peaks were fit with mixed Lorentzian and Gaussian profiles to obtain accurate 2θ values from which the unit cell dimensions were refined.
8. The normalization procedure involves multiplying the ^{18}O magnetic data by a factor so that the ^{16}O and ^{18}O curves intersect at 2 K. This factor is typically between 1.0 and 1.2. The low temperature magnetic susceptibilities of the ^{18}O samples are always smaller, and we do not understand the origin of the small reduction of the Meissner effect after ^{18}O substitution. There is no apparent correlation of this phenomenon with x.
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6 August 1990; accepted 11 October 1990

Realized Reproductive Success of Polygynous Red-Winged Blackbirds Revealed by DNA Markers

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Hypervariable genetic markers, including a novel locus-specific marker detected by a mouse major histocompatibility complex probe, reveal that multiple paternity is common in families of polygynous red-winged blackbirds (*Agelaius phoeniceus*). Almost half of all nests contained at least one chick resulting from an extra-pair fertilization, usually by a neighboring male. Genetically based measures of reproductive success show that individual males realize more than 20% of their overall success from extra-pair fertilizations, on average, and that this form of mating behavior confounds traditional measures of male success. The importance of alternative reproductive tactics in a polygynous bird is quantified, and the results challenge previous explanations for the evolution of avian polygny.

AN IMPORTANT CONTROVERSY IN studies of avian mating systems is the degree to which observed social relationships of breeding males and females accurately predict patterns of gene transfer across generations (*1*). The observation of copulations between individuals not paired with each other (*2*) and parentage analyses using allozymes (*3*) imply a significant lack of congruence between the behavioral and genetic mating systems of many species and argue that alternative reproductive tactics such as extra-pair fertilizations by males and egg-dumping by females may be important components of individual reproductive success (*4*). If significant, such alternative reproductive behaviors would also raise serious questions about traditional measures of reproductive success used in studies of avian mating systems (*5*).

Theory (*6*) suggests that extra-pair fertilizations (EPFs) may be particularly important sources of male success in polygynous birds like the red-winged blackbird (*Agelaius phoeniceus*) where males provide little parental care. In this species, field experiments have shown that females nesting on the territories of vasectomized males often produced fertile eggs (7), and there is evidence that nonterritorial "floater" males are capable of fertilizing females (*8*). We used a combination of traditional and novel hypervariable genetic markers to assess the extent of extra-pair fertilizations in families of red-winged blackbirds and to identify the males responsible. Our results show that some males gain a substantial portion of their reproductive success through EPFs and provide a detailed analysis of realized male and female reproductive success in a natural population of a polygynous bird. They also demonstrate that EPFs severely confound traditional measures of male success and hence call into question previous tests of explanations for the evolution of polygyny in these birds (*9*).

To study parentage in wild blackbirds, we collected blood samples from adults and 6-day-old nestlings from one low-density (0.1 territorial males per hectare) and two high-density (0.4 males per hectare) marshes near the Queen's University Biology Station, Chaffey's Lock, Ontario, in 1986. We successfully collected samples from 75% (21 of 28) of all territorial males in these three marshes and from all chicks in 90% (36 of 40) of nests on the territories of sampled males that had nestlings old enough to be bled. In total the 36 families we sampled consisted of 111 nestlings, 21 putative male, and 31 putative female parents; for 31 of these families we captured both male and female parents, whereas for five nests we only had samples from the presumed male parent (*10*).

