Determining genetic monogamy in American Kestrels (*Falco sparverius*) via RAPD-PCR DNA fingerprinting

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Who's your Daddy?

ABSTRACT

RAPD-PCR techniques were used to determine genetic monogamy in a local population of American Kestrels (*Falco sparverius*). Three family groups nesting on the Ridgefield National Wildlife Refuge were studied. Familial comparisons were performed using two RAPD primers chosen to see which provided optimal differentiation between individuals. Initial results indicate that in all cases studied, PCR products from progeny samples correlated to combinations of PCR products observed in the resident female parent and the putative male parent. Results using primer 3 (5'-d[GTAGACCCGT-3) also provided better overall differentiation. Therefore, preliminary data would suggest genetic monogamy in this population of American Kestrels rather than extra-pair fertilization. Additional research screening a larger number of family groups and an examination of the breeding density may also yield important information.

INTRODUCTION

While most species of raptor are listed as monogamous, it is unclear whether these are genetically monogamous (genetically exclusive parings for the season to rear young) or socially monogamous parings (for the season to rear young, with extra pair fertilization). For a review of extra pair paternity in birds see Westneat and Stewart (1) and Griffith *et al*. (2). Genetic monogamy would imply an established system that passes genes of the mating pair to ensure the biological fitness of that pair; their genes are passed on to their offspring. Social monogamy, on the other hand, leads to the theoretically best genes being passed on to the next generation, yielding young that have an increased biological fitness. The question can then be posed, if a female is trying to pass on the most fit genes to the next generation, what is the incentive for a male to support offspring that may not contribute to his overall fitness?

Many passerines that were once believed to be genetically monogamous have been confirmed as socially monogamous. Limited data is available for raptors (3). Genetic monogamy has been confirmed in Black Vultures (*Caragyps atratus*)(4) and Long-eared Owls (*Asio otus*)(5). Recent work indicates that American Kestrels (*Falco sparverius*) are also genetic monogamists (6) but this has not been extensively studied across its range in local populations. Westneat and Stewart (1) indicated there is within and between population variation as to the amount of extra pair paternity in birds. The literature further documents extra pair copulations and non-breeding season copulations in American Kestrels (6,7,8,9,10,11). The evolutionary trade-off is a paradox of this question; pass on the best genes from the fittest male or select a mate that may be less fit but is likely to help raise offspring and lead to overall nesting success. Currently there are a variety of proposed theories explicitly formulated to explore the influences of population level characteristics on specific mating behaviors such as extra-pair copulations (EPC), extra-pair fertilizations (EPF) that lead to extra-pair paternity (EEP) as well as strategies employed that would assure paternity assurance; mate guarding and high within-pair copulation rates (WPC)(3,1,2,12).

Our research was designed examine paternity patterns from an American Kestrel population nesting in Clark County, Washington. Studies from several locations are needed to help to build the overall picture as to whether this species is genetically monogamous or socially monogamous. We hypothesize this species is indeed genetically monogamous across all populations (11,12,13). If this is the case, all offspring studied will be genetically related to both the maternal attendant and putative male at the nestbox. If American Kestrels are socially monogamous, a preponderance of the young should still be related to the male attendant which should ensure that male's continued nesting duties (13). Familial status of the female, resident male and chicks was determined by genetic analysis using random amplified polymorphic DNA (RAPD) PCR to perform a type of DNA fingerprinting.

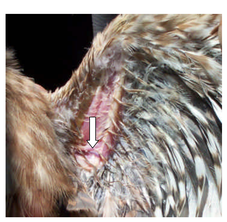

Figure 2. Exposed under-wing of an American kestrel with and arrow indicating location of the brachial vein.

METHODS

Field Methods

Ten nestboxes suitable for American Kestrels were placed at the Ridgefield National Wildlife Refuge (WA) in 2004 (some nestboxes were relocated prior to the breeding season in 2006)(Fig. 1). Nestboxes were monitored for occupation in the spring of each year and kestrel pairs were observed during the breeding season. Adult birds were trapped via a *bal-chatri* trap or within the nestbox; chicks were sampled prior to fledging. All subjects were banded with U.S. Fish and Wildlife Service bands to allow for the identification of individual birds and standard morphometric data was collected for all individuals (14). Blood samples (0.05 ml) were collected from the brachial vein (Fig. 2). All samples were collected in accordance with *Guidelines to the use of wild birds in research* (15).

Figure 1.Nestbox locations for the 2006 American Kestrel Study at the Ridgefield National Wildlife Refuge, Ridgefield, WA.

Sample Storage

After collection, blood samples were preserved by either of two methods: 1) 50µl whole blood diluted in 1.0 ml Queens lysis buffer (16) or 4.0 µl whole blood diluted in 600 µl of Cell Lysis Solution (GenomicPrep Blood Isolation Kit, Amersham Biosciences). After collection and dilution, samples were stored frozen at -20^{0}C.

DNA Purification

DNA was purified using a commercial kit following the manufacturer's protocol for nucleated whole blood (GenomicPrep Blood DNA Isolation Kit, Amersham Biosciences). In addition, optional protocols for RNase A treatments were performed on cell lysates and samples were incubated on ice prior to the addition of "Protein Precipitation Solution" to enhance protein precipitation.

