

**Polygyny and extra-pair paternity in a population of southwestern willow
flycatchers (*Empidonax traillii extimus*)**

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Abstract

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Microsatellite genetic markers were used to detect extra-pair offspring in clutches from monogamous and polygynous pairs of southwestern willow flycatchers (*Empidonax traillii extimus*). These markers were also used to determine paternity for extra-pair offspring. Both polygynous and monogamous males engaged in extra-pair fertilizations; however, polygynous males were more likely to be cuckolded than monogamous males. This apparent cost of polygyny was offset by overall higher reproductive success of polygynous males. Variation in the number of polygynous males and their reproductive success suggests that a polygynous mating strategy may not always be advantageous. When resources are sparse, making mate and territorial guarding more difficult, and/or paternal care more important to offspring survival, polygynous males may become more susceptible to cuckoldry and have lower average reproductive success than monogamous males. Correlating reproductive output to demographic and environmental data can illuminate the conditions under which different breeding strategies are most effective, and thus provide further understanding of selective pressures and constraints in the evolution of this social mating system.

Acknowledgments

I would like to thank my advisor, Paul Keim, and committee members Tad Theimer and Mark Sogge for their support and suggestions throughout this study. I would also like to thank Joseph Busch, William Young, James Schupp, and Lance Price for technical suggestions, and Eldon Palmer for his assistance in the laboratory. Throughout this study, I received much love, support, and encouragement from my family. My brother Amartey Pearson provided me with custom analysis software and I received many helpful suggestions from my wife Kristen Pearson. This work would not have been possible without blood samples and behavioral data collected by Mary Whitfield and her research assistants.

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CHAPTER 1

Introduction

Most North American bird species in the genus *Empidonax* are monogamous (Skutch 1960); however, polygyny has been reported in acadian (*E. virescens*) (Mumford 1964), least (*E. minimus*) (Briskie and Sealy 1987), and willow flycatchers (*E. traillii*) (Prescott 1986). Polygyny can provide males an opportunity for increased reproductive output and is therefore generally assumed to be beneficial for them (Verner and Wilson 1966). On the other hand, females that mate with polygynous males can potentially incur costs that reduce their reproductive fitness: male involvement in paternal care of offspring and in predator defense may be reduced (Webster 1991, Sandell et al. 1996), and/or females may face competition for resources by other females within a harem (Orians 1961, Orians 1969, Weatherhead and Robertson 1979). Males are therefore likely to pursue a polygynous mating strategy while females are expected to mate with a polygynous male only if her fitness would be greater or equal to her expected fitness if she were to mate monogamously (Verner and Wilson 1966, Orians 1969). Extra-pair fertilizations are common in birds (Birkhead and Møller 1995), and provide further opportunity for males to increase their reproductive output, however, they also face the risk of being cuckolded (Hasselquist and Bensch 1991). Benefits of extra-pair copulations for females may include increased parental help, material benefits (Gray 1997), and/or genetic benefits (Smith and von Schantz 1993, Dunn et al. 1994, Wetton and Parkin 1991, Whittingham and Lifjeld 1995) to offspring, but they risk a reduction in parental care from the resident male (Delehanty et al. 1998). The resulting mating system

may be the outcome of conflicting sexual strategies of both females and males instead of a consequence of male or female choice (Davies 1989).

Observational data on mating and social relationships do not necessarily indicate genetic parentage because extra-pair copulations are rarely observed; therefore, recent studies of mating patterns have used genetic markers to aid in parentage determination (see for example Gilbert et al. 1991, Travis et al. 1996, Questiau et al. 1999). This study is the first to use genetic markers to determine parentage in willow flycatchers. Extra-pair offspring were also detected, thus providing a quantitative assessment of individual reproductive success. The aims of this study were to use molecular marker data and behavioral observations from 1995-2001 to: (1) compare reproductive success of individuals engaged in monogamous and polygynous mating strategies; (2) assess the role of extra-pair paternity as a selective force behind the evolution of this mating system and; (3) discuss the effects of this mating strategy on the genetic diversity of this endangered population.

A population of southwestern willow flycatchers (*E. t. extimus*) in Kern County, California provided an important opportunity to study the ramifications of different mating systems on small, facultatively polygynous bird populations. Genetic sampling of individuals was made easier by the philopatric nature of this population: sampled nestlings returned as breeders in subsequent seasons, reducing the need to sample adults. The decline of this population has prompted researchers to investigate causes and possible management tools (see for example Harris 1991, Whitfield et al. 1999). Implementations of the latter have not resulted in a population increase. As with all critically small populations, concerns about genetic diversity (see for example Saccheri et al. 1998, Westemeier et al. 1998)) arose, stimulating a need to understand the manner by

which behavior affects reproductive success and genetic diversity. The polygynous nature of this species is of additional interest for evolutionary ethologists because willow flycatchers do not exhibit the morphological characteristics normally associated with polygynous species. Little sexual dimorphism and no secondary sexual characteristics suggest a lack of sexual selection on willow flycatchers; however, the potential for high reproductive variation amongst males through polygyny and extra-pair fertilizations indicate favorable conditions for this type of selection (Kirkpatrick et al. 1990).

CHAPTER 2

Methods

1) Study species and study area

The southwestern willow flycatcher (*Empidonax traillii extimus*) is an endangered subspecies of the willow flycatcher (*Empidonax traillii*) (Federal Register 60 [38]:10694) that breeds at approximately 110 known sites scattered throughout the southwestern United States (Unitt 1987, Marshall 2000). Nesting habitat for this neotropical migrant is restricted to densely vegetated riparian areas containing slow moving or still water (Unitt 1987, Sogge et al. 1997). In the southwest, riparian habitat has declined by 95% due to channelization of water, forest destruction, and habitat fragmentation (Johnson and Haight 1984, Katibah 1984), resulting in a dramatic loss and fragmentation of flycatcher breeding habitat (Unitt 1987). Southwestern willow flycatcher populations may be isolated due to a strong tendency to return to a particular breeding site or territory each breeding season after spring migration (Whitfield 1990, Whitfield and Strong 1995, Whitfield and Enos 1996, Paxton et al. 1997, Netter et al. 1998), however a small amount of gene flow between breeding populations does occur (Busch et al. 2000).

