuckoldry (Hoogland and Sherman 1976; Wittenberger and Hunt 1985). Our allozyme data are consistent with the prediction that brood parasitism might increase with colony size. The 1100-nest colony had a higher frequency of families with non-kin offspring than the two smaller colonies (of 140 and 340 nests), although the differences between sites were not statistically significant. Other data (e.g., Brown 1984; Brown and Brown 1988, 1989) also indicate an effect of colony size on incidence of brood parasitism. The exclusion data reported here revise upward the estimate of brood parasitism's frequency in cliff swallows based on observational data, but do not change our conclusions about the risk-spreading advantages of parasitism in this species (Brown and Brown 1989).

The limitations of using electrophoretic protein analyses to study parentage have been well described (Mumme et al. 1985; Westneat 1987b; Westneat et al. 1987; Wrege and Emlen 1987; Hoffenberg et al. 1988). Our results underscore these limitations. We had only seven weakly polymorphic loci upon which to base our analyses, and we undoubtedly missed detecting many additional cases of multiple parentage. Most of our exclusions were ambiguous with respect to either the mother or father. Nevertheless, despite the relatively low

level of detectable genetic variability in cliff swallows, we still found 35 parental exclusions. We had a higher frequency of detectable non-kin offspring than in previous studies on colonial birds and a frequency comparable to that of Westneat's (1987b) for a noncolonial bunting. If used with theoretical models (Westneat et al. 1987) to predict expected distributions of exclusions and in populations without high degrees of relatedness among potential breeders (cf. Mumme et al. 1985), we are optimistic that genetic exclusion analyses may have much value and applicability to many species of animals in which alternative reproductive strategies are suspected to occur.

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Appendix

Electrophoretic genotypes of non-kin nestling cliff swallows and their putative parents, total number of nestlings per family, and colony of occurrence. Colony A consisted of 140 total nests; Colony B of 340 total nests; and Colony C of 1100 total nests. Loci at which parental exclusions occurred are shown in **boldface**. Numbers for each locus refer to the relative mobility of proteins on starch gel and hence the two putative alleles at that locus.

| Colony | Family | Total no. nestlings in family | Individual | Genotype | | | | | | |
|--------|--------|-------------------------------|------------|----------|-----------|-----|------|-----|-----------|-----------|
| | | | | Gpi | Mpi | Pgd | Mdh1 | Idh | PepGI | Mdh2 |
| A | 187 | 4 | mother | 14 | 11 | 11 | 12 | 11 | 11 | 11 |
| | | | father | 11 | 11 | 11 | 11 | 11 | 11 | 11 |
| | | | nestling | 11 | 11 | 11 | 12 | 11 | 15 | 11 |
| A | 282 | 4 | mother | 11 | 11 | 11 | 11 | 11 | 11 | 11 |
| | | | father | 11 | 11 | 11 | 11 | 11 | 11 | 11 |
| | | | nestling | 11 | 11 | 11 | 11 | 11 | 12 | 11 |
| A | 222 | 4 | mother | 11 | 11 | 11 | 11 | 11 | 12 | 11 |
| | | | father | 11 | 11 | 11 | 11 | 11 | 12 | 11 |
| | | | nestling | 11 | 11 | 11 | 11 | 11 | 12 | 12 |
| A | 192 | 4 | mother | 11 | 11 | 11 | 11 | 11 | 11 | 11 |
| | | | father | 11 | 11 | 11 | 11 | 11 | 11 | 11 |
| | | | nestling | 11 | 11 | 11 | 11 | 11 | 13 | 11 |
| B | 3 | 4 | mother | 11 | 11 | 11 | 11 | 11 | 11 | 11 |
| | | | father | 11 | 11 | 11 | 11 | 11 | 11 | 11 |
| | | | nestling | 11 | 11 | 11 | 11 | 11 | 13 | 11 |
| B | 229 | 3 | mother | 11 | 14 | 11 | 11 | 11 | 11 | 11 |
| | | | father | 11 | 11 | 11 | 11 | 11 | 11 | 11 |
| | | | nestling | 11 | 11 | 11 | 11 | 11 | 13 | 11 |
| B | 363A | 3 | mother | 11 | 11 | 11 | 11 | 11 | 11 | 11 |
| | | | father | 11 | 11 | 11 | 11 | 11 | 14 | 11 |
| | | | nestling | 11 | 11 | 11 | 11 | 11 | 12 | 11 |
| B | 367 | 5 | mother | 11 | 11 | 11 | 11 | 13 | 11 | 11 |
| | | | father | 11 | 11 | 11 | 11 | 11 | 11 | 11 |
| | | | nestling | 11 | 11 | 11 | 11 | 11 | 12 | 11 |
| C | 824 | 3 | mother | 11 | 11 | 11 | 11 | 11 | 11 | 11 |
| | | | father | 11 | 11 | 11 | 11 | 11 | 11 | 11 |
| | | | nestling | 11 | 11 | 11 | 11 | 11 | 13 | 11 |
| | | | nestling | 11 | 11 | 11 | 11 | 11 | 12 | 11 |