PCR Reactions

PCR was performed using commercially supplied Ready-To-Go RAPD Analysis Beads (Amersham Biosciences). After initial screening of the six 10-mer primers included with the kit, two primers were chosen for sample analysis: primer 2 – (5'-d[GTTTCGCTCC]-3'), primer 3 – (5'-d[GTAGACCCGT]-3'). Corresponding DNA fragments were amplified using a PTC-100 Programmable Thermocycler (MJ Research, Inc.) using the following cycling conditions: 1 cycle at 95^{0}C for 5 min followed by 45 cycles of 95^{0}C for 1 min, 36^{0}C for 1 min, 72^{0}C for 2 min. *E. coli* BL21 (DE3) and C1a DNA, also supplied with the kit, were used as positive controls.

Electrophoresis

DNA fragments were separated on 20 cm long agarose gels (2% w/v) made with 1x TBE buffer and containing 0.5 µg/ml ethidium bromide. Electrophoresis using 5 µl of PCR products was performed at a constant 150 volts (BIO-RAD 300X computer controlled power supply) for approximately 3 hours. Finished gels were photographed using a UVP ultraviolet transilluminator (model TS-15E) and a Polaroid Photo-Documentation Camera (FB-PDC-34) using a Tiffen 40.5mm deep yellow 15 filter.

RESULTS

During the 2006 breeding season, three of four occupied nest boxes produced viable clutches (one nest was abandoned). The three adult pairs and all six fledgling chicks were trapped and sampled (Table 1). Observation of initial results indicate that for the family groups studied, PCR products from progeny samples correlated to combinations of PCR products observed in the parents attending the nest.

For family group NB13 using RAPD primer 2, 1300 and 500 bp bands present in both parents were also present in both chicks (Fig. 3). A 480 bp band observed in the paternal parent (933) was also observed in chick 937. A band of approximately 490 bp not corresponding to either parent was observed in both chicks. Use of RAPD primer 3 resulted in a band at 310 bp for all family members, a 1000 bp band observable in the paternal parent and both siblings and a maternal band of 1400 bp also observable in both siblings. A band of 1500 bp not corresponding to those observed in either parent was observed for both siblings.

NB14 family members, when evaluated using RAPD primer 2, all showed a common band at 280 bp (Fig. 4). Both siblings also shared bands at approximately 560 bp with the maternal parent and at 550 bp with their paternal parent. Results using primer 3 provided bands at 380 and 310 bp for all family members. A paternal band at 410 bp correlated with both siblings while a paternal 350 bp band was observed only in sibling 939. Additional PCR products not observed in either parent were present only in chick 939.

Family group NB15 using RAPD primer 2 was difficult to evaluate due to poor resolution of parental samples (Fig. 5). With primer 3, all family members produced bands of 420, 350 and 310 bp. Additionally, bands of 410 and 390 bp were present paternally and in both chicks; maternal bands of 1000 and 1300 bp were present in both chicks. Two PCR products not corresponding to either parent were noted for chick 934 and were approx. 1500 and 650 bp in length.

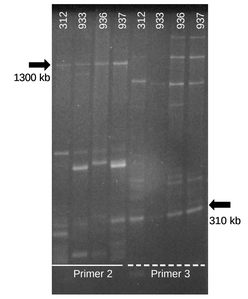

Figure 3. RAPD-PCR Products from family group occupying nestbox 13 using primers 2 and 3. The pair, 312 resident female and putative male 933, produced offspring 936 and 937.

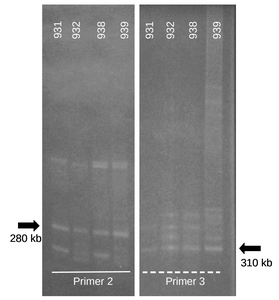

Figure 4. RAPD-PCR Products from family group occupying nestbox 14 using primers 2 and 3. The pair, 931 resident female and putative male 932, produced offspring 938 and 939.

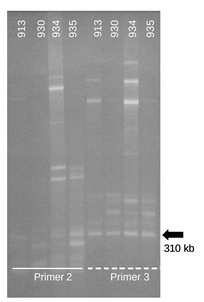

Figure 5. RAPD-PCR Products from family group occupying nestbox 15 using primers 2 and 3. The pair, 913 resident female and putative male 930, produced offspring 934 and 935.

Table 1. Identification of individuals within family groups examined during the 2006 breeding season.

	Nestbox		
Individual	NB13	NB14	NB15
Resident female	312	931	913
Putative male	933	932	930
Chicks	936	938	934
	937	939	935

Chicks 937, 938 were female; all others were male.

DISCUSSION/CONCLUSION

Our initial findings support the status of American Kestrels as being genetically monogamous. This is consistent with findings of other studies (6, 7, 8, 9, 10, 11) that kestrels do engage in extra-pair copulations prior to the fertile period but are genetically monogamous. While the RAPD-PCR method did prove effective in determining paternity, work still needs to be done to improve PCR product resolution. Our study tested packaged primers singly; primers in combination may yield more distinctive results. Further we recognize the small sample size of our study, three family groups, needs to be expanded before firm conclusions about either the effectives of the RAPD-PCR method or the breeding patterns of kestrels can be firmly established. To this end, we hope to increase the number of family groups in the coming breeding season and test primers in combination.

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