The southwestern willow flycatcher population in the Kern River Valley in California was one of the largest known breeding populations of this subspecies in the mid to late 1990s (Marshall 2000). From 1989 to 1998, this population consisted of approximately 27-45 breeding pairs, but has since declined to as few as 10 pairs (Whitfield et al. 1999, Whitfield pers. comm.). Breeding behavior in the South Fork Kern River Valley population has been extensively monitored in the past twelve years (Whitfield unpublished data), and over 80% of the population has been marked with both

U.S. Fish and Wildlife Service aluminum bands and a unique combination of plastic color bands.

Willow flycatcher males may sometimes be polygynous (Sedgwick and Knopf 1989, Whitfield and Enos 1996) and recent observations suggest extra-pair fertilizations occur (Paxton unpublished data, Whitfield unpublished data, Pearson pers. obs.). Males usually defend a territory that contains one or more females and are thought to mate with all of the females in their territory and, occasionally, with females in adjacent territories. For the purposes of this study, and because of a lack of appropriate terminology, females paired with socially monogamous males are referred to as monogamous females, and those paired with socially polygynous males are referred to as polygynous females.

2) Field Procedures

Individual birds, territories, and nests were monitored, and birds were captured and banded as documented in Whitfield and Enos (1996). Blood samples were obtained from most birds captured between 1995-2001 by clipping a toenail above the quick (Busch et al. 2000). In the field, samples were stored on ice in lysis buffer (10mM Tris, 1mM EDTA, 1% SDS, 100mM NaCl, pH 8) and then at -80° in the lab for later DNA extraction and analysis.

3) Laboratory Procedures

a) DNA extraction and sample storage

DNA was extracted from blood samples using the procedure described by Mullenbach et al. (1989). DNA was resuspended in 50 μ l of TE. Serial dilutions of each sample were made in water for final DNA concentrations of 10, 1, and 0.1ng/ μ l. Samples were stored at -20° C.

b) Development of microsatellite molecular markers

Microsatellite markers were identified using an enrichment protocol (Li et al. 1997). Microsatellite repeats in the genome were enriched by selective hybridization to biotinylated oligonucleotide probes consisting of (ACG)₅, (AAAG)₆, (CTT)₅, and (GATA)₆ repeat motifs. Genomic microsatellites were recovered by linking the biotinylated probe to the streptavidin-coated surface of magnetic beads (Dynabead™ M280 by Dynal). A magnet was used to separate the hybridized microsatellites from unwanted genomic DNA. The magnetic beads were then removed. The microsatellites were inserted into pGEM plasmid vectors, transformed into *E. coli* to create clone libraries, screened for size differences using PCR, then sequenced to characterize flanking sequences and to create microsatellite probes. Of the 82 clones that were sequenced, primers were designed for 29 that contained both microsatellite repeats and suitable flanking sequence for primer designation. Ten of the resulting primer pairs yielded polymorphic amplicons across individuals (Table 1). Approximate allelic size ranges were determined and the forward primers were each labeled with one of three different bioluminescent dyes such that all PCRs for each individual could be pooled and run on one lane of an acrylamide gel using an ABI 377 automated sequencer. Two of the ten markers did not amplify consistently during PCR and thus were not used for analysis.

PCR was performed in 10µl reactions with final concentrations of 1x PCR Buffer, 3mM MgCl₂, 0.2mM dNTPs, 10pM primers, 0.5U *Taq* DNA Polymerase, and 0.2ng template DNA. The complete thermal profile (94° C for 20 s; 15 s

annealing; and 72° C for 15 s), preceded by a 2 minute incubation time at 94° C, was repeated 35 times (Table 1).

Table 1. Primer loci, sequences, repeat sequences, amplicon size from clone, annealing temperatures, and number of alleles found across 5 cassin’s kingbirds (*Tyrannus vociferans*), and 5 acadian flycatchers (*Empidonax virescens*).

Locus Dye Label ^a	Primer Sequences	Repeat Sequences	Size (bp)	T _m (°C)	CAKI	ACFL
GATA2 FAM	f:aatgagagtgataattatctg tgag r:aagcctaagattccactac ttgacatttccttc	(GATA) ₁₀ (TG) ₃ (TA) ₂	253	65	1	2
GATA5 HEX	f:aggcttatttcaagacaagc aaatggaaacat r:gaaggattccttggcttca attatcacctatctt	(GATA) ₇ GACA (GATA) ₂	202	65	4	4
GATA6 FAM	f:tgcccaaaattcctcaga r:atcaaagcagcatagtc	(GATA) ₁₂	424	65		
GATA7 HEX	f:ctcctcgagctggattgta ttact r:ctaaaattcctcgcgaaaac aacctcttctta	(CTAT) ₁₆ CATCAT (CTAT) ₂	434	65	0	0
GATA9 FAM	f:gaggcaggtctctttattct r:tcgagcaagttttcctgg atttgagtgtg	(CTAT) ₄ CTAC (CTAT) ₁₂	353	65	0	5
ACG1 FAM	f:cgcataaacaaccaacct r:gctcagctccgaattctca	(GCT) ₁₆	160	65		2
ACG2 NED	f:cattacaagtgccatgtgct r:ctgcttgccttctctacgtg	(AGC) ₆	175	65	1	3
ACG5 HEX	f:tgccgatgggaagcaccag agc r:gtccgtgagcagaagccct aaaacacagagt	(GCT) ₄ GGT(GCT) ₃ (GCT) ₃ CCT(GCCT) ₃	128	65	1	6
GATA1 FAM	f:atttgaagtgccagagaatt accagaatattactatgctc r:actttgcctggaaaacaga gattttaaccatc	(CTAT) ₁₈	319	65	1	0
GATA3 NED	f:gactgaagtgccaaggca aaatgagtatatctc r:gtacccgggaagcttgctt tgaataatt	(CTAT) ₂₁	385	65	0	3

