

Adaptive Advantages of Cooperative Courtship for Subordinate Male Lance-Tailed Manakins

Emily H. DuVal*

Department of Integrative Biology and Museum of Vertebrate Zoology, University of California, Berkeley, California 94720

Submitted July 18, 2006; Accepted November 27, 2006;
Electronically published February 6, 2007

ABSTRACT: Male lance-tailed manakins (*Chiroxiphia lanceolata*) cooperate in complex courtship displays, but the dominant (alpha) partner monopolizes mating opportunities. This raises the question of why subordinates (betas) cooperate. Three nonexclusive hypotheses explain the adaptive basis of helping behavior by subordinate males: cooperation may increase (1) subordinates' immediate reproductive success, (2) the reproductive success of close relatives, or (3) subordinates' chances of future reproduction. I demonstrated that beta males rarely sired chicks and were unrelated to their alpha partners but received delayed direct benefits from cooperation; betas had an increased probability of becoming an alpha when compared to males that had not been betas. To investigate the mechanism by which betas attain these adaptive benefits, I examined betas' success in replacing their alpha partners both in natural turnover events and when alphas were experimentally removed. Beta males did not consistently inherit alpha roles in the same territories where they served their beta tenure, arguing that queuing for status does not fully explain the benefits of cooperation for betas. Instead, betas may be apprenticing to develop effective and appropriate displays that enhance their subsequent success as alphas. Complex social affiliations appear to mediate selective pressure for cooperation in this species.

Keywords: lance-tailed manakin, *Chiroxiphia*, cooperative courtship, relatedness, delayed direct benefits.

Cooperative reproduction is a classic paradox in evolutionary biology because cooperating individuals apparently sacrifice their own reproductive potential to assist the reproductive efforts of others. Much theory about cooperation is informed by studies of cooperative breeding, in

which certain individuals delay reproduction to help others raise offspring (Brown 1987; Cockburn 1998; Koenig and Dickinson 2004). In many cases, the paradox of cooperation is resolved by inclusive fitness theory (Hamilton 1964): cooperators help close relatives and benefit through the increased production of nondescendant kin (Griffin and West 2003). However, cooperators may reap direct benefits as well (e.g., Richardson et al. 2002; Dickinson 2004). As cooperators most frequently live in groups of close relatives, it can be difficult to separate the influence of direct and indirect genetic benefits in selecting for cooperative behavior (Clutton-Brock 2002). Species and situations in which helping is decoupled from the immediate family group are therefore of particular interest in investigating the evolution of cooperation.

Although animals are also known to form cooperative alliances in contexts other than helping at the nest, these have received relatively little empirical attention (Cockburn 1998). Cooperative alliances in animal societies may form to increase foraging efficiency (*Parabuteo unicinctus*: Coulson and Coulson 1995; *Phalacrocorax carbo sinensis*: Van Eerden and Voslamber 1995), acquire or defend territories (*Psophia leucoptera*: Sherman 1995; *Alouatta seniculus*: Pope 1990; *Panthera leo*: Grinnell et al. 1995; Heinsohn et al. 1996), or attract or defend mates (*Tursiops* sp.: Krützen et al. 2004; *Meleagris gallopavo*: Watts and Stokes 1971; Krakauer 2005; *Philomachus pugnax*: van Rhijn 1973; Lank et al. 2002; *Chiroxiphia* manakins: Sick 1967). Ecological benefits from cooperative foraging or territoriality are shared among members of a group; in contrast, the reproductive benefits of alliances formed to attract mates are not usually distributed evenly among members of the coalition (Kokko and Johnstone 1999). Such reproductive coalitions are therefore ideal for investigating the complex series of costs and benefits that interact to determine whether subordinate individuals cooperate.

Manakins in the genus *Chiroxiphia* are a particularly interesting example of reproductive coalitions. Male *Chiroxiphia* form long-term cooperative alliances of a dominant alpha male and one or more subordinate beta partners to perform tightly synchronized courtship dances in

* Present address: Max Planck Institute for Ornithology, Department of Behavioural Ecology and Evolutionary Genetics, Postfach 1564, Haus Nr. 5, D-82319 Seewiesen, Germany; e-mail: ehduval@gmail.com.

a dispersed lek mating system (Gilliard 1959; Foster 1977, 1981). A series of previous studies on long-tailed manakins (*Chiroxiphia linearis*) have indicated that beta males do not benefit by helping relatives (McDonald and Potts 1994), and copulations by subordinate males are exceedingly rare (Foster 1981; McDonald 1993). These results contradict findings in other species with cooperative courtship that suggest that individuals receive inclusive or direct fitness benefits from cooperative behavior (Lank et al. 2002; Krakauer 2005). Instead, male *Chiroxiphia* manakins are believed to benefit by eventually inheriting a breeding position from their alpha partner (McDonald and Potts 1994). Until now, however, the genetic measures of direct fitness and experimental assessments of territory inheritance necessary to evaluate definitively the major adaptive hypotheses about subordinate cooperation have been lacking.

To investigate the adaptive benefits of male cooperative courtship, I studied cooperative behavior in the lance-tailed manakin (*C. lanceolata*). I tested the predictions of three classic, nonexclusive hypotheses regarding the types of benefits that betas may obtain through their cooperative behavior: (1) direct genetic benefits: if beta-ranked males receive direct fitness benefits from mating with the females they help to attract, then beta copulations will be detected by genetic paternity testing; (2) inclusive fitness benefits: if beta males receive inclusive fitness benefits by increasing relatives' reproductive success (Hamilton 1964), betas will be more related to their partners than to other males selected at random from the population; and (3) delayed direct benefits: if beta males gain future direct fitness benefits from cooperation, betas should become alphas with greater frequency than do nonbetas. In addition, if delayed direct benefits result from queuing for status (Wiley and Rabenold 1984), betas should ascend to alpha status on the display area where they cooperate.