| Colony | Family | Total no. nestlings in family | Individual | Genotype | | | | | | |
|--------|--------|-------------------------------|------------|-----------|-----------|-----|-----------|-----------|-----------|------|
| | | | | Gpi | Mpi | Pgd | Mdh1 | Idh | PepGl | Mdh2 |
| C | 358 | 3 | mother | 11 | 11 | 11 | 11 | 11 | 11 | 11 |
| | | | father | 11 | 11 | 11 | 11 | 11 | 11 | 11 |
| | | | nestling | 11 | 13 | 11 | 11 | 11 | 15 | 11 |
| C | 351 | 4 | mother | 11 | 11 | 11 | 11 | 11 | 11 | 11 |
| | | | father | 11 | 11 | 13 | 11 | 11 | 11 | 11 |
| | | | nestling | 11 | 14 | 11 | 12 | 11 | 11 | 11 |
| C | 224 | 4 | mother | 11 | 11 | 11 | 11 | 11 | 11 | 11 |
| | | | father | 11 | 13 | 11 | 11 | 11 | 11 | 11 |
| | | | nestling | 11 | 11 | 11 | 11 | 11 | 22 | 11 |
| | | | nestling | 11 | 11 | 11 | 11 | 11 | 12 | 11 |
| | | | nestling | 11 | 11 | 11 | 11 | 11 | 12 | 11 |
| | | | nestling | 11 | 11 | 11 | 11 | 11 | 22 | 11 |
| C | 777 | 2 | mother | 11 | 11 | 11 | 11 | 11 | 11 | 11 |
| | | | father | 13 | 11 | 11 | 11 | 11 | 12 | 11 |
| | | | nestling | 11 | 13 | 11 | 11 | 11 | 11 | 11 |
| | | | nestling | 11 | 13 | 11 | 11 | 11 | 11 | 11 |
| C | 381 | 3 | mother | 12 | 11 | 11 | 11 | 11 | 11 | 11 |
| | | | father | 11 | 11 | 11 | 11 | 11 | 11 | 11 |
| | | | nestling | 11 | 11 | 11 | 11 | 13 | 11 | 11 |
| | | | nestling | 11 | 11 | 11 | 11 | 13 | 11 | 11 |
| C | 496 | 3 | mother | 11 | 11 | 11 | 11 | 11 | 11 | 11 |
| | | | father | 11 | 11 | 11 | 11 | 11 | 11 | 11 |
| | | | nestling | 14 | 11 | 11 | 11 | 11 | 11 | 11 |
| C | 675 | 3 | mother | 11 | 11 | 11 | 11 | 11 | 12 | 11 |
| | | | father | 11 | 14 | 11 | 11 | 11 | 15 | 11 |
| | | | nestling | 11 | 12 | 11 | 11 | 11 | 12 | 11 |
| C | 684 | 4 | mother | 11 | 11 | 11 | 11 | 11 | 11 | 11 |
| | | | father | 11 | 11 | 11 | 11 | 11 | 11 | 11 |
| | | | nestling | 11 | 11 | 11 | 11 | 13 | 11 | 11 |
| | | | nestling | 11 | 11 | 11 | 11 | 11 | 12 | 11 |
| C | 516 | 3 | mother | 11 | 11 | 11 | 11 | 11 | 11 | 11 |
| | | | father | 11 | 11 | 11 | 11 | 11 | 11 | 11 |
| | | | nestling | 11 | 11 | 11 | 11 | 11 | 12 | 11 |
| | | | nestling | 11 | 11 | 11 | 11 | 11 | 13 | 11 |
| C | 227 | 3 | mother | 11 | 11 | 11 | 11 | 11 | 11 | 11 |
| | | | father | 11 | 14 | 11 | 11 | 11 | 11 | 11 |
| | | | nestling | 11 | 11 | 11 | 11 | 11 | 12 | 11 |
| C | 681 | 4 | mother | 11 | 11 | 11 | 11 | 11 | 15 | 11 |
| | | | father | 11 | 11 | 11 | 11 | 13 | 11 | 11 |
| | | | nestling | 11 | 12 | 11 | 11 | 11 | 11 | 11 |
| C | 712 | 4 | mother | 11 | 11 | 11 | 11 | 11 | 11 | 11 |
| | | | father | 11 | 11 | 12 | 11 | 11 | 11 | 11 |
| | | | nestling | 11 | 11 | 11 | 11 | 11 | 15 | 11 |
| C | 347 | 2 | mother | 11 | 11 | 11 | 11 | 11 | 11 | 11 |
| | | | father | 11 | 11 | 11 | 11 | 11 | 22 | 11 |
| | | | nestling | 11 | 11 | 11 | 11 | 11 | 11 | 11 |