^a PE Biosystem’s 5’ fluorescent phosphoramidite dyes: 6-Fam, Hex, and Ned

c) Determination of parentage

Parentage was determined based on either behavioral observations or genotypic analysis. For behavioral data, maternity was assigned to the individual that constructed the nest, incubated the eggs, and/or cared extensively for the young. The male that defended the territory in which the nest was contained was considered to be the father. Therefore, based on behavioral data, all nestlings in a nest were assigned to a single parental male and female. Genetic parentage was determined by performing a pair-wise comparison across 8 loci in all individuals to generate a list of probable parents or offspring for each individual. The resulting list of parents and their offspring was then compared to a similar list based on behavioral observations. To avoid overestimating extra-pair fertilization rates resulting from genotyping errors, only nestlings whose genotypes were found to differ by 3 or more alleles from the “behavioral” father were considered to be sired by a different male through extra-pair fertilizations. A probable genetic parent could be detected for 45% of offspring that were the result of extra-pair fertilizations.

d) Data analyses

To compare reproductive success between monogamous and polygynous females, the number of offspring produced by individual adults in each category was calculated. The total number of fledglings per season was used for behavioral comparisons of reproductive success using all behavioral data collected between 1995 and 2001. For comparisons of reproductive success from the subset of individuals for which genetic samples were obtained, the number of nestlings from

which blood samples were taken was counted. Student's t-tests were used to compare reproductive success between treatments.

Finally, a simple mathematical model was constructed to illuminate possible effects of variation in parameters that determine the overall reproductive success of polygynous and monogamous males. These parameters were: average apparent reproductive success based on behavioral observations (RS_A), average number of offspring lost to cuckoldry (EPF_L), and average number of offspring gained from extra-pair fertilizations (EPF_G). Therefore, true reproductive success (RS_T) = $RS_A + EPF_G - EPF_L$. Determination of the sensitivity of behavioral parameters to manipulation can be used to estimate the degree of change necessary to invoke a qualitative shift in the comparative reproductive success of monogamous and polygynous males (to cross the polygyny threshold where average reproductive success is equal for monogamous and polygynous males).

CHAPTER 3

Results

1) Mating strategies

Between 1995 and 2001, the proportion of polygynous males (counting only those males that defended territories with nests and whose identity could be determined) ranged from 0 to 0.57 ($\bar{X} = 0.21 \pm 0.7$ S.E). The proportion of females that paired with polygynous males ranged from 0 to 0.62 ($\bar{X} = 0.35 \pm 0.09$ S.E). For polygynous males with more than one year of data during this time period, 54% were polygynous for more than one year.

2) Effects of mating strategies on reproductive success

Behavioral data were gathered for 220 offspring from 131 nests between 1995 and 2001. For 134 of these offspring from 54 nests, genetic data were also obtained. Behavioral analysis of the sub-set used for genetic analysis showed the same pattern as the full data set, suggesting that the sub-set provided a representative sample (Fig. 1).

Based on behavioral data, polygynous males averaged more offspring than monogamous males (two-tailed Student's t-test using full data set: $t = 3.24$, d.f. = 36, $P = 0.003$, for genetic sub-set: $t = 1.77$, d.f. = 16, $P = 0.096$), but approximately the same number of offspring per pair (for full data set: $t = -0.26$, d.f. = 121, $P = 0.80$, for genetic sub-set: $t = 0.38$, d.f. = 49, $P = 0.70$). These patterns were the same even when only offspring that were genetically related to resident males were used in the comparison (Fig. 2): polygynous males averaged more offspring than monogamous males ($t = 1.68$, d.f. = 23, $P = 0.11$), but approximately the same number of offspring per pair ($t = -0.49$, d.f. = 49, $P = 0.62$). Due to small sample sizes and the difficulty in determining parentage of extra-pair