Similar to other *Chiroxiphia* manakins, pairs of male lance-tailed manakins form multiyear partnerships to execute a stereotyped courtship display that includes both a coordinated duet song and a dance display (DuVal 2007a). Females move freely among display areas in a dispersed lek to assess potential mates. Each display area is typically attended by one alpha, one beta, and on average 10 other adult- and subadult-plumage males that do not display for females (DuVal 2007b). Only alphas appear to have the opportunity to mate because only alphas perform the solo displays that immediately precede copulation (DuVal 2007b), raising the question of why beta males cooperate. This study combines behavioral, genetic, and experimental evidence to assess the adaptive basis of cooperative courtship for subordinate males in lance-tailed manakin coalitions.

Methods

Field Methods

I studied lance-tailed manakins on 46 ha of Isla Boca Brava, Chiriquí, Panamá (8°12'N, 82°12'W). Using mist nets, I captured a total of 457 postfledging individuals during 1999–2004. All birds were color-banded with unique band combinations, and approximately 20 μ L of blood were taken from the brachial wing vein and stored in lysis buffer (DuVal and Nutt 2005). Behavioral observations were conducted in March–July 2000–2002, March–May 2003, and March–April 2004.

Nests were located by daily searches of understory vegetation. Lance-tailed manakins lay a maximum of two eggs per clutch (mean 1.88 ± 0.33 eggs, $n = 170$ nests). A total of 218 nestlings from 130 broods were genetically sampled between 2000 and 2003. The adult female attending a nest was assigned as the female parent of the chicks in that nest.

Male lance-tailed manakins displayed on a dispersed lek consisting of approximately 25–30 display areas spread throughout the study site. Display areas ranged from 525 to 4,500 m² in size and were in auditory but not visual contact (DuVal 2007a). The study site is bordered on two sides by ocean and on the third by cattle pasture that is generally unsuitable for lance-tailed manakin display areas, but remnant forest along the coastline did host additional male territories that females nesting on the study site may have sampled. Each display area was attended by multiple adult and subadult males, though only one alpha male and usually one beta male displayed for females at each area (see “Behavioral Definitions”; DuVal 2007b). Individual behavior at display areas was monitored at 16–28 display areas per year (4,146 h total observation) during 1- or 2-h sessions of all-occurrence sampling (Altmann 1974). Courtship behaviors included “pip flight” attraction displays performed when females approach the display area (DuVal 2007a), “leapfrog” displays by two males, and nine other display elements that are characterized elsewhere (DuVal 2007a). Observers recorded identities of all birds seen as well as frequencies and durations of all display behaviors.

Behavioral Definitions

The alpha and beta partners were the most common duet singers and the only males on the display area to perform displays for females. The alpha in each partnership (1) was present for the greatest proportion of observation sessions, (2) gave the “eek” call ending a bout of display for females, (3) performed solo pip flights, and (4) performed solo slow-flight displays for females. The distinction here of displays “for females” specifically denotes a female present

Table 1: Characteristics of dinucleotide microsatellite loci, based on genotypes of 308 postfledging individuals used to calculate allele frequencies for relatedness analyses

Locus	No. alleles	Size range ^a	Heterozygosity ^b		T_a^c	Null frequency ^d	Genotyping error rate ^e	Reference ^f
			H_o	H_e				
Lan10	5	192–212	.689	.710	54	+.017	.025	DuVal and Nutt 2005
Lan15	2	202–206	.134	.137	50	+.008	.004	DuVal and Nutt 2005
Lan20	8	105–151	.721	.748	54	+.018	.010	DuVal and Nutt 2005
Lan21	6	198–214	.725	.742	52	+.008	.010	DuVal and Nutt 2005
Lan22	7	143–165	.543	.509	55	–.048	.009	DuVal and Nutt 2005
LTM8	4	137–145	.521	.537	54	+.014	.008	McDonald and Potts 1994
LTM15	2	174–194	.387	.448	52	+.072	.039	McDonald and Potts 1994
Lox1	3	397–409	.351	.352	50	+.006	.009	Piertney et al. 1999a
Man3	8	193–225	.767	.752	50	–.016	.015	Piertney et al. 2002

^a Size range of alleles detected at each locus, in base pairs.

^b Observed (H_o) and expected (H_e) proportion of heterozygotes.

^c Annealing temperature for polymerase chain reaction (PCR).

^d Per-locus frequency of null alleles, as calculated by CERVUS (Marshall et al. 1998). No locus was found to deviate from Hardy-Weinberg expectations.

^e Per-locus genotyping error was estimated from 57 to 108 randomly selected individuals that were repeat-genotyped in independent PCR reactions. Individuals were typed on average 2.3 ± 0.98 times. Of 1,848 total PCR reactions, 26 resulted in incorrect allele calls, giving an overall error rate of 0.014 per reaction.

^f Reference in which locus or loci were found.

on the display perch in close proximity to displaying males; similar male behaviors also occurred when no females were present, but these were not interpreted as status indicators. Using these criteria, I assigned alpha and beta status to 13–21 pairs per year (85 display area–years). A logistic regression model for assigning male status, detailed elsewhere, was used to confirm qualitative status assignments for 2001–2004 (DuVal 2007b). All alpha or beta males (and the majority of all individuals) sighted after 2001 were banded. Behavioral data comprised only observations in which all interacting individuals were identified. In each year, 30.7 ± 5.8 males were identified as alphas or betas, but 73.7 ± 18.6 additional adult-plumage males were also present at observed display areas ($n = 4$ years; DuVal 2007b). To characterize the source of males replacing alpha and beta males, I defined two further status classes of adult males: floaters and affiliates. Floater males were adult males that were sighted at three or more display areas but were not consistently present in any one area. Affiliates were adult males that were consistently present at one display area and performed duet songs and participated in dance displays when no females were present but that were neither alpha nor beta at that display area.

