

Cooperative Display and Relatedness Among Males in a Lek-Mating Bird

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Long-tailed manakins mate in leks and cooperate in multiyear male-male partnerships. An alpha male is responsible for virtually all mating, whereas a beta male assists in the courtship displays. Such altruism by the beta male poses a problem for evolutionary theory because most theoretical treatments and empirical examples of cooperative behavior involve kin selection or reciprocity. Here it is shown that alpha and beta partners are not relatives and that reciprocity is not involved. Instead, direct, though long-delayed benefits to beta males are demonstrated, which include rare copulations, ascension to alpha status, and female lek fidelity. These benefits maintain this unusual form of male-male cooperation.

Dual-male courtship display in long-tailed manakins, *Chiroxiphia linearis* (Aves, Pipridae), is a particularly puzzling form of cooperation (1) for two reasons. First, it occurs in a lek mating system, in which males cluster in traditional lek arenas for courtship display that is subject to female choice. Such systems are characterized by intense sexual selection in which males compete vigorously for mates (2). Second, the benefits of the joint display appear to be virtually unilateral; during partnerships between alpha and beta males that may persist for many years (3), only the alpha normally copulates (259 of 263). Reciprocity, therefore, is not involved. Further, the beta is generally heavier than the alpha (3), which suggests that size does not promote the highly skewed dominance interaction. One resolution of the puzzle would be if partners were relatives, with the noncopulating beta males benefiting through the indirect component of inclusive fitness (kin selection) (4). Alternatively, both males might benefit directly from the partnership, with the payoff to the beta offset in time.

Discussion of intraspecific cooperation has focused largely on nonparental kin selection and reciprocity (5, 6), but Brown (5) pointed out the possibility of intraspecific mutualisms in which both parties gain without requiring reciprocity. He used the term by-product mutualism to describe such cases. Theoretical and empirical discussion of mutualism has been largely restricted to interspecific interactions (7). Here we illustrate a mutualism from which both parties benefit but in which the benefits are highly skewed in time. Alpha males have the op-

portunity for immediate benefits, whereas the benefit for beta males is offset from the initiation of cooperation by months or years.

A marked study population in Monteverde, Costa Rica (10°18'N, 84°48'W), has been monitored since 1982 (3, 8, 9). Males form multimale teams of as many as 13 members, with an alpha and beta male that do the bulk of the obligate dual-male, unison song duets (*toledo* calls) and dual-male dance displays (8, 10). The cooperative displays require immense effort by beta males, who can give more than 3 million *toledo* calls and engage in more than 1000 hours of dual-male leapfrog hops and labored *butterfly* flight during their multiyear tenure as betas.

To test the hypothesis that kin selection might largely or partially explain the evolution of this extreme form of male-male cooperation, we assessed the relatedness of 33 pairs of cooperating males (11) using polymorphic microsatellite loci (Table 1). The mean relatedness coefficient, R , of partnered males was negative ($R = -0.14$; jackknifed SE = 0.10), with 95% confidence limits of -0.35 and 0.07 (12). The fact that the upper confidence limit for

relatedness is approximately equivalent to that between second cousins ($R = 0.0625$) argues against the kin selection hypothesis. Further, if partnerships formed among relatives, as expected under the kin selection hypothesis, the majority of partners should be positively related. Instead, 17 of the 33 R coefficients were negative whereas 16 were positive, a result consistent with partnerships forming randomly with respect to relatedness (13) but contrary to the kin selection hypothesis.

Indirect inclusive fitness effects could still be a force if certain conditions were met. (i) Partnerships among relatives might be more successful than partnerships among unrelated individuals. This was not the case. Of the 11 partnerships that resulted in one or more copulations by alphas, seven showed negative relatedness and four showed positive relatedness. Of the nine pairs of males that were seen to dance more than 10 times for females, three were positively related and six were negatively related. (ii) Males might associate with the most closely related among potential partners actually available to them. A test of this hypothesis required genetic data on two or more males of approximately equivalent age and status that were observed to have interacted with the target male at a lek arena. Of males for whom sufficient data existed, the primary partner was the most closely related in only one of 13 cases. The genetic results are therefore inconsistent with the hypothesis that indirect inclusive fitness benefits play a role in male-male cooperation in this species.