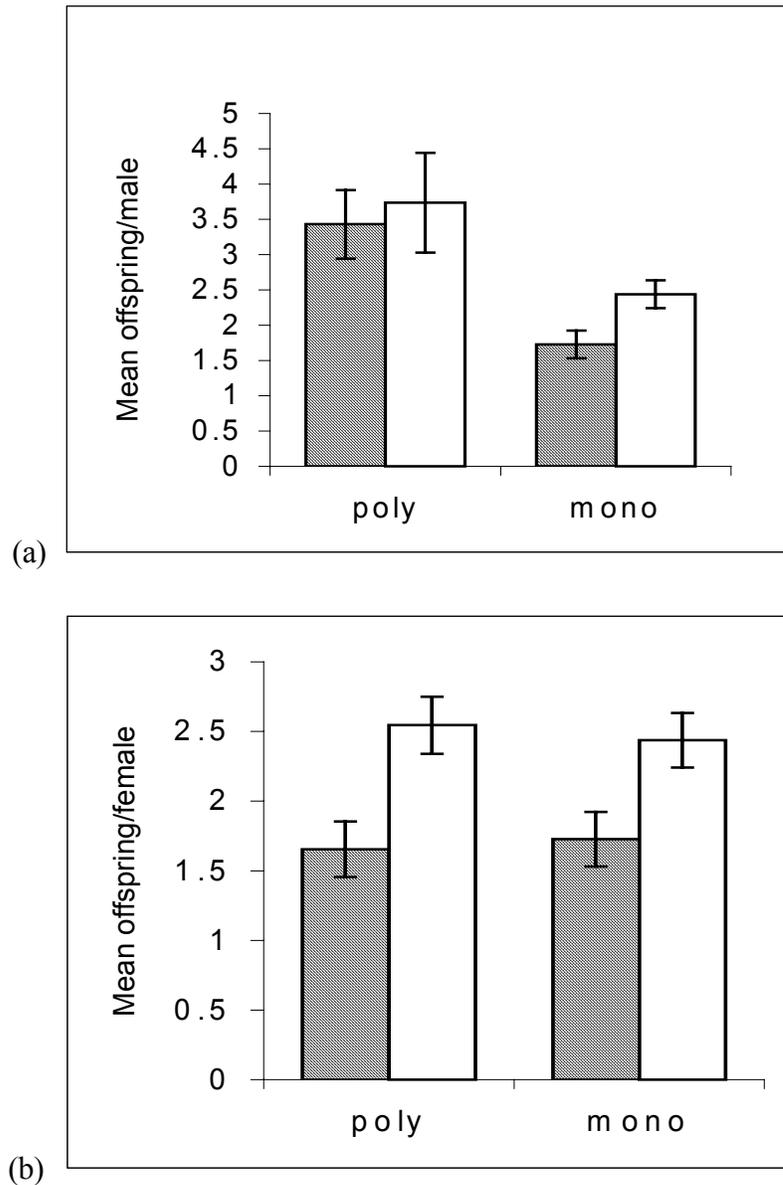


Figure 1. Mean (\pm SE) reproductive success of monogamous and polygynous (a) males, and (b) females based on behavioral observations. Hatched bars show data derived from the full data set, and open bars show data from the sub-set for genetic analysis. For the full data set, data include $N = 28$ polygynous males, $N = 66$ monogamous males (and pairs), and $N = 58$ polygynous females. Genetic sub-set data include $N = 15$ polygynous males, $N = 32$ monogamous males (and pairs), and $N = 22$ polygynous females.

offspring, the average numbers of extra-pair offspring sired by individuals were not used to calculate reproductive success.

Genetic analyses, however, revealed that polygynous males lost an average of almost three times as many offspring to cuckoldry compared to monogamous males, and females paired with polygynous males were approximately twice as likely to engage in cuckoldry than females paired with monogamous males (Fig. 3). The differences between polygynous and monogamous male susceptibility to cuckoldry ($t = 0.97$, d.f. = 15, $P = 0.35$) and susceptibility differences between polygynous and monogamous females to extra-pair fertilizations ($t = 0.97$, d.f. = 28, $P = 0.34$) were not statistically significant, probably because of great variation among polygynous males. Nonetheless, these differences suggest a cost to polygyny through a reduction in male reproductive success.

Genetic analyses of data from 1998 revealed that both monogamous males ($\bar{X} = 0.42$) and polygynous males ($\bar{X} = 0.67$) sired extra-pair offspring (Fig. 4). Due to the small sample size ($n = 7$: 2 offspring sired by polygynous males, and 5 by monogamous males), this comparison provided only a qualitative estimate of the success of polygynous and monogamous males in procuring extra-pair fertilizations. Data from other years were not used because parentage could either not be determined, or was assigned to unmated males. In 1998, parentage was assigned to 7 or 11 extra-pair offspring.

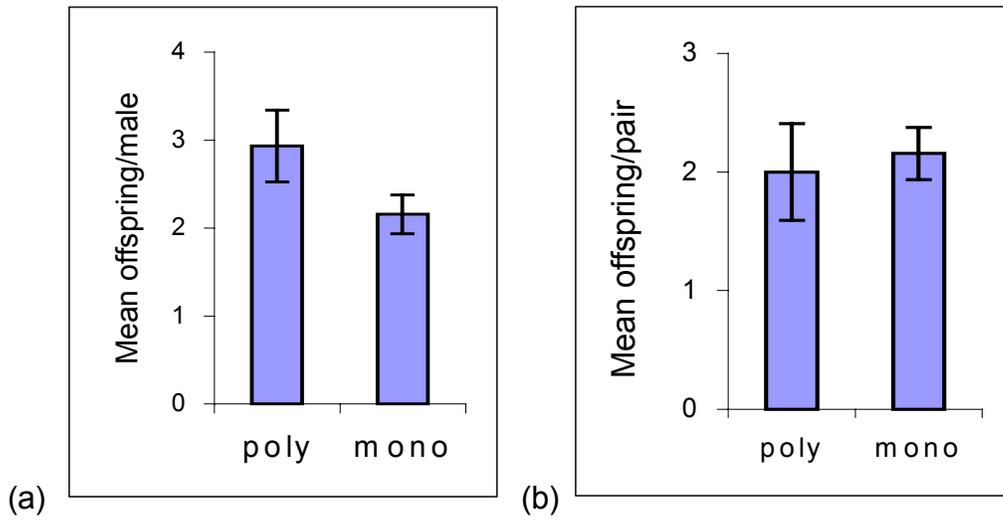


Figure 2. Mean (\pm SE) reproductive success of polygynous and monogamous (a) males, and (b) females based on genetic analysis of offspring. Genetic sub-set data include N = 15 polygynous males, N = 32 monogamous males (and females), and N = 22 polygynous females.