DNA Extraction and Genotyping

Genomic DNA was extracted from blood using the DNeasy Tissue Kit (Qiagen). Individuals were genotyped at nine variable microsatellite loci with a combined paternity exclusion power of 0.98 (table 1). Loci showed no evidence of linkage disequilibrium, as determined by GENEPOP, version 3.1d (Raymond and Rousset 1995), using Bon-

ferroni correction of observed P values ($\alpha = 0.001$). Fluorescent-labeled alleles were amplified using polymerase chain reaction (PCR) under the following reaction conditions: 5 min at 94°C; 32 cycles of 30 s at 94°C, 30 s at T_a (table 1), and 45 sec at 72°C; and a final extension of 20 min at 72°C. Reactions were 10 μ L total volume with 5–20 ng of genomic DNA; 0.5 U Taq DNA polymerase (Invitrogen); 1 μ L Taq DNA polymerase PCR reaction buffer (10 mM Tris-HCl, 1.5 mM MgCl₂, 50 mM KCl, pH 8.3); 0.2 μ L each of forward and reverse primers (10 μ M) and 0.2 μ L dNTPs (40 mM). PCR products were visualized on an Applied Biosystems 3730 DNA analyzer.

Analysis of Genetic Data

Genetic estimates of reproductive success were generated by determining paternity of chicks during 2000–2003. Genotypes of all chicks were compatible with their behaviorally assigned mother (see “Field Methods”). To avoid assigning paternity to males that matched offspring by chance (Type I error), paternity was assigned through a combination of maximum likelihood analysis (CERVUS, ver. 2.0; Marshall et al. 1998) and genotypic exclusion. In this approach, a male was assigned as a chick’s father only if he matched a chick with $\geq 95\%$ confidence and was the only perfect genotypic match among the candidate males with positive likelihood scores. Confidence levels for paternity assignment are calculated in CERVUS via a simulation employing population allele frequencies, the number of candidate males, and sampling success. This simulation returns a “delta score” used to resolve the likelihood of paternity between candidate males at a given

confidence level. All simulations were conducted using 10,000 cycles and a 1% genotyping error based on the empirical per-locus genotyping error (table 1). Year-specific allele frequencies and proportion of loci typed (0.98 in all years) were calculated from the genotypes of postfledging individuals that were captured or resighted during the year in question. Separate paternity analyses were performed for each study year, with all alpha and beta males present on the study area designated as candidate fathers. In cases in which one alpha performed courtship displays and duets with two adult males at approximately equal rates, both males were treated as beta partners and as candidate parents in paternity analyses. The proportion of candidates sampled was defined as the number of identified alpha or beta males divided by two times the number of display areas for which behavioral observations were performed (table 2). Not every display area active on the study site was observed in every year, so this is likely an overestimate of candidate male sampling success. Decreasing this sampling parameter decreased the number of chicks assigned to fathers but did not change the relative success of alpha versus beta males.

Paternity analyses assigned 63 of 218 tested chicks (representing 52 of 130 broods) to a known-status male (table 2). Four additional chicks were assigned to three males known to be either alpha or beta at observed display areas but whose exact status was undetermined; these chicks were excluded from tests of alpha versus beta reproductive success. Remaining chicks were unassigned for one of three reasons: mismatches with all males (which may represent mutations, genotyping error, or failure to sample the true father; $n = 42$); complete genotypic matches with multiple candidate males ($n = 87$); or low statistical power to assign paternity ($n = 22$). While genotyping at additional loci may resolve paternity of these chicks, there was no indication that chicks that were assigned paternity were not a random, representative sample of the young produced.

Pairwise relatedness (r) between alpha and beta partners

was calculated using the program Relatedness, version 5.0 (Queller and Goodnight 1989), with background allele frequencies estimated from 308 postfledging individuals. The r values of 58 known alpha-beta pairs were compared with those of 58 artificial pairs drawn at random from observed alpha and beta males. To validate r values for known pairs of close kin, I calculated mother-offspring relatedness using one randomly selected chick for each sampled mother and nestmate relatedness using all two-chick broods.

Alpha Male Removal Experiment

Alpha males were mist-netted and removed from eight randomly selected display areas (four each in 2002 and 2003, removals separated by ≥ 10 days). Removed males were relocated to other islands ($n = 5$) or collected under license as museum specimens ($n = 3$). The two relocated males transferred to the nearest sites (0.75 and 1 km from the study site) returned to their territories (after 17 and >24 days, respectively) and were excluded from analysis of long-term results. Males transferred to more distant sites (>5.25 km from the study site) were not resighted and thus were considered permanently removed. Observations were conducted for 2–6 h/day for the 10 days following each removal to determine when a new male assumed alpha status. To determine the short-term effect of alpha removal on beta behavior, I analyzed the proportion of 10 observation sessions in which each beta performed status-linked behaviors (DuVal 2007b) before (≤ 29 days) and after (≤ 10 days) alpha removal. Proportions were arcsine transformed and compared in a linear mixed effects model with year and display area as random effects in the program R (Venables and Ripley 2002). Because the outcomes of natural turnovers were usually not detected until the following breeding season, I continued to monitor experimental territories in the following field season (10–12 months after manipulation).

Table 2: Summary of paternity analyses by year: CERVUS input parameters and paternity assignment success

	Analysis parameters			No. chicks assigned paternity	Reasons that chicks were not assigned paternity ^a		
	No. candidate males	Proportion candidates sampled	95% Δ		Low confidence, one match	Mismatch most likely male	Match multiple males
2000	40	.83	2.17	10	6	13	10
2001	38	.97	1.36	15	4	13	23
2002	56	.95	1.65	25	7	9	23
2003	60	.95	1.67	17	5	7	31
Totals				67	22	42	87

^a “Match” indicates that all paternal alleles in an offspring’s genotype (determined by subtracting the known maternal alleles) were also identified in the candidate father’s genotype. Exceptions to this at one or more loci were considered “mismatches.”

