COSTS AND BENEFITS OF VARIABLE BREEDING PLUMAGE IN THE RED-BACKED FAIRY-WREN

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Abstract.—The red-backed fairy-wren is a socially monogamous passerine bird which exhibits two distinct types of breeding male, bright males that breed in bright red and black plumage and dull males that breed in dull brown plumage. Most males spend their first potential breeding season in dull plumage and subsequent breeding seasons in bright plumage, but a relatively small proportion of males develop bright plumage in their first breeding season. This study quantifies morphology, behavior, and reproductive success of dull and bright males to assess the adaptive costs and benefits of bright plumage while controlling for age. Older bright males (two years of age or older) attempted to increase their reproductive success via copulations with extrapair females, whereas younger (one-year-old) bright males and dull males did not. Thus, older bright males spent less time on their own territories, intruded on neighboring groups with fertile females more frequently, gave more courtship displays, and had larger sperm storage organs than did younger bright males and dull males. Microsatellite analyses of paternity indicate that the red-backed fairy-wren has extremely high levels of sexual promiscuity, and that older bright males had higher within-brood paternity than dull males or younger bright males. Regardless of age, bright males were more attractive to females in controlled mate choice trials than were dull males, and both age classes of bright males obtained higher quality mates earlier in the breeding season than did dull males, when nesting success was higher. In conclusion, although it appears that bright plumage increases access to higher quality mates, age also plays a central role in determining a male's overall reproductive success because of the high levels of sexual promiscuity exhibited by the red-backed fairy-wren.

Key words.—Delayed plumage maturation, *Malurus melanocephalus*, red-backed fairy-wren, sexual promiscuity, sexual selection.

The evolution of bright plumage in male birds has been a subject of intense interest and research in evolutionary biology from the field's inception (Darwin 1871; Wallace 1889) to the present day (reviewed in Anderson 1994). A central theme in evolutionary studies of bright male plumage has been identifying an adaptive benefit to a trait which carries such obvious costs (reviewed in Butcher and Rohwer 1989). In this paper, I characterize and quantify the adaptive costs and benefits of bright breeding plumage in the red-backed fairy-wren *Malurus melanocephalus*, a bird which exhibits two distinct phenotypic classes of breeding male (Schodde 1982; Rowley and Russell 1997).

Red-backed fairy-wrens are small, insectivorous passerines distributed across northern, tropical Australia. The species is socially monogamous, with males and females forming multiyear pair bonds. As with other *Malurus* species, it is likely that red-backed fairy-wrens are sexually promiscuous, with a mating system in which females copulate with males from other groups (extrapair copulations, or EPCs, resulting in extrapair fertilizations, or EPFs). Red-backed fairy-wrens live in sedentary, temporally stable social groups consisting of an adult male and an adult female. Occasionally a breeding pair is joined by an adult male ''helper'' in dull plumage, which has postponed dispersal from its natal territory to assist its parents with subsequent reproductive efforts (Schodde 1982; Rowley and Russell 1997).

Male red-backed fairy-wrens display one of two distinct breeding plumages: ''bright'' plumage, which is glossy black with a large scarlet patch on the back, and ''dull'' plumage, which is a light brown color similar to that of females. For the remainder of the paper, I will refer to males which attempt to breed in bright plumage as ''BRT males'' and males which attempt to breed in dull plumage as ''DULL males.'' Most males spend their first potential breeding season in dull plumage and all subsequent breeding seasons in bright plumage, although some males attain bright plumage in their first breeding season. Thus, there are three cooccurring classes of breeding male: DULL males (all of which are one year of age); BRT-1 males (one-year-old males in bright plumage); and BRT-2 males (bright males two years of age or older). All three classes of male are reproductively capable, and all defend territories, obtain mates, and successfully rear young. As mentioned above, DULL males without mates remain on their natal territories as helpers rather than defending a territory. Dull helpers are reproductively capable, and often obtain breeding territories during the course of the breeding season (thereby changing in status from helpers to breeders) due to female immigration or mortality of established breeders.

The presence of two distinct male phenotypes, one very similar to that of females and one very different, provides an ideal venue in which to test hypotheses about the costs and benefits of bright plumage and the evolution of sexual dimorphism. The fact that one of the plumage types occurs in two age classes allows specific comparisons of the relative importance of age and plumage. This paper tests hypotheses for the current adaptive significance, if any, of bright plumage and age by quantifying and comparing differences between BRT-1, BRT-2, and DULL males in morphology, behavior, attractiveness to females, and reproductive success.