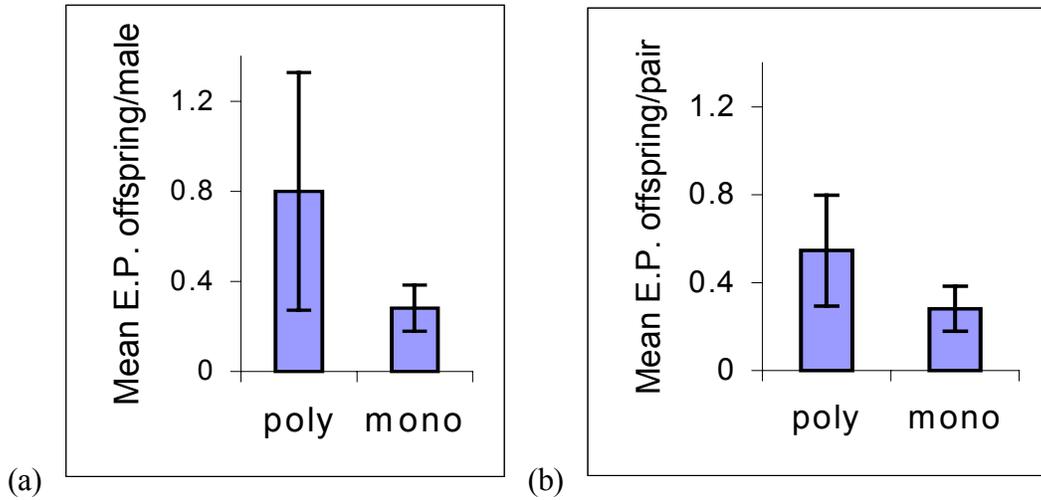


Figure 3. Mean (\pm SE) number of extra-pair offspring in nests of monogamous and polygynous (a) males, and (b) pairs. Data include N = 15 polygynous males, N = 32 monogamous males (and females), and N = 22 polygynous females.

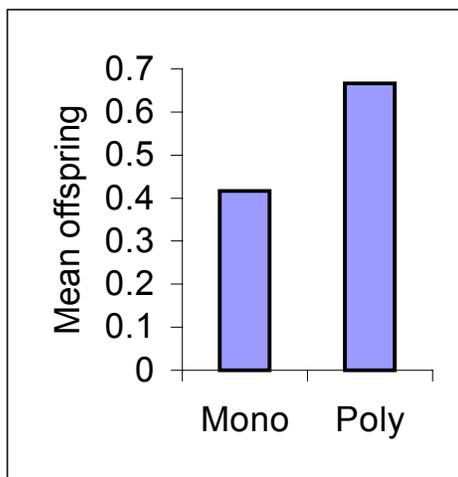


Figure 4. Mean number of extra-pair offspring sired by monogamous and polygynous males in 1998 based on genetic analyses. Data include N = 7 (2 offspring sired by polygynous males, and 5 by monogamous males).

3) Effects of population parameters on polygyny and extra-pair fertilization rates

The percentage of polygynous males increased with total population size, total number of females, and with the female to male ratio (Fig. 5). As the population size increased, and especially as the number of males rose, so too did the percentage of extra-pair fertilizations (Fig. 6).

4) Effects of extra-pair fertilization rates on reproductive success

In this population of willow flycatchers, 32% of mated males were considered polygynous and sired 42% of offspring based on behavioral observations. Genetic analyses of paternity indicated that polygynous males in fact sired only 33% of offspring, losing 9% to cuckoldry. Monogamous males were the genetic parents of 51% of offspring and lost 7% to extra-pair fertilizations. Therefore, in the Kern population, approximately 16% of all offspring are born to cuckoldry (Fig. 7). Not all offspring that were the result of extra-pair fertilizations could be matched to both parents, therefore calculations to determine actual reproductive success of individuals included only losses to cuckoldry, but not gains from extra-pair relations. True reproductive success might therefore be approximately 40% for polygynous males.

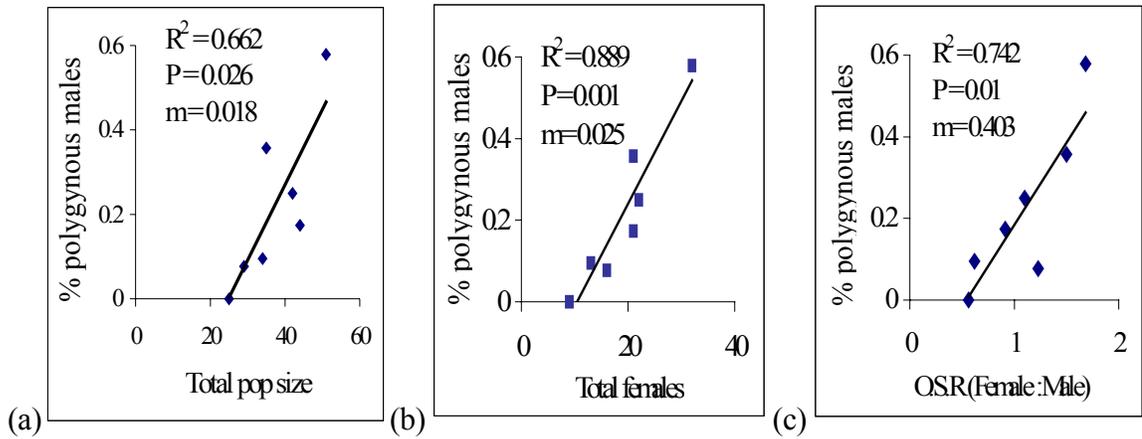


Figure 5. Linear regression of percent polygynous males with (a) total population size, (b) total number of females, and (c) the operational sex ratio.

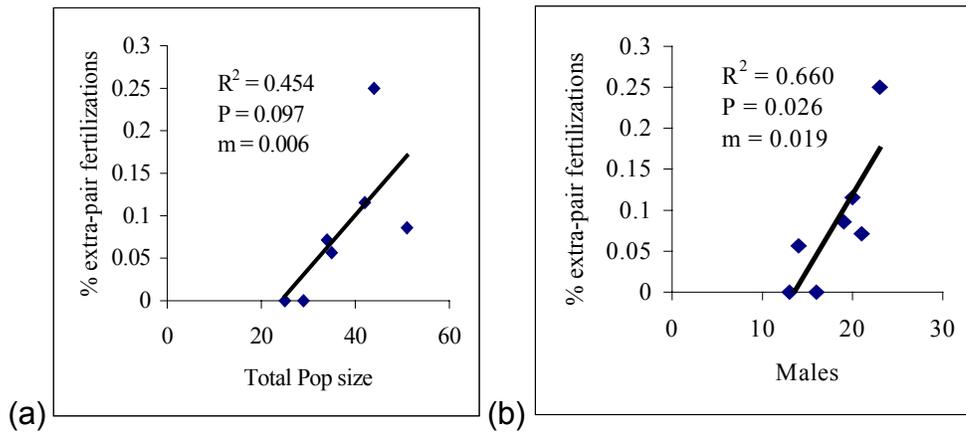


Figure 6. Linear regression of the percentage of extra-pair fertilizations with (a) total population size and (b) total number of males.