Four kinds of direct, though long-postponed, benefits to beta males existed (Table 2). First, a rare benefit to beta males was through copulations late in their tenure as betas. Of 263 copulations during the study, four were by beta males in the temporary absence of the alpha (9). In at least one case, the alpha male had recently copulated with the female. Most beta males

Table 1. Attributes of microsatellite loci used to assess relatedness. Detailed laboratory procedures are provided in (20). The few modifications used are outlined in (21). The mean of the heterozygosity values is 0.402.

Locus	PCR conditions		Fragment size (base pair range)	Allelic frequencies				Heterozygosity
	Temperature (°C)	Mg concentration (mM)		a	b	c	d	
SJ133	55	1.5	134 to 142	0.701	0.179	0.015	0.104	0.448
LTR8	53	1.5	140 to 148	0.545	0.440	0.015		0.552
LTR6	55	1.5	188 to 190	0.806	0.194			0.269
LTR15	48	2.0	189 to 201	0.743	0.257			0.338

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Table 2. Direct benefits to beta male from cooperating in dual-male courtship display. Spearman rank correlation, r_s .

Benefit	Selective mechanism	Delay (years)
Copulations during beta tenure (4 of 263; 1983 to 1992)	Direct reproductive success	4 to 8 (to beta status)
Succession to alpha role (11 of 11 documented turnovers)	Male-male interactions	5 to 13 (1 to 5 years as beta)
Female site fidelity (16 of 27 females site-faithful) (10 of the 16 mated with replacement alpha)	Female choice	5 to 13 (1 to 5 years as beta)
Mating success correlated with predecessor's* ($r_s = 0.71$ for visits; $r_s = 0.83$ for copulations)	Partial phenotype continuity (plus female choice effects)	5 to 13 (1 to 5 years as beta)

*See Table 3 and text.

were 8 years or older, and none was younger than 4 years.

Second, males formed orderly queues for social status (14), such that all cases of succession to the alpha role by banded males were by beta males ($n = 11$). In no case was a lower ranking male seen to supersede another in moving to the alpha role. Females appeared to enforce the orderliness of queues (leks) by choosing queues with high levels of cooperative display (8) and by discriminating against queues that threatened to become disorderly (as a result of male-male antagonism) (14). Female choice among queues produced a high variance of mating success restricted to a few older ($\bar{x} = 10.1$ years) males (9), which meant that few options existed for young males (≤ 8 years). Such a lack of options should further favor orderly queuing (15) and acts as the constraint on present reproduction that favors delayed reproduction by beta males.

Third, patterns of female visitation benefited beta males. Female visitation correlated with output of unison song duets, and copulation success correlated with performance of the dual-male dance display (8). Of banded females with multiyear copulation records, 16 of 27 showed site fidelity to lek arenas between years. Site fidelity was apparent ($n = 10$) even after alpha male turnover within or between years. All 11 females known to have switched away from

a lek arena did so after several months or a season of low levels of performance and switched into lek arenas with higher performance levels. During 7556 hours of simultaneous observations at 4 to 10 lek arenas over a 10-year period, females were seen to visit multiple lek arenas and to copulate repeatedly at various intervals at the same arena but never to copulate at two different arenas in the same season.

Finally, measures of mating success for beta males and their predecessors (Table 3) were significantly correlated both for female visitation ($r_s = 0.71$, $P < 0.05$) and for copulations ($r_s = 0.83$, $P = 0.01$). The robustness of the correlations was assessed with a randomization procedure (16). Beta males that helped establish a reputation of high performance for a lek arena, therefore, stood to benefit when they became alphas from the return of females that they helped attract during their tenure as betas. The benefit from cooperative display was thus due in part to a male's past as well as present performance. The benefits accrued from both direct male-male competition and female choice as well as from the unusual feature of a dual phenotype chosen by females (alpha plus beta), half of which persisted after a turnover of the alpha male (Table 2).