Results

Current Direct Fitness

If current direct fitness selects for cooperation in lance-tailed manakins, then subordinate males must reproduce during their beta tenure. However, 100% of observed copulations ($n = 34$) were performed by alpha males. Thirty-nine percent (17/44) of alpha males were observed copulating with females at display perches versus none of 52 betas ($\chi^2 = 24.4$, $df = 1$, $P < .001$).

Since observed copulations may not reflect actual mating success (Hughes 1998), I also evaluated beta reproductive success using genetic analyses of paternity. Genetic data showed that beta males did sometimes engage in successful copulations (fig. 1). The demonstration here of successful fertilization by subordinate male lance-tailed manakins confirms that rare copulations may be an important source of direct fitness for some betas. However, very few beta males sired chicks (<4%, $n = 2$ of 52 betas), suggesting that other adaptive benefits are more important in selecting for cooperation in this species.

Indirect Fitness

The indirect fitness hypothesis asserts that beta males partner with closely related alphas, thereby benefiting from cooperation via the increased production of non-descendant kin. This predicts that betas will be more closely related to their partners than expected from random pairing in the population. Contrary to this, dyads of alpha and beta males displaying together were not more closely related than randomly created pairs of males (fig. 2). Observed alpha and beta male dyads were related at $r = -0.01 \pm 0.28$ (95% confidence interval [CI] = -0.08 to 0.06 ; $n = 58$), a level not significantly different from relatedness between randomly drawn males ($r = 0.00 \pm 0.27$, 95% CI = -0.07 to 0.07 , $n = 58$; two-tailed t -test, $t = -0.16$, $df = 1, 114$, $P = .87$). Relatedness values of mother-chick and of nestmate pairs agree with values predicted by their pedigree relationships (mother-offspring, $r = 0.49 \pm 0.17$, 95% CI = 0.45 to 0.53 , $n = 84$; nestmate, $r = 0.45 \pm 0.27$, 95% CI = 0.40 to 0.51 , $n = 86$; two-tailed $t = 1.05$, $df = 1, 168$, $P = .29$), confirming that low r values between alphas and betas reflect a lack of genetic kinship.

Relatedness could nevertheless play a role in selecting for beta cooperation if partnerships composed of more closely related males are more successful in attracting females than are partnerships composed of unrelated males. However, male pairs in which alphas sired chicks were not more closely related than pairs for which no paternity was detected (successful pair, $r = 0.00 \pm 0.26$, 95% CI = -0.13 to 0.13 , $n = 20$; unsuccessful pair,

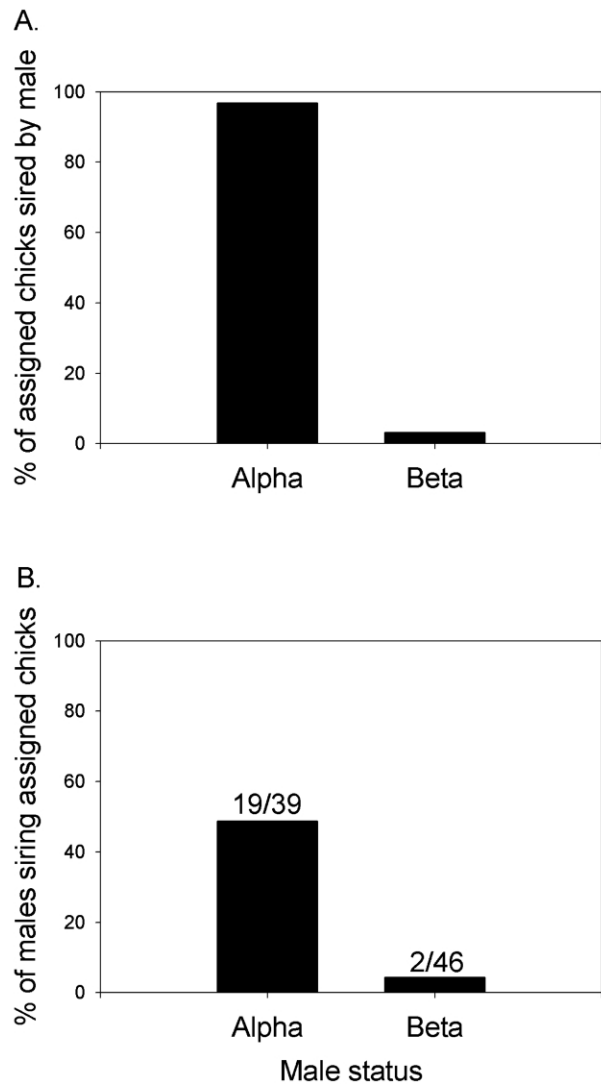


Figure 1: Genetically determined reproductive success of males in different status classes. Of 63 chicks for which paternity could be assigned, all but two (97%) were sired by alpha males ($\chi^2 = 39.8$, $df = 1$, $P < .001$; A). Based on the assigned offspring, 49% of alpha males sired chicks in 2000–2003 versus only 4% of betas (B). Significantly more alpha than beta individuals sired chicks ($\chi^2 = 22.3$, $df = 1$, $P < .001$). These data represent 52 broods by 42 females; chicks sired by betas were from different mothers.

$r = -0.02 \pm 0.30$, 95% CI = -0.11 to 0.08 , $n = 38$; two-tailed $t = -0.20$, $df = 56$, $P = .84$; means \pm SD throughout).

Collectively, these data fail to provide evidence that indirect fitness benefits influence male cooperative courtship in lance-tailed manakins. These results are similar to findings for the long-tailed manakin (McDonald and Potts 1994), suggesting that cooperation in the genus *Chiroxi-phia* cannot be explained by indirect fitness benefits.