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METHODS

General Field Methods

All field research and experiments were conducted in forests surrounding the Moomin Reservoir, 5 km outside the town of Herberton, on the Atherton Tableland in Queensland Australia (145°25′E, 17°23′S). This study spanned three breeding seasons (October–February) from 1997–2000. During this time I color banded and monitored the population biology of all red-backed fairy-wrens within a contiguous area of 2.5 × 1 km area. I captured over 1000 individuals, each of which I weighed, measured, scored for plumage brightness, and provided with a unique combination of colored leg bands. For individuals measured multiple times within a breeding season, median values for morphological parameters were calculated and used for subsequent analyses. I used an ANCOVA comparing masses (with tarsus length as covariate) as a measure of male condition.

As in most malurids, male red-backed fairy-wrens have a cloacal protuberance where sperm is stored (Mulder and Cockburn 1993; Tuttle et al. 1996). I used three measures of the cloacal protuberance: length (L), or the distance from the anterior portion of the cloacal opening to the posterior edge; maximum depth (D); and maximum width (W), and calculated volume as $\pi \times D/2 \times W/2 \times L$ (Mulder and Cockburn 1993; Tuttle et al. 1996).

To quantify plumage brightness, I scored each male's head, back, tail, belly, and chest (primary and secondary wing feathers remain brown in all birds) on a scale from 1–10 (for a maximum score of 50) according to the proportion of each region covered in bright plumage. This score was doubled to arrive at an overall plumage brightness score, which ranged from 0 (completely dull)–100 (completely bright). For individuals measured multiple times within a breeding season, median values for plumage scores were calculated and used for subsequent analyses. A male was considered BRT if it had a plumage score of 80 or greater, and DULL if it had a plumage score of 20 or lower. A small number of males with intermediate plumage scores (>20 and <80, $n = 14$) were excluded from analyses presented in this paper.

I determined age and survival of birds by multiple retraps and resightings of banded individuals. Individuals were classified by an exact age if I had initially banded them as nestlings or fledglings, or a minimum age if I initially banded them as adults. For most analyses, individuals were classified as either one year of age or two years of age or more.

All successful and most unsuccessful nesting attempts by groups within the study area were monitored each breeding season. Territories were searched and active nests were checked at least once per week to determine clutch size, nesting status and fates of nests. Analyses of nesting success were based on fledging date of each nest, which was estimated for nests which failed prior to fledging based on known lengths of egg incubation and nestling feeding stages (J. Karubian, unpubl. data). A nest was considered to be successful if it fledged one or more live young.

Behavioral Observation

Behavior was quantified in focal animal samples that averaged 28.2 ± 0.12 min (range 15–45 min; goal was 30 min observation periods). Data were collected both as instantaneous scan samples at 60 sec intervals (presence/absence on territory, distance from mate, and activity) and on a continuous basis (displays, departures from territory to intrude on neighboring groups, intrusions onto territory by neighboring males, and vocalizations). If an observer lost sight of the focal individual for more than four min at any point, the sample was terminated and the data were not used. Only breeding DULL males which had obtained a mate and breeding territory are included in behavioral analyses (i.e., helpers are excluded from analysis).

Behavioral data from focal samples were transformed to rate per 30 min, and these rate data were used for subsequent analyses. Average values for each behavioral parameter were calculated for each male in each breeding season, and these values were used in subsequent analyses (t-tests, ANOVA's, and χ^2 tests). Only males with four or more focal samples in a given field season were included in analyses.

Feeding rates at nests with hatched young were recorded in 180 min observation periods by researchers hidden in blinds. Data from nest watches were transformed to rate per 60 min, and these rate data were used for subsequent analyses. The presence or absence of helpers at the nest had no effect on male or female feeding rates (J. Karubian, unpubl. data). Feeding rate for each adult was averaged across all observation periods (usually two) for a given nesting attempt and across multiple nesting attempts by the same individual within the same year.

Molecular Analyses

I collected blood samples (ca. 25 μl) for genetic analyses from all birds captured in mist nets and from nestlings that survived at least five days after hatching. All embryos visible to the unaided eye found in unhatched eggs were also collected for analysis. I analyzed a total of 51 full broods: one (2%) in 1997, nine (17%) in 1998, 24 in 1999 (46%), eight (35%) in 2000, for a total of 144 young. The average clutch size for broods I analyzed was 2.8 ± 0.14 (range 1–4). I analyzed all broods collected from DULL and BRT-1 males, but only a random subset of broods (22%) collected from BRT-2 males. Among the broods analyzed, there was no difference in the relative proportion from BRT-1, BRT-2, or DULL males across 1999 or 2000 (Fisher Exact test; df = 2, $P > 0.5$; there were no known broods from BRT-1 males in 1997 or 1998). Also, there was no difference in the overall proportion of extrapair young across years (ANOVA, $F_{2,0.89}$, $P = 0.4$).