For parentage analyses we followed procedures described by Seutin *et al.* (*11*) to extract DNA from the samples and used Southern Blot techniques to carry out pedigree analyses on the families using two types of hypervariable DNA probes. First, a mouse class II major histocompatibility complex (MHC) cDNA probe from the I-alpha locus (*12*) that hybridizes to a locus-specific hypervariable region in the blackbird genome; second, two multi-locus mini-satellite probes, Jeffreys 33.15 (*13*) and the M2.5 repetitive sequence from the *per* gene in mice (*14*). The MHC probe (*15*) detects restriction fragment length polymorphisms in Eco RI–cut genomic DNA that have the genetic characteristics of a single hypervariable locus with many co-dominant alleles ($n = 36$) at low frequencies (mean allele frequency = 2.2%). As a result the probability of false inclusion (*16*) for this locus in blackbirds equals 0.07, which is equivalent to that of many human single locus variable number of tandem repeat (VNTR) markers (*17*). Both mini-satellite probes have been used in parentage analyses of wild birds (*18, 19*); each detects complex individual-specific band patterns in blackbirds (Fig. 1).

Our approach was to use both types of

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probes in such a way as to apply the relative strengths of each for parentage analysis (for example, the simplicity and scoreability of the single-locus marker versus the high resolving power of the complex genetic profile generated by the multi-locus probes). To identify nestlings whose genotypes did not match those of their parents, we initially screened all families with the MHC probe and identified chicks with alleles with sizes (in kilobases) three or more standard deviations different from the size of any parental allele (*17*). We then used at least one of the mini-satellite probes to confirm the MHC results by comparing band-sharing coefficients between all chicks and their putative parents (*20*). In all cases, results from both types of markers were in agreement in confirming either genetic matches or mismatches between parents and offspring.

For parentage assignments, we adopted a two-step procedure in which we first used the simple single-locus genotypes to screen for possible parents at the population level and then a mini-satellite probe to choose a parent from this select group of adults. More specifically, with data from the initial MHC analysis, we calculated the size of the stray MHC allele in the mismatched chick and then identified all adults in the population with MHC alleles within 10% of the stray allele's size. We then used one of the mini-satellite probes to identify which adult of this subset (usually three or fewer individuals), if any, had a genetic profile that matched that of the chick and hence could be assigned as the true genetic parent.