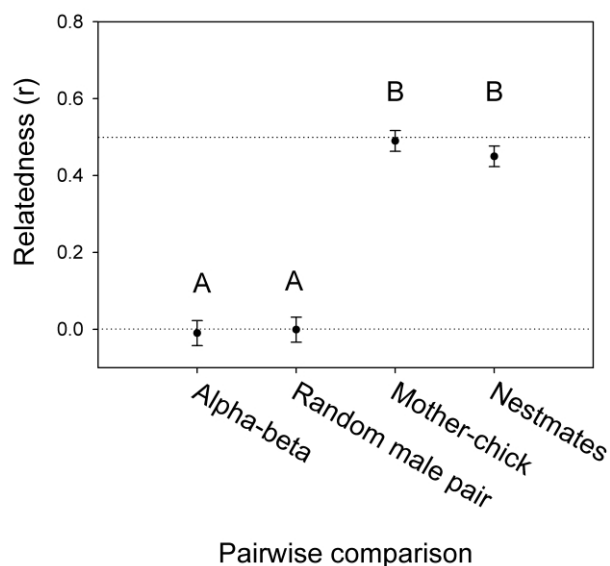


Figure 2: Mean relatedness values of observed alpha-beta partners. Dotted lines indicate expected average r for full siblings or parent-offspring comparisons (0.5) and unrelated individuals (0). Bars indicate standard errors. Common letters denote groups that are not statistically different in relatedness but are significantly different from groups marked by different letters (two-tailed t -test: alpha-beta vs. nestmate $t = -10.03$, $df = 1,142$; alpha-beta vs. mother-chick $t = -13.13$, $df = 1,140$; both $P < .001$).

Delayed Direct Fitness

The critical prediction of the delayed direct fitness hypothesis is that beta males become alphas more frequently than do other, nonbeta adult males. Observations of male status over years support this prediction; of all adult males observed in one year and resighted in the following year, betas became alphas in 10 of 67 cases, while nonbeta adults became alpha in only 6 of 164 cases (15% vs. 4%, $\chi^2 = 6.94$, $df = 1$, $P = .008$).

This finding raises questions regarding the mechanisms that provide cooperating betas with increased opportunities to attain alpha status. Queuing for dominance within a social group is a common mechanism of attaining status in a variety of species (Alberts et al. 2003; Cockburn et al. 2003; Buston 2004), and strict linear queues apparently regulate status succession in the long-tailed manakin (McDonald and Potts 1994). If queuing for alpha status is the primary benefit of cooperative behavior, beta lance-tailed manakins should consistently ascend to dominant status when their alpha partner is lost. To test this prediction, I monitored natural territory turnovers by determining male status at display areas in consecutive breeding seasons. The beta male succeeded his alpha partner in only six (46%) of 13 cases in which an identified alpha male

disappeared in the following breeding season (table 3). Males replacing alphas came from a variety of sources and were not limited to the display area where the replacement occurred (table 4). At the majority of display areas where an alpha or beta was replaced, new males had no known history of affiliation at the display area where the vacancy occurred (58% of 33 replacement events), suggesting that males do not form strictly linear queues for status within display areas. However, queuing may still be an important mechanism for ascending to alpha status, as six (60%) of 10 betas that took over alpha positions did so through succession. Furthermore, local betas are clearly more likely than a randomly chosen male to succeed their alpha partners: the probability of randomly selecting the local beta from the minimum of 80 adult-plumage males present in each year is 0.013, whereas the observed probability of local beta succession (0.46) is 35 times this value.

Alpha Removal Experiment

To explore the extent of queuing for status experimentally, I randomly selected eight display areas (four each in 2002 and 2003) and removed the alpha males from these sites. During the 10 days following the alpha removals, seven of eight beta males (88%) began to exhibit alpha-specific behaviors (fig. 3). Thus, immediately following removals, beta males were significantly more likely to inherit alpha status than expected given the proportion of beta males that inherit following natural losses of alphas (beta inheritance after experimental manipulation = 0.88, expected beta inheritance from natural status changes = 0.46; $G_{(1)} = 6.03$, $P < .02$). To determine whether experimentally promoted betas retained alpha positions over a biologically significant timescale, I revisited six of eight manipulated display areas during the breeding season following the alpha removal (see "Methods"). Of four experimentally promoted beta males that were resighted during the following breeding season, only one had retained alpha status. The remaining three males were each beta to a new alpha partner, either at different display area ($n = 2$) or on the original manipulated territory ($n = 1$). This result strongly suggests that betas that initially took over vacated alpha positions did not typically maintain this status long enough to gain substantive direct fitness benefits.

Discussion

This study suggests that delayed direct fitness is the primary adaptive advantage attained through cooperation by beta male lance-tailed manakins. Both behavioral and genetic evidence confirmed that beta males had a low probability of reproducing during their beta tenure, so current

Table 3: Details of natural transitions in dominance status by display area

Years	No. display areas alpha and beta identified in consecutive years ^a	Changes in alpha or beta individuals at display areas			
		No change	Both replaced	Alpha only replaced ^b	Beta only replaced ^b
2000–2001	11	7	2	0	2
2001–2002	16	5	3	2 (1)	6 (3)
2002–2003	18	12	2	1	3 (1)
2003–2004	14	7	0	2	5
Totals	59	31	7	6 ^c	20 ^d

^a Natural changes in alpha and beta status occurred primarily between field seasons (85%, $n = 33$ changes), and so male status at a given display area had to be assigned in two consecutive years to identify partnership changes. Display areas manipulated in the removal experiment were excluded from this analysis in the season following manipulation but included in subsequent years.

^b Numbers in parentheses represent changes that occurred during a field season in the second of the two consecutive years.

^c A majority of replaced alphas (10 of 13) were never seen again, and these males were presumed dead. Three alphas were resighted following their replacement; these individuals were never seen more than twice and were therefore classified as being of unknown status.