Cooperation is perhaps most difficult to explain when the cooperators forego reproduction. Discriminating between the rela-

tive importance of indirect inclusive fitness benefits and direct benefits has been difficult in such systems, because all other well-studied examples (cooperative-breeding birds and mammals, eusocial insects, naked mole rats, and termites) occur in inherently family-structured social systems (5, 17). In these cases, helpers are sometimes unrelated, but the cooperative behavior is not an obligate feature of the social system and helping by unrelated individuals is often directed toward a potential mating partner or future helper (18). Such a return for the investment in helping has been termed a form of reciprocity. In long-tailed manakins, no confounding effect of kinship exists. Clearly, alpha male long-tailed manakins, to whom virtually all copulations accrue during their tenure, do not reciprocate.

The cooperative behavior of long-tailed manakins is significant because it combines the following characteristics. (i) The cooperation involves foregoing reproduction for many years, but relatedness is not a contributing factor. (ii) The cooperative behavior is not a form of reciprocity, because it is not directed toward an individual that may later repay the effort expended. (iii) The benefit to the beta male is usually displaced from the cooperative behavior by several years. (iv) The benefits do not increase with increasing group size over some range of group sizes, unlike most examples of social grouping involving foraging, vigilance, or thermoregulation (19). (v) Parental care is not involved (1). (vi) Cooperation is an obligate feature of courtship display subject to female choice. (vii) Female choice among partnerships of males appears to enforce cooperation within partnerships (9). Thus, cooperation by nonbreeding beta males has evolved, despite the intense competition expected among males and despite the absence of indirect inclusive fitness benefits or reciprocity.

Table 3. Mating success of beta males after ascension to alpha status and that of their predecessors. Mating success was measured in the season or partial season after (beta ascending to alpha) or before (predecessor) a turnover of alpha males. Sample sizes over which means were calculated varied, but none was less than 50 hours of scheduled 2-hour observation periods. Female visits were the mean number of seconds females were present during scheduled 2-hour observation periods.

Beta band	Predecessor band	Turnover	Female visits per 2 hours (s)		Copulations per 2 hours	
			Beta	Predecessor	Beta	Predecessor
3595	4790	1989 to 1990	7.34	6.02	0.000	0.000
2120	1965	1991 to 1992	83.29	120.24	0.086	0.087
1935	3570	1992 to 1993	89.34	98.84	0.014	0.045
3570	3315	1991 to 1992	98.84	173.28	0.045	0.111
4990	1805	1991	157.69	290.45	0.143	0.136
1820	4990	1991 to 1992	304.22	157.69	0.217	0.143
1805	4685	1990 to 1991	290.45	420.64	0.136	0.327
1585	4690	1984 to 1985	324.00	260.30	0.350	0.290

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11. Genotypes were assessed for 68 males, of which 28 were involved in one or more of 33 partnerships that were successful in attracting at least one female to a lek arena for dual-male dance display. Relatedness coefficients, $R_{i=1}^{33}$, for each of 33 male partnerships were calculated from the four loci with the method of D. C. Queller and K. F. Goodnight [*Evolution* **43**, 258 (1989)]. With this method, relatedness coefficients can range from -1.0 to 1.0. A negative coefficient means that the individuals share fewer genes than the ambient level of sharing in the population. As with pedigree-based schemes, the expected relatedness among full siblings is 0.5. To assess the mean relatedness coefficient over all partnerships, the genotypes of the 40 unpartnered males were repeated 300 times as a background against which relatedness could be assessed. Only males were used for the analyses, because male and female Wright's inbreeding coefficient (F_{IS}) values differed slightly. All four loci conformed to Hardy-Weinberg expectations ($P \geq 0.25$).
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13. Binomial test, $P = 0.36$ for 16 or more positive relatedness coefficients, on the assumption of an equal probability of positive or negative relatedness; $P < 0.05$ would require at least 22 positive coefficients.
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16. For each of the measures of mating success, 2000 sets of eight values from the beta and predecessor columns of Table 3 were drawn randomly, with replacement. The drawing was constrained so that the paired value was neither self nor partner. Spearman rank correlation coefficients were calculated for each set of eight paired values. Of the 2000 sets drawn for female visitation, 23 coefficients (1.15%) exceeded the correlation coefficient between partners (0.71). Of the 2000 sets drawn for copulatory success, five coefficients (0.25%) exceeded the correlation coefficient between partners (0.83).
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21. Genomic DNA from long-tailed manakin and Florida scrub jay was digested with either Bam HI and Eco RI or Pst I and Sst I, as was the plasmid vector Puc 19. Genomic fragments between 200 and 700 base pairs (bp) were excised from agarose gels and cloned into Puc 19 to create size-selected genomic libraries for these two species. These libraries were screened simultaneously with radiolabeled (CA)_n and (CT)_n polynucleotides (Pharmacia). Positive clones were sequenced, and polymerase chain reaction (PCR) prim-