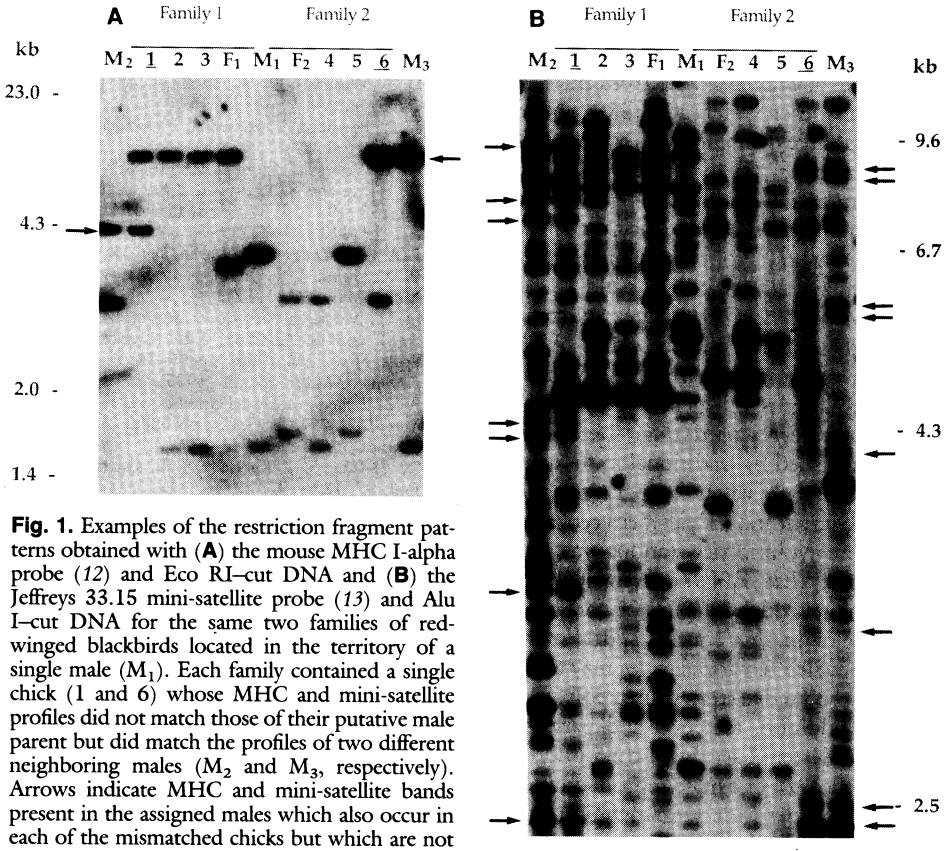

Fig. 1. Examples of the restriction fragment patterns obtained with (**A**) the mouse MHC I-alpha probe (*12*) and Eco RI–cut DNA and (**B**) the Jeffreys 33.15 mini-satellite probe (*13*) and Alu I–cut DNA for the same two families of red-winged blackbirds located in the territory of a single male (M_1). Each family contained a single chick (1 and 6) whose MHC and mini-satellite profiles did not match those of their putative male parent but did match the profiles of two different neighboring males (M_2 and M_3, respectively). Arrows indicate MHC and mini-satellite bands present in the assigned males which also occur in each of the mismatched chicks but which are not found in the resident male. The figure also shows a case where the mini-satellite probe confirms a match between a putative male parent and his offspring that was ambiguous based on the MHC data alone. The paternal MHC allele in chick 4 is shared by both the resident territorial male (M_1) and a neighbor (M_3). Band-sharing coefficients (*D*) (*20*) between the chick and each of the two males confirm that the resident male is its true father [$D(M_1$ and 4) = 0.52; $D(M_3$ and 4) = 0.12]. Methods used for the MHC analysis are described elsewhere (*15*). Protocols for the mini-satellite analyses were the same with the following exceptions: 4 μg of genomic DNA was digested with Alu I or Hae III, run through 30 cm 0.8% agarose gels at 1.2 v/cm for 48 h, blotted onto Hybond-N+ nylon membrane in 20 × saline sodium citrate (SSC), hybridized with purified inserts of one of the mini-satellite probes in 0.263*M* Na_2PO_4, 7% SDS, 1 m*M* EDTA (pH 8.0), and 1% BSA (*30*), and then washed in 2 × SSC; 0.1% SDS at 65°C. The mean frequency of alleles detected by the MHC probe in blackbird DNA cut with Eco RI is 0.022; hence, the probability of two unrelated individuals sharing the same genotype is 4.8×10^{-4} (*15*). For the two mini-satellite probes, the number of bands detected per bird and the proportion of bands shared between unrelated individuals depended on the probe-enzyme combination used: for the same 11 unrelated individuals run on a single gel the number of scoreable bands (*n*) in the 2.5- to 25-kb size range varied from 17.72 ± 0.35(SE) (*per*/Hae III) to 23.3 ± 0.37 (33.15/Hae III), whereas the proportion of bands shared between individuals (*D*) based on visual comparisons ranged between 0.11 ± 0.008 (*per*/Alu I) to 0.23 ± 0.012 (33.15/Hae III). Because data from sib-ships indicate that individual bands segregate independently (*31*), the probability of two unrelated individual blackbirds having the same fingerprint profile (D^n) (*18*) is $< 2 \times 10^{-15}$ for any of the probe-enzyme combinations used here.