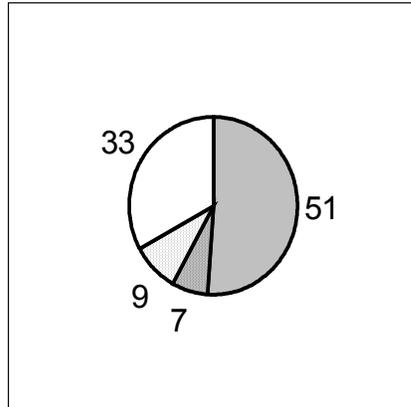


Figure 7. Percentage of offspring in the Kern population sired by polygynous (open) and monogamous (shaded) males, and resulting from extra-pair fertilizations (dotted).

Sensitivity analyses of parameters influencing reproductive success

Mean true reproductive success (RS_T) is influenced by average apparent reproductive success (RS_A), based on behavioral observations, average number of offspring lost to cuckoldry (EPF_L), and average number of offspring gained from extra-pair fertilizations (EPF_G) such that $RS_T = RS_A + EPF_G - EPF_L$. Because the average number of offspring gained from extra-pair fertilizations could not be quantified, EPF_G was not included in the calculation of RS_T . For polygynous males, $RS_A = 3.7$ and $EPF_L = 0.8$, therefore, $RS_T = 2.9$. For monogamous males, $RS_T = 2.1$ (2.4 minus 0.3). Under the environmental conditions from this seven-year period, selection for polygyny was expected because polygynous males sired more offspring than monogamous males (an average of 0.8 more).

Variation in RS_A , EPF_L , EPF_G , and environmental conditions suggests that monogamy may sometimes be favored over polygyny. If, for example, polygynous males were more susceptible to cuckoldry and/or their apparent reproductive success decreased, the true reproductive success of monogamous males may be greater than for polygynous males. The manner in which these parameters influence true reproductive success was determined by manipulating RS_A and then EPF_L while the other was held constant. The combined effect of these two parameters was also determined by manipulating RS_A and EPF_L simultaneously. The degree of change for each parameter necessary for monogamy to be more beneficial than polygyny in terms of true reproductive success is shown in Figure 8. The intersection point of the lines with the x-axis show the percentage that parameters must be changed in order for average true reproductive success to be equal for monogamous and polygynous males (the polygyny threshold). RS_A has the greatest influence on true

reproductive success because small variations in RS_A can decrease the benefit of polygyny below the polygyny threshold where monogamous males have higher true reproductive success (Figure 8). EPF_L appears to be a fairly robust parameter in that monogamous males would need a much lower value of EPF_L than polygynous males in order to have a higher reproductive success. The inclusion of EPF_G (not shown) would act as a buffer to the system, requiring greater manipulation of all other parameters in order to give monogamous males a greater RS_T than polygynous males.

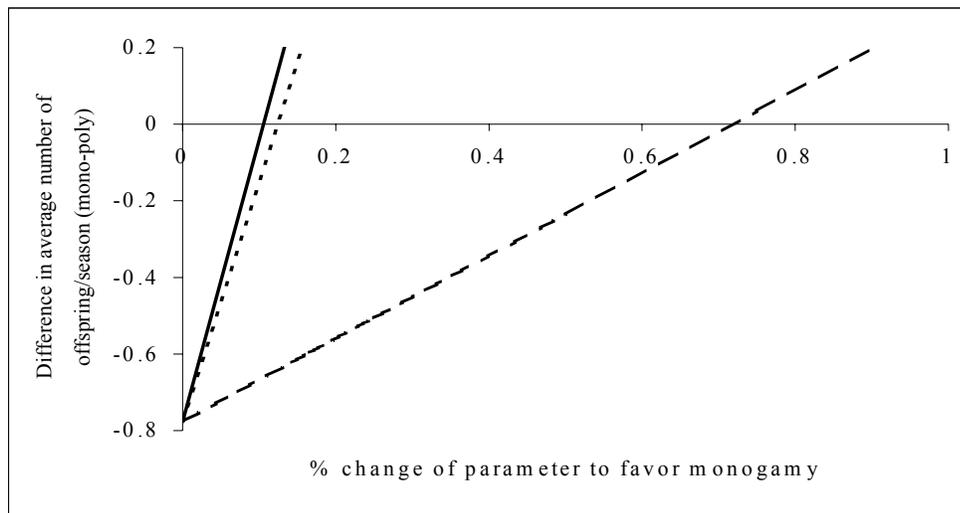


Figure 8: Effect of manipulating $R.S_A$ (dotted), EPF_L (dashed), and both $R.S_A$ and EPF_L (line) to show the degree of change needed for average reproductive success to be greater for monogamous males than for polygynous males.

CHAPTER 4

Discussion

In the last decade, the application of DNA-based methods to determine parentage in birds has allowed detection of extra-pair offspring, and better estimates of true reproductive success. Extra-pair fertilizations have since been accepted as common and widespread in avian species, casting doubt on the reliability of traditional methods of determining reproductive success (Mock 1983). Estimates of apparent reproductive success are most unreliable for species where extra-pair fertilizations are not distributed evenly across the population. In red-winged blackbirds (*Agelaius phoeniceus*), estimates of apparent reproductive success for males can be inaccurate because of the high frequency and unequal distribution of extra-pair fertilizations: males that were most successful on their own territories were also most successful at siring extra-pair offspring (Gibbs et al. 1990, Weatherhead and Boag 1997). Extra-pair fertilizations are also common in monogamous species and, where variability of male success in extra-pair fertilizations is great (see for example Hill et al. 1994, Kempenaers, et al. 1997, Richardson and Burke 1999), estimates of apparent reproductive success do not accurately reflect true reproductive success. On the other hand, genetic methods have confirmed qualitative estimates of observed reproductive success in species with very low rates of extra-pair parentage (Gyllensten et al. 1990), and in species where gains and losses in extra-pair paternity are more evenly distributed across males (Leisler et al. 2000).