^d Nine of the replaced betas were resighted after they were replaced. Eight moved to a different display area and were identified as alpha (four males), beta (three males), or present as a nondisplaying male (one male). One replaced beta remained at the original display area as a nondisplaying male. Two of four betas that left one display area to become alpha at another had been affiliated simultaneously with both involved display areas in the year before the partnership change.

direct benefits had a relatively small potential to select for cooperation in this species. Male partners were not more closely related than randomly selected pairs of males, and therefore, betas did not receive indirect fitness benefits from their cooperative behavior. This finding is particularly important in light of recent evidence that relatedness influences cooperative courtship (Krakauer 2005) and is related to spatial associations of several lekking birds (Hög-lund et al. 1999; Petrie et al. 1999; Pierney et al. 1999b; Shorey 2002; but see also Gibson et al. 2005). Betas did, however, become alphas more often than nonbeta males, providing a clear benefit to cooperative behavior.

This study confirms previous findings that cooperating partners in *Chiroxiphia* manakins are unrelated (McDonald and Potts 1994) and that factors selecting for cooperation in this genus are distinct from those detected in other species with cooperative courtship displays. Subordinate males in coalitions of wild turkeys cooperate with close relatives and benefit through an increase in indirect fitness (Krakauer 2005), while male ruffs of both cooperating morphs mate with females that they attract with paired displays (Lank et al. 2002). There are, therefore, three emerging pathways for male-male cooperation, each with its own balance of costs and benefits. Courtship behavior in ruffs, wild turkeys, and *Chiroxiphia* manakins apparently represents a case of convergence in which different selective environments result in similar patterns of cooperative male behavior.

In addition to the adaptive benefits addressed here, co-

operating individuals may benefit through reciprocal altruism (Trivers 1971), in which the helper receives a direct benefit from help in kind by the recipient of his altruistic behavior. Reciprocal altruism cannot apply in this system, as alpha and beta partners never switched behavioral roles. However, cooperation by beta male lance-tailed manakins may be viewed as a form of pseudoreciprocity (Connor 1995) or “stakeholder altruism” (Roberts 2005), in which helpers receive future by-product benefits from their beneficiary’s selfish behavior. In the lance-tailed manakin sys-

Table 4: Source of males replacing alphas and betas after natural transitions

	Prior display area same or different from where re- placement occurred?	New alphas	New betas	Total
Alpha	Same	...	0	0
Beta	Same	6	...	6
Affiliate ^a	Same	0	8	8
Alpha	Different	2	0	2
Beta	Different	1	2	3
Affiliate	Different	0	5	5
Floater	Not applicable	2	7	9
Unknown ^a	Not applicable	2	11	13
Total		13	33	46

^a Unknown males were not observed more than five times between banding and assuming alpha or beta status.

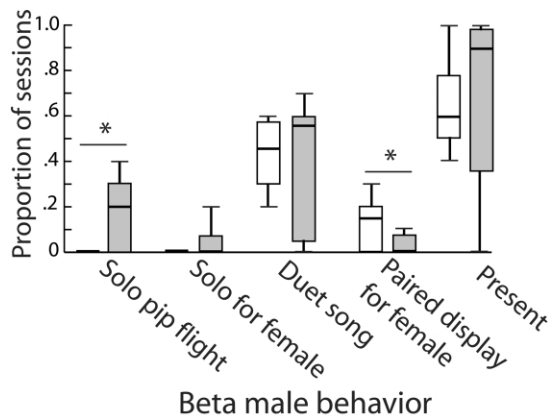


Figure 3: Behavioral changes by beta males in the 10 days following experimental removal of their alpha partners. Bars indicate the proportion of observation sessions in which betas performed behaviors before (*white*) and after (*shaded*) the removal of their alpha partner ($n = 8$ beta males). Horizontal lines indicate median values, boxes surround the 25%–75% intervals of the data, and vertical lines show data within 1.5 interquartile ranges of this interval. Betas performed only solo pip flight (attraction) displays after their partners were removed (linear mixed effects [LME]: $t = -3.12$, $df = 7$, $P = .02$). Several betas also performed solo displays for females on the dance perch, but this increase in display rate was not significant (LME: $t = 1.48$, $df = 7$, $P = .18$). In general, betas continued to perform duet songs and were present for the majority of observation sessions following the removal of their alpha partner (LME: $t = 0.14$ for duets and -0.67 for presence, $df = 7$, $P > .5$ for both). While some males also formed new partnerships and performed two-male displays for females on the dance perch ($n = 3$ former betas), the overall rate of paired displays decreased when compared with display rate when the removed alpha was present (LME: $t = 2.72$, $df = 7$, $P = .03$).

tem, this would be the case if the alpha's current reproductive success increases his beta partner's later reproductive success. Female fidelity to display sites provides one mechanism by which this might occur; in long-tailed manakins, females return to the same display sites to copulate, even when the attending alpha male is replaced (McDonald and Potts 1994). However, such a mechanism would predict high site fidelity by beta males as well, which was not the case in this study. Pseudoreciprocity could also result if betas benefit from interactions with alphas by learning skills involved in successful courtship displays. By helping alphas attract females, betas witness and participate in more successful courtship displays, which may improve their own display competence when they later become alphas. More comprehensive analyses of male reproductive success and female mate choice in multiple years offer one means of testing these possibilities.