Genetic analyses revealed a high level of extra-pair fertilizations in these birds. For nests for which both putative parents had been sampled, 14 of 31 nests (45%) had at least one chick whose MHC and DNA fingerprint genetic profiles did not match with one of its parents. Adding the results from the five male-only nests increases this ratio to 17 of 36 nests (47%). Of all chicks sampled for both types of nests, 28% (31 of 111) had genotypes not compatible with one parent. In all cases the excluded adult was the putative male parent (Fig. 1). Territorial male density within a marsh was positively correlated with the overall frequency of illegitimate chicks: the proportion of illegitimate chicks was significantly higher in the two marshes with relatively high male densities [frequency of EPFs: 32% (30/95 chicks)] as compared to the marsh with lower male density [frequency of EPFs: 6% (1/16 chicks); $G = 5.82$; $P \leq 0.05$]. The proportion of nests with one or more mismatched chicks also increased with male density [52% (16 of 31 nests) versus 6% (1 of 5 nests)], but not significantly ($G = 1.68$; $P > 0.05$). Thus, contrary to previous reports (*21*), EPFs due to male cuckoldry are frequent in this species. In contrast, and unlike certain other birds (*4*), there is no evidence for intraspecific brood parasitism by females.

Previous studies have rarely been able to identify the genetic parents of mismatched young. Our two-step paternal assignment procedure demonstrates that cuckolding males are often neighbors who hold territories on the marsh (Fig. 2). For this analysis, we concentrated on a single high density population (Barb's Marsh) for which we had the most complete samples of nestlings and adults. We were able to identify the true genetic father of 26 of the 28 chicks that resulted from an EPF (*22*). All sampled resident males were excluded as genetic fathers of the two remaining mismatched chicks, both from the same nest. Their fathers could have been one of the two resident males for which we had no blood samples (Fig. 2) or one of the nonresident floater males that are abundant in this population (*23*). In 20 of the 26 (77%) successful paternal assignments, the true father held a territory bordering on the territory containing the mismatched chick's nest (Fig. 2). In nests with illegitimate chicks, the maximum number of males fathering young was two, usually the resident and one other male. In one case, two neighbors each fathered a single chick in a nest with only two young. Multiple neighbors did, however,

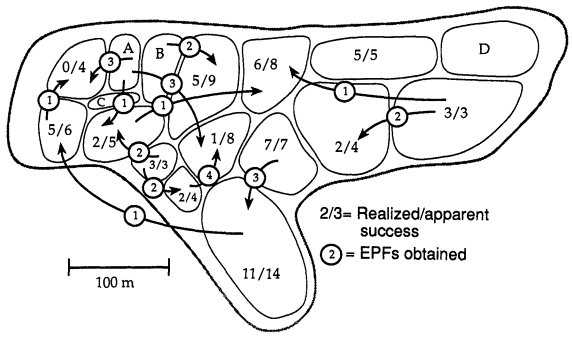

Fig. 2. Realized reproductive success of male red-winged blackbirds holding territories on Barb's Marsh. The fraction in each territory represents the number of chicks sired by a resident male over the number sampled from nests on his territory. Arrows describe instances of extra-pair fertilizations: the origin of the arrow shows the identity of the cuckolding male; the arrowhead, the territory in which he fertilized chicks; and the number in the circle, the number of extra-pair chicks he sired. Only the resident males were sampled on territories A and B; territory C contained an unsampled male and no nesting females; and territory D had a male and nesting females, none of which were sampled.

father chicks in separate nests within the same resident male's territory.

On the basis of this parentage analysis, we calculated realized reproductive success (sampled chicks fathered through both within- and extra-pair fertilizations) for 13 male blackbirds resident on the intensively studied marsh and compared it with estimates of their apparent reproductive success (chicks from sampled nests on a male's territory). Seven of 13 males obtained one or more EPFs; chicks sired in this fashion made up an average of 21% (range 0% to 67%) of a male's total realized success. There was also a significant positive correlation between the realized paternity in a male's own nests (percentage of young sired by the resident male) and the number of chicks fathered on other males' territories [Kendall rank correlation, tau = 0.405 (corrected for ties), $P = 0.05$]. Thus, the gains and losses of paternity by individual males did not balance each other (*24*); rather, individuals who were successful in fertilizing females breeding on their own territories were also successful in gaining matings with females on other territories. As a consequence, the standardized variance in male reproductive success [variance/(mean)^2] (*25*) was 56% higher when based on realized success (0.39) as compared to apparent success (0.25). This increase, however, was not significant when tested by a randomization procedure (*26*), likely because of the small number of males in the sample. Finally, we found no correlation whatsoever between apparent and realized male reproductive success [Kendall rank correlation, tau = 0.126 (corrected for ties), $P = 0.59$] suggesting that the traditional measure of male success (nestlings produced) used in field studies of birds (*27*) may be inaccurate in species where EPFs commonly occur. In contrast, apparent measures of female reproductive success in this species are not affected by uncertain maternity.