Polygyny versus monogamy

For the Kern River Valley population of southwestern willow flycatchers, DNA fingerprinting has validated qualitative patterns of reproductive success made on the basis of

behavioral observations: polygynous males sire more offspring than monogamous males, yet there is no difference in reproductive output between polygynous and monogamous females. Behavioral observations suggested that extra-pair relations were possible, and genetic comparisons of nestlings to putative parents revealed the extent and slightly unequal distribution of extra-pair progeny among polygynous and monogamous males and females. Because offspring from females paired with polygynous males are more likely to be sired by extra-pair males than offspring from monogamous females, the benefits of polygyny are reduced to some degree by this apparent cost.

The cost of polygyny may seem high because offspring from polygynous males are almost three times as likely to be sired by an extra-pair male than offspring from monogamous males. Likewise, females associated with polygynous males are twice as likely to produce extra-pair offspring than those associated with monogamous males. However, the actual number of extra-pair offspring remains small. Furthermore, both polygynous and monogamous males sire extra-pair offspring. Therefore, large relative differences in probability of being cuckolded do not translate into a statistically significant difference in average reproductive success between monogamous and polygynous males. For monogamy to be favored over polygyny, polygynous males must suffer from many more extra-pair fertilizations and/or gain fewer offspring from cuckoldry than monogamous males; neither seems to be the case. The rates of extra-pair fertilizations and male involvement in cuckoldry presented here indicate that selection would likely favor polygyny when, on average, polygynous males sire (by behavior) more than 52% of offspring raised in their territory.

Because the overall average reproductive success of polygynous males is 15% greater than monogamous males, the benefits of polygyny can be substantial. Despite this advantage of polygyny, only an average of 21% of males in the Kern population are polygynous. The variances associated with the parameters that determine overall reproductive success suggest that selection could sometimes favor monogamy over polygyny. Indeed, the sensitivity of RS_A to manipulation indicates that this is likely. The prevalence of monogamy is likely due to a combination of factors that reduce either the environmental potential for polygyny or the ability of males to exploit any existing polygyny potential (Emlen and Oring 1977). For example, when females are not able to meet the demands of chick rearing, male care may become more important for nest success (Davies 1991). Biparental care is not mandatory for willow flycatchers (Sedgwick and Knopf 1997), however, the degree of paternal involvement may become correlated with reproductive success during periods of sub-optimal conditions. In house wrens, for example, when males were removed from nests, nest success dropped only during periods of poor weather and food scarcity (Bart and Tornes 1989). Such conditions would favor monogamy if polygynous males were less capable of providing sufficient paternal care, and were therefore unable to exploit any existing polygyny potential. Furthermore, polygynous males may not be able to invest as much time in territorial and mate defense if resources are more difficult to obtain, thus increasing their susceptibility to cuckoldry (Davies 1991, Hasselquist and Bensch 1991). Willow flycatcher males forage, protect mates and territories, and guard against predators more or less simultaneously from various perches surrounding their territories. This low-energy time budget allows for a significant amount of “loafing” time that may act as a buffer against periods of harsh conditions, giving males the

flexibility to increase reproductively beneficial behavior (Ettinger and King 1980). Because of this ability, it is likely that only extreme conditions may limit exploitation of polygyny potential by willow flycatcher males. For example, food resources and or suitable nesting sites may not be clumped, forcing females to become more scattered and thus reducing the ability of males to monopolize them (Verner and Wilson 1966, Hannon and Dobush 1997). In the Kern River valley, food and nesting resources for willow flycatchers are usually clumped, but plentiful, thus not eliminating the possibility of scattered territories especially in years of small population sizes. Perhaps the greatest constraint to polygyny is the adult sex ratio. When the ratio is skewed towards more females, as in the Kern population, competition over females would likely decrease, increasing the likelihood of female monopolization and the potential for polygyny (Emlen and Oring 1977, Smith et al. 1982, Arcese 1989). It is probable that male southwestern willow flycatchers are opportunistically polygynous; however, the influence of factors that may affect the polygyny potential and the ability of male exploitation of this potential needs more attention. Furthermore, the role of females in determining the mating system needs to be investigated.

Mate choice for females in polygynous systems is expected to be influenced by mate and territorial quality, such that female benefits in reproductive success offset potential costs of reduced paternal care that may be incurred by pairing with a polygynous male (Emlen and Oring 1977). If female mate choice is based on the polygyny threshold model (Orians 1969), whereby females choose a polygynous mate if they expect to have a higher fitness than by mating with a monogamous male in a poorer territory, then males would be expected to preferentially choose territories that were polygynous in the previous year (provided that either habitat quality is consistent across years or females favor previous

territories). Willow flycatcher habitat in the South Fork Kern River valley is potentially highly variable due to inconsistent rates of annual flooding that may then affect prey availability. Despite the potential for such variability, female flycatchers often return to previous territories; therefore, it is possible that previously polygynous territories would remain polygynous in subsequent years, benefiting males that choose these territories. Further support for the polygyny threshold hypothesis would require: (1) a correlation between polygynous territories and some measure of habitat quality, (2) a correlation between quality of breeding situation (habitat and mate quality) and female fitness, and/or (3) evidence linking territorial settlement order to territorial quality (Davies 1989). A comparison of reproductive success between monogamous and polygynous females (here, polygynous females did not fare better than monogamous females) cannot be used as evidence for the polygyny threshold model since average fitness for polygynous females could be greater, equal, or less than average fitness for monogamous females (Davies 1989).