Importantly, both natural and experimental changes in male status indicate that territory inheritance does not occur in a linear queue, as previously suggested for the congeneric long-tailed manakin (Foster 1977). Though

queuing cannot be ruled out as a mechanism of attaining alpha status in lance-tailed manakins because local betas were more likely than random males to succeed their alpha partners, the observed results suggest that territory inheritance is modified by factors in addition to male status and presence. Departure from a queuing system could be explained if some betas are unable to maintain alpha status during prolonged interactions with other, competitively superior males. Alternatively, there are three mechanisms by which beta males may obtain benefits from associating with alphas in the absence of a strict queuing system. First, cooperation may provide opportunities to assess territory quality (Boulinier et al. 1996; Hatchwell et al. 1999). However, adult males that do not display for females are frequently observed at display areas (e.g., $38.3\% \pm 15.4\%$ of observation sessions in 2002, $n = 22$ display areas) and are tolerated by the alpha and beta males, suggesting that cooperation in displays is not necessary to gather information about territory quality. Second, cooperative interactions with a variety of males may help betas develop affiliations with a future subordinate partner. However, beta males affiliate almost exclusively with their alpha partner for cooperative duets and dance displays (DuVal 2007b), thereby limiting the time that betas can spend developing affiliations with other males. Third, cooperation may increase skill in display performance (Trainer et al. 2002). Lance-tailed manakin courtship displays are long and complex (DuVal 2007a), and interactions with experienced males may be a critical component of learning display behavior (Collis and Borgia 1993). In accord with this hypothesis, beta males are generally younger than their alpha partners (DuVal 2007b). Consistent performance of courtship displays with a successful alpha partner may allow betas to develop effective and appropriate displays that enhance their subsequent success as alphas. In systems such as this, in which factors other than kinship select for complicated cooperative behavior, long-term strategies to maximize future fitness may depend on social affiliations that reinforce the evolution of complex social structure.

Acknowledgments

I thank J. Dale, J. Dickinson, R. Gibson, M. Hauber, B. Kempnaers, W. Koenig, A. Krakauer, P. Palsbøll, S. Pruett-Jones, and especially E. A. Lacey for helpful comments on this manuscript. D. McDonald offered advice and encouragement in the early stages of this research. Field assistance was provided B. Carter, K. Janaes, R. Lorenz, J. Lorion, K. Manno, E. Reeder, and M. Westbrook. Frank and Y. Köhler kindly allowed field site access. Funding for this research was provided by the National Science Foundation (predoctoral fellowship and dissertation improvement grant); the University of California, Berkeley, Mu-

seum of Vertebrate Zoology, Department of Integrative Biology, and Vice Chancellor for Research fund; Animal Behavior Society; American Ornithologists' Union; American Museum of Natural History; Sigma Delta Epsilon Graduate Women in Science; Manomet Bird Observatory Kathleen Anderson award; Sigma Xi; and the Smithsonian Tropical Research Institute. All protocols followed Animal Behavior Society guidelines for research on animals and were approved by the University of California, Berkeley, Animal Care and Use Committee and Autoridad Nacional del Ambiente, Panamá.

Literature Cited

- Alberts, S. C., H. E. Watts, and J. Altmann. 2003. Queuing and queue-jumping: long-term patterns of reproductive skew in male savannah baboons, *Papio cynocephalus*. *Animal Behaviour* 65:821–840.
- Altmann, J. 1974. Observational study of behavior: sampling methods. *Behaviour* 48:227–265.
- Boulinier, T., E. Danchin, J. Y. Monnat, C. Doutrelant, and B. Cadiou. 1996. Timing of prospecting and the value of information in a colonial breeding bird. *Journal of Avian Biology* 27:252–256.
- Brown, J. L. 1987. Helping and communal breeding in birds: ecology and evolution. Princeton University Press, Princeton, NJ.
- Buston, P. M. 2004. Territory inheritance in clownfish. *Proceedings of the Royal Society B: Biological Sciences* 271(suppl.):252–254.
- Clutton-Brock, T. 2002. Breeding together: kin selection and mutualism in cooperative vertebrates. *Science* 296:69–72.
- Cockburn, A. 1998. Evolution of helping behavior in cooperatively breeding birds. *Annual Review of Ecology and Systematics* 29:141–177.
- Cockburn, A., H. L. Osmond, R. A. Mulder, D. J. Green, and M. C. Double. 2003. Divorce, dispersal and incest avoidance in the cooperatively breeding superb fairy-wren *Malurus cyaneus*. *Journal of Animal Ecology* 72:189–202.
- Collis, K., and G. Borgia. 1993. The costs of male display and delayed plumage maturation in the satin bowerbird (*Ptilonorhynchus violaceus*). *Ethology* 94:59–71.
- Connor, R. C. 1995. Altruism among non-relatives: alternatives to the "Prisoner's Dilemma." *Trends in Ecology & Evolution* 10:84–86.
- Coulson, J. O., and T. D. Coulson. 1995. Group hunting by Harris' hawks in Texas. *Journal of Raptor Research* 29:265–267.
- Dickinson, J. L. 2004. A test of the importance of direct and indirect fitness benefits for helping decisions in western bluebirds. *Behavioral Ecology* 15:233–238.
- DuVal, E. H. 2007a. Cooperative display and lekking behavior of the lance-tailed manakin (*Chiroxiphia lanceolata*). *Auk* (forthcoming).
- . 2007b. Social organization and variation in cooperative alliances among male lance-tailed manakins. *Animal Behaviour*, doi: 10.1016/j.anbehav.2006.05.017.
- DuVal, E. H., and K. J. Nutt. 2005. Isolation and characterization of polymorphic microsatellite loci in the lance-tailed manakin (*Chiroxiphia lanceolata*). *Molecular Ecology Notes* 5:112.
- Foster, M. S. 1977. Odd couples in manakins: a study of social organization and cooperative breeding in *Chiroxiphia linearis*. *American Naturalist* 111:845–853.
- . 1981. Cooperative behavior and social organization of the swallow-tailed manakin (*Chiroxiphia caudata*). *Behavioral Ecology and Sociobiology* 9:167–177.
- Gibson, R., D. Pires, K. Delaney, and R. Wayne. 2005. Microsatellite DNA analysis shows that greater sage grouse leks are not kin groups. *Molecular Ecology* 14:4453–4459.
- Gilliard, E. T. 1959. Notes on the courtship behavior of the blue-backed manakin (*Chiroxiphia pareola*). *American Museum Novitates* 1942:1–19.
- Griffin, A. S., and S. A. West. 2003. Kin discrimination and the benefit of helping in cooperatively breeding vertebrates. *Science* 302:634–636.
- Grinnell, J., C. Packer, and A. E. Pusey. 1995. Cooperation in male lions: kinship, reciprocity or mutualism? *Animal Behaviour* 49:95–105.
- Hamilton, W. D. 1964. The genetical evolution of social behaviour. I, II. *Journal of Theoretical Biology* 7:1–52.
- Hatchwell, B. J., A. F. Russell, M. K. Fowlie, and D. J. Ross. 1999. Reproductive success and nest-site selection in a cooperative breeder: effect of experience and a direct benefit of helping. *Auk* 116:355–363.
- Heinsohn, R., C. Packer, and A. E. Pusey. 1996. Development of cooperative territoriality in juvenile lions. *Proceedings of the Royal Society B: Biological Sciences* 263:475–479.
- Höglund, J., R. V. Alatalo, A. Lundberg, P. T. Rintamäki, and J. Lindell. 1999. Microsatellite markers reveal the potential for kin selection on black grouse leks. *Proceedings of the Royal Society B: Biological Sciences* 266:813–816.
- Hughes, C. 1998. Integrating molecular techniques with field methods in studies of social behavior: a revolution results. *Ecology* 79:383–399.
- Koenig, W. D., and J. L. Dickinson. 2004. Ecology and evolution of cooperative breeding in birds. Cambridge University Press, New York.
- Kokko, H., and R. A. Johnstone. 1999. Social queuing in animal societies: a dynamic model of reproductive skew. *Proceedings of the Royal Society B: Biological Sciences* 266:571–578.
- Krakauer, A. 2005. Kin selection and cooperative courtship in wild turkeys. *Nature* 434:69–72.
- Krützen, M., L. M. Barré, R. C. Connor, J. Mann, and W. B. Sherwin. 2004. "O father: where art thou?"—paternity assessment in an open fission-fusion society of wild bottlenose dolphins (*Tursiops* sp.) in Shark Bay, Western Australia. *Molecular Ecology* 13:1975–1990.
- Lank, D. B., C. M. Smith, O. Hanotte, A. Ohtonen, S. Bailey, and T. Burke. 2002. High frequency of polyandry in a lek mating system. *Behavioral Ecology* 13:209–215.
- Marshall, T. C., J. Slate, L. E. B. Kruuk, and J. M. Pemberton. 1998. Statistical confidence for likelihood-based paternity inference in natural populations. *Molecular Ecology* 7:639–655.
- McDonald, D. B. 1993. Demographic consequences of sexual selection in the long-tailed manakin. *Behavioral Ecology* 4:297–309.
- McDonald, D. B., and W. K. Potts. 1994. Cooperative display and relatedness among males in a lek-mating bird. *Science* 266:1030–1032.
- Petrie, M., A. Krupa, and T. Burke. 1999. Peacocks lek with relatives even in the absence of social and environmental cues. *Nature* 401:155–157.
- Piertney, S. B., M. Marquiss, and R. Summers. 1999a. Characterization of tetranucleotide microsatellites in the Scottish crossbill (*Loxia scotica*). *Molecular Ecology* 7:1247–1263.