Our results have a number of important technical and evolutionary implications. We used a cloned heterospecific MHC locus to detect a hypervariable genetic marker suitable for parentage analysis in a wild bird. This suggests that the large number of MHC loci already available from mice, humans, and other vertebrates (*28*) may be a valuable source of single-locus hypervariable genetic markers for parentage analysis in wild vertebrates that precludes the time-consuming procedure of isolating species-specific markers from genomic libraries. Such locus-specific markers would be especially valuable for parentage assignment studies that require the screening of large numbers of potential parents as the simple banding patterns of such markers can be more accurately scored than the complex profiles revealed by traditional mini-satellite probes, especially when comparing the profiles of different individuals between blots.

From an evolutionary standpoint, our results argue that EPFs are an important source of reproductive success for at least some males in this species and show the extent to which territorial males may adopt a mixed reproductive strategy in a polygynous bird. In addition, the level and pattern of EPFs we detected seriously confounds traditional measures of male reproductive success. This argues for a reexamination of the results of past studies in this area and underlines the need to use genetic parentage techniques based on DNA markers to augment simple measures of male reproductive success. For example, in studies of avian polygyny, the number of females a male attracts is often used as a measure of male mating success on the basis of the assumption that females mate exclusively with the owner of the territory on which they nest (*9*). Consistent with these studies, we indeed found a significant positive relation between apparent male success and harem size [measured as the maximum number of reproductively active females on a male's territory; Kendall rank correlation, tau = 0.679 (corrected for ties), $P = 0.0012$]. However, the relation is markedly nonsignificant [tau = 0.217 (corrected for ties), $P = 0.83$] when realized male success, which incorporates the effects of EPFs on paternity, is used. This result reinforces the view that for this and other species, the choice by a female of where to nest may be distinct from her choice of which male she mates with (*1*); this may explain why attempts to find support for general models for the evolution of polygyny in birds have been unsuccessful (*29*). Because males that were most successful at gaining fertilizations on their own territories were also most successful in gaining fertilizations on other males' territories, the next question should be what phenotypic attributes, if any, confer high fitness on this select group of individuals.

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20. Band similarity coefficients (D) were calculated as $2(n_{ab})/(n_a + n_b)$, where n_{ab} is the number of scored bands shared by birds a and b and n_a and n_b are the number of bands scored in birds a and b, respectively [J. H. Wetton, R. E. Carter, D. T. Parkin, D. Walters, *Nature* **327**, 147 (1987)]. Values of D of roughly 0.5 or greater are expected between parents

and offspring, whereas, from estimates of band-sharing between unrelated individuals (Fig. 1), D values of less than 0.25 should identify adults that can be excluded as parents. The mean D value (±SE) for nestlings not excluded as offspring of their putative parents on the basis of their MHC genotypes was 0.52 ± 0.009 for male parents and 0.48 ± 0.009 for female parents. Mean D values for excluded chicks were 0.04 ± 0.007 (male parents) and 0.48 ± 0.013 (female parents). These values confirm the MHC results that only males and not females are excluded as the parents of the mismatched chicks. The probability of false inclusion of an unrelated male (assuming correct maternity) is approximately $X^{y/2}$ [U. B. Gyllensten, S. Jakobsson, H. Temrin, *ibid.* **343**, 168 (1990)] where X is the proportion of shared fragments and $y/2$ is the minimum number of paternal specific bands. For males in this population this value is always less than 2×10^{-6}, depending on the probe-enzyme combination used to generate the data.
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27 June 1990; accepted 17 September 1990