If females choose polygynous territories as an act of “desperation” when all other suitable sites are taken (Orlans 1961), or on the basis of male quality such that an immediate reduction in reproductive success would be offset by long-term fitness of offspring (“sexy son” hypothesis) (Weatherhead and Robertson 1979), reproductive success of secondary females would be expected to be lower than for monogamous females. In this study, no distinction was made between primary and secondary females of polygynous males. The lack of a significant difference in average reproductive success between polygynous and monogamous females does not preclude the possibility of high average fitness for primary females reduced by a low average fitness for secondary females. A further prediction of the “desperation” hypothesis is that only after all territorial males are mated will polygyny

occur. This is unlikely for Kern River flycatchers since unmated males are frequently observed when other males are polygynous. Even though no comparisons of lifetime reproductive success were made between monogamous and polygynous females, the “sexy son” model is not likely to apply to willow flycatchers because the lack of apparent physical differences between monogamous and polygynous males renders male quality assessment by females unlikely.

A fourth model that predicts female settlement patterns is based on the neutral-mate-choice hypothesis (Lightbody and Weatherhead 1987). Unlike the previous three models of female choice, the neutral-mate-choice model predicts that females settle independently of male and territory quality (Lightbody and Weatherhead 1988, Hartley and Shepherd 1995). In this model, females settle randomly on suitable territories occupied by males, maximizing their fitness by avoiding difficult or costly predictions of mate or territorial quality. Perhaps female willow flycatcher settlement patterns best fit this model for the following reasons: (1) there appears to be little variation in territory quality, (2) neither food nor nest sites are limited, and (3) there is no difference in reproductive success between monogamous and polygynous females. Acceptance of this model for willow flycatchers would require verification of the above observations and evidence that female reproductive success or settlement patterns are not affected by the behavior of other females.

Extra-pair fertilization

Extra-pair fertilizations are beneficial to males (but see Hannon and Dobush 1997) because they provide increased reproductive output; however, benefits for females are not always clear. Cuckoldry has only recently been confirmed in willow flycatchers (Paxton unpublished data, this study). The level of extra-pair offspring in this population is fairly

high and accounts for approximately 16% of nestlings. No behavioral observations have been published, which makes it impossible to know the extent to which females actively solicit extra-pair copulations; a potentially important clue in determining benefits for females. It is commonly assumed that females benefit from extra-pair copulations either indirectly, through genetic benefits, and/or directly, by procuring male help or gaining access to resources. Genetic benefits for females may include diversifying offspring genotypes, thus increasing the probability of survival in a variable environment (Smith and von Schantz 1993, Dunn et al. 1994), insurance against mate infertility (Wetton and Parkin 1991), and procuring “good genes” for offspring (Whittingham and Lifjeld 1995, Kempenaers et al. 1997, Richardson and Burke 1999). Direct benefits for females that engage in extra-pair copulations have been shown to include foraging privileges on extra-pair territories, help in predator defense (Gray 1997), and/or paternal aid in caring for offspring. Potential costs for females that seek extra-pair copulations may range from none to male abandonment (Delehanty et al. 1998).

There is no evidence that male willow flycatchers reduce the amount of paternal care when their mate engages in extra-pair copulations; however, since paternal care is not essential to successful fledging of young, the costs to females may be low or non-existent. The genetic variability hypothesis predicts that extra-pair fertilizations should be common and relatively evenly distributed throughout the population. This model is not likely to explain female benefits for willow flycatchers since extra-pair young are found only in 24% of nests and are not evenly distributed throughout the population. It is unlikely that female flycatchers engaged in extra-pair copulations to insure against infertile mates, as has been shown in house sparrows (*Passer domesticus*; Wetton and Parkin 1991) because all

cuckolded males also successfully sired offspring. It is also unlikely that female willow flycatchers engage in extra-pair copulations with superior males to procure “good genes” for their offspring. It is commonly assumed that polygynous males are superior to monogamous males; however, polygynous males were cuckolded more often than monogamous males.

Direct benefits to female willow flycatchers that engage in extra-pair copulations seem unnecessary because food resources do not appear to be limited, and biparental care of offspring does not appear to be necessary. However, there have been occasional observations of extra-pair males feeding nestlings (Pearson pers. observ., Whitfield unpublished data). Such behavior may be an attempt by females to decrease their own parental effort, thus increasing their own long-term fitness (Lessels 1991). It is unlikely that benefits to lifetime reproductive success provide the sole motivation behind the relatively extensive rate of extra-pair fertilizations that occur in this population, however further research is needed to illuminate the costs, benefits, and repercussions of extra-pair copulations.

Implications of breeding behavior and population size on genetic diversity

Breeding behavior plays a vital role in the genetic diversity of populations. For endangered species with small population sizes, genetic diversity may be especially important for population persistence (Lynch et al. 1995, Saccheri et al. 1998, Westemeier et al. 1998), and maximizing genetic diversity has therefore been the goal of many conservation efforts (see for example Longmire et al. 1992, Hedrick and Miller 1992). The extent to which mating patterns maximize genetic diversity is therefore an important consideration. Maximum genetic diversity occurs when a large population is totally

panmictic and all breeders contribute equally to the gene pool. In polygamous populations where few individuals dominate the breeding system, genetic diversity is reduced. In the small polygynous population of flycatchers studied here, polygyny may not reduce genetic diversity. When the population size is small, and thus most vulnerable to a reduction in genetic diversity, there are few or no cases of either polygyny or extra-pair fertilizations, thus the potential for all breeders to contribute equally to the gene pool is maximized. This potential may be reduced at high population levels because the prevalence of polygyny increases. However, because extra-pair fertilization rates also increase with population size, more males may be able to contribute to the gene pool. Therefore higher rates of polygyny when combined with higher rates of extra-pair fertilizations may not significantly decrease genetic diversity.

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