- Piertney, S. B., A. D. C. MacColl, X. Lambin, R. Moss, and J. F. Dallas. 1999b. Spatial distribution of genetic relatedness in a moorland population of red grouse (*Lagopus lagopus scoticus*). *Biological Journal of the Linnean Society* 68:317–331.
- Piertney, S. B., L. Shorey, and J. Höglund. 2002. Characterization of microsatellite DNA markers in the white-bearded manakin (*Manacus manacus*). *Molecular Ecology Notes* 2:504–505.
- Pope, T. R. 1990. The reproductive consequences of male cooperation in the red howler monkey: paternity exclusion in multi-male and single-male troops using genetic markers. *Behavioral Ecology and Sociobiology* 27:439–446.
- Queller, D. C., and K. F. Goodnight. 1989. Estimating relatedness using genetic markers. *Evolution* 43:258–275.
- Raymond, M., and F. Rousset. 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity* 86:248–249.
- Richardson, D. S., T. Burke, and J. Komdeur. 2002. Direct benefits and the evolution of female-biased cooperative breeding in Seychelles warblers. *Evolution* 56:2313–2321.
- Roberts, G. 2005. Cooperation through interdependence. *Animal Behaviour* 70:901–908.
- Sherman, P. T. 1995. Social organization of cooperatively polyandrous white-winged trumpeters (*Psophia leucoptera*). *Auk* 112:296–309.
- Shorey, L. 2002. Genetic structuring and the evolution of lekking behaviours in the white-bearded manakin, *Manacus manacus*. PhD diss. thesis. Uppsala University.
- Sick, H. 1967. Courtship behavior in manakins (Pipridae): a review. *Living Bird* 6:5–22.
- Trainer, J. M., D. B. McDonald, and W. A. Learn. 2002. The development of coordinated singing in cooperatively displaying long-tailed manakins. *Behavioral Ecology* 13:65–69.
- Trivers, R. L. 1971. The evolution of reciprocal altruism. *Quarterly Review of Biology* 46:35–57.
- Van Eerden, M. R., and B. Voslamber. 1995. Mass fishing by cormorants *Phalacrocorax carbo sinensis* at Lake IJsselmeer, The Netherlands: a recent and successful adaptation to a turbid environment. *Ardea* 83:199–212.
- van Rhijn, J. G. 1973. Behavioral dimorphism in male ruffs, *Philomachus pugnax* (L.). *Behaviour* 47:153–229.
- Venables, W. N., and B. D. Ripley. 2002. *Modern applied statistics with S-plus*. Springer, New York.
- Watts, C. R., and A. W. Stokes. 1971. The social order of turkeys. *Scientific American* 224:112–118.
- Wiley, R. H., and K. N. Rabenold. 1984. The evolution of cooperative breeding by delayed reciprocity and queuing for favorable social positions. *Evolution* 38:609–621.

Associate Editor: Elizabeth Adkins-Regan
 Editor: Michael C. Whitlock