ers were developed for clones containing repeats with $n > 7$. Primer sequences for the four loci used in this study [three from the long-tailed manakin library (LTMR) and one from the Florida scrub jay library (SJR)] are as follows: SJR133, 5'-CATGCTCATG-GCTCAGTTCA-3', and 5'-TGTGGCAAGTGTGG-GTGAT-3'; LTMR6, 5'-GCCATGCCACAGGAGT-GAGTC-3', and 5'-AGTCATCTCCATCAAGG GCAT-3'; LTMR8, 5'-AATGACACCCACATTCAGT-3', and 5'-TGCCAAATAGCAAAGGAACC-3'; LTM-R15, 5'-CATTATCCATAGTGCAAAGC-3', and 5'-AACAGGTGCATCACTAAGCAG-3'. PCRs were performed in 25- μ l volumes with the thermostable Taq-Polymerase, under conditions recommended by the supplier (Boehringer Mannheim, Indianapolis, IN) and 100 ng of template DNA. Thirty-five cycles of amplification were performed on a 9600 Perkin-Elmer thermocycler with 30 s at 94°C, 30 s at the annealing temperatures provided above, and 30 s at 72°C for each cycle. An initial 2-min denaturation at 94°C and a

final extension of 7 min at 72°C were included. PCR products were mixed with 0.1% by volume glycerol dye, resolved on a 7% native acrylamide gel (30 cm by 45 cm), and visualized with ethidium bromide staining.

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Molecular Maps of Red Cell Deformation: Hidden Elasticity and in Situ Connectivity

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Fluorescence-imaged micropipette aspiration was used to map redistribution of the proteins and lipids in highly extended human red blood cell membranes. Whereas the fluid bilayer distributed uniformly (± 10 percent), the underlying, solidlike cytoskeleton of spectrin, actin, and protein 4.1 exhibited a steep gradient in density along the aspirated projection, which was reversible on release from deformation. Quantitation of the cytoskeletal protein density gradients showed that skeletal elasticity is well represented by a grafted polymer network with a ratio of surface dilation modulus to shear modulus of approximately 2:1. Fractionally mobile integral proteins, such as band 3, and highly mobile receptors, such as CD59 as well as glycophorin C in protein 4.1-deficient cells, appeared to be squeezed out of areas dense in the underlying network and enriched in areas of network dilation. This complementary segregation demonstrates patterning of cell surface components by cytoskeletal dilation.

Cell deformation is an integral feature of biological function even for passive capsules like red blood cells in the circulation. Such deformation can cause major rearrangements of structural proteins and lipids and thereby affect biological activity. The distributions of components in deformation provide significant clues to both strong structural connections and the susceptibility of those connections to gradients in chemical potential. A method has now been developed to map molecular distributions in well-defined states of deformation. Though this method was designed to be widely applicable to many types of cells, we used the human red blood cell as a prototypical system to demonstrate how extreme deformations act differentially on proteins and lipids in cell membranes supported by

cytoskeletal networks. The results reveal a highly durable network that can sustain large chemical gradients with little relaxation over long periods (~ 30 min) of deformation.

The erythrocyte is a highly deformable membrane capsule with most of its mechanical features (with the exception of initial shape) largely decoupled from intracellular metabolism. Although many of the cell's component proteins, lipids, and carbohydrates have been identified (1), the functions of and physical connections between molecules in the assembled membrane during deformation are less clear. Key aspects of the deformability of the membrane arise from the underlying cytoskeleton (2), a prototypical assembly of structural proteins (3). Ultrastructural studies on membranes prepared for electron microscopy suggest, for example, that the in situ cytoskeleton is a highly organized, two-dimensional network of spectrin filaments interconnecting $\sim 35,000$ nodes or junctional complexes (3). The isolated junctional complexes consist primarily of three highly conserved and pervasive structural proteins: spectrin, ac-

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