A Trans-Acting Factor That Binds to a GT-Motif in a Phytochrome Gene Promoter

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The regulatory photoreceptor, phytochrome, controls the expression of numerous genes, including its own *phyA* genes, which are transcriptionally repressed in response to light. Functional analysis of a rice *phyA* gene promoter, by means of microprojectile-mediated gene transfer, indicates that a GT motif, GCGGTAATT, closely related to elements in the promoters of a number of other light-regulated genes, is critical for expression. Partial complementary DNA clones have been obtained for a rice nuclear protein, designated GT-2, that binds in a highly sequence-specific fashion to this motif. Mutational analysis shows that the paired G's are most crucial to binding. GT-2 has domains related to certain other transcription factors. Northern blot analysis shows that GT-2 messenger RNA levels decline in white light although red and far red light pulses are ineffective.

PHYTOCHROME IS A BINARY MOLECULAR switch that regulates plant gene expression in response to light (*1–3*). This photoreceptor is reversibly interconvertible between its inactive Pr form and its active Pfr form by red (R) and far red (F) light. However, the molecular mechanism by which the regulatory signal is transduced from Pfr to responsive genes remains unknown (*3–7*).

We have focused on the genes for phytochrome itself to study this transduction mechanism. Transcription of *phyA* genes in monocots is repressed within 5 min of Pfr formation, and repression occurs in the absence of protein synthesis (*8–11*). These data indicate that all components necessary for transduction exist in the cell before light perception. Similarities between sequence motifs in different monocot *phyA* promoters (*8, 11, 12*) suggest that these motifs could be functionally important. These motifs include tandem GT elements related to the motif GTGTGGTTAAT that has been implicated in the phytochrome-regulated expression of a pea *rbcS* gene and that binds to a factor, designated GT-1, detected in crude nuclear extracts (*3, 5, 6*). Indeed, a factor in rice nuclear extracts binds to a rice *phyA* promoter fragment containing tandem GT elements, and the binding is completed by an oligonucleotide with the sequence GTGTGGTTAAT (*11*). We have therefore examined here the potential role of GT motifs in the autoregulated expression of the rice *phyA* gene. We also report the characterization of a partial cDNA clone encoding a protein that binds in a highly sequence-specific manner to one of two GT motifs in this promoter.

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The activity of the rice *phyA* promoter was assayed by means of microprojectile-mediated gene transfer (*13, 14*) into etiolated rice seedlings (Fig. 1). Deletion analysis showed that the −364 to +111 bp fragment used here supports the same level of expression as a fragment from −2 kb to +111 bp upstream (*15*). The wild-type promoter (Fig. 1, plasmid 702) responded reversibly to R and F light, indicating that this fragment contains the sequences necessary for autoregulation of *phyA* genes in rice cells. The TATA element alone (plasmid 717) supported expression at ~25% of the maximum wild-type level with no significant effect of light. Thus, sequences that determine differences in transcriptional activity between high-Pfr and low-Pfr cells reside between −364 and −31 bp in the rice *phyA* promoter. Deletion of both GT elements (plasmid 711), or linker-substitution mutation (*16*) of the 3′ element (plasmid 712), showed that at least the 3′ motif (−228 to −219 bp) functions as a positive element in transcriptional activation in cells with low Pfr levels (Fig. 1). The alternative, that this motif functions as a negative element that causes repression in response to high Pfr levels and derepression upon Pfr depletion, is unlikely because removal of this sequence did not lead to derepression in the presence of high Pfr levels (plasmids 711 and 712).

An oligonucleotide containing both of the tandem GT motifs from the rice *phyA* promoter (−242 to −219 bp) was used to isolate cDNA clones producing proteins that bound to these elements (*17, 18*). The insert from the largest positive clone (designated λGT-2) was used for overexpression of the protein product, GT-2, which was then examined for specificity of bind-