To quantify rates of sexual promiscuity, I used four hypervariable microsatellite loci developed for congeners (Table 1; Double et al. 1997; S. Pruett-Jones and M. Webster, unpubl. data). The four loci used for analysis ranged in allele number from 10–14, and in observed heterozygosity from 0.61–0.89 (Table 1). Genomic DNA was amplified with chemo-illuminescently tagged primers (Perkin Elmer Life Sciences, Boston, MA) in standard 25 μl PCR reactions containing distilled water, PCR buffer solution with the final concentration: 50mM KCl, 1.5 mM MgCl$_2$, 10 mM Tris-HCl (Promega Corp., Madison, WI), 0.15 mM of each nucleotide (Promega), 0.5 μM of each primer, 1 unit of Taq polymerase

TABLE 1. Characterization of four microsatellite loci used to assess paternity.

Locus	Annealing temp. (°C)	No. of alleles	Allele size range (bp)	Observed hetero-zygosity	Prob. of paternal exclusion (P_{ej})
Mcyμ7	60	10	91–113	0.61	0.42
Mcyμ4	55	10	142–160	0.89	0.81
Mcyμ3	50	14	228–263	0.82	0.82
Mspμ4	67	12	105–145	0.71	0.80
				Combined (P_{et}) = 0.997	

(Promega), and approximately 100 ng genomic DNA. This reaction was subjected to 30 cycles of 94°C for 60 sec, $\bar{x}$°C for 60 sec, and 72°C for 45 sec (annealing temperatures, $\bar{x}$°C, for each locus are given in Table 1).

I separated products by size by running them out on 4.5% polyacrylamide gels on 36-cm plates for 120 min at constant voltage on an ABI 377 Automated Sequencer (Applied Biosystems, Foster City, CA). I analyzed the results of these runs using the program Genescan (Applied Biosystems), which determines length of amplified alleles by comparing them to an internal size standard (Rox500, Applied Biosystems). For each individual, I scored each amplified locus and entered the length into a dataset. Once fragment lengths had been amplified and collected for all individuals, I established ranges, or ''bins,'' for each allele of each locus that ranged ± 1 base pair from the median length of the allele. I used these ''binned'' values for each allele in subsequent comparisons of allele length between individuals.

I determined parentage by comparing an offspring's paternal allele (i.e., the allele it did not share in common with its social mother) to the alleles of its putative father. If neither of the social father's alleles matched the offspring's paternal allele, the social father was excluded as a potential sire at that particular locus. Putative fathers excluded as sires did not match the offspring's paternal allele at an average of 2.4 ± 0.24 of the four loci. For all cases in which putative fathers were excluded on the basis of mismatches at ≤2 loci, I reran and reanalyzed both the young and parents at least once. I assumed that social mothers were also biological mothers because I only encountered one brood with mismatches between offspring and social mothers that could not be explained by the presence of null (nonamplifying) alleles (see below). Although it is possible that this brood represented a rare case of intraspecific brood parasitism, I decided not to include the brood in analyses presented here because the possibility that vials were mislabeled could not be excluded.

To characterize the microsatellite loci, I calculated the frequency of each allele from all individuals genotyped ($n = 230$ individuals) and used these frequencies to calculate the expected frequency of heterozygotes (h_e) for each locus. The value h_e was compared to the observed frequency of heterozygotes (h_o) to determine if any null (i.e., nonamplifying) alleles were present. I also calculated the average probability of paternal exclusion (P_{ej}), which is the probability that a randomly selected nonsire male will not match the paternal alleles of an offspring at a given locus (Jamieson 1994). The combined probability of paternal exclusion (P_{et}) for all four loci was high (0.997), making it unlikely that unrelated males would spuriously be assigned as sires. Because red-backed fairy-wrens exhibit a high degree of male philopatry (J. Karubian, unpubl. data), however, P_{et} may overestimate the utility of loci to exclude false sires (Double et al. 1997).

I tested for the presence of null (i.e., nonamplifying) alleles by comparing (h_e) and (h_o) using a standard goodness of fit (χ^2) test. One of the loci (MSPμ4) showed evidence of a null allele ($\chi^2_1 = 10.3$, $P < 0.01$). The frequency of the null allele (r) under the assumption of no null homozygotes was 0.07 (Brookfield 1996). To account for the presence of a null allele at Mspμ4, a putative father and nestling were considered to match if the male and the nestling both appeared homozygous for the maternal allele. The null allele did not alter the analysis of paternity, but there were two broods in which females did not match offspring at Mspμ4. However, these offspring were homozygous for a paternal allele and I assumed that these mother/offspring mismatches were due to the presence of the null allele.

For males with multiple broods in a single year, I calculated mean proportion of extrapair young across all broods by that individual in that year and used that value for subsequent statistical analyses. Two of the broods I genotyped lacked a DNA sample from the female. I included both these broods in my analyses because the putative fathers did not match offspring alleles at ≥2 loci for any of the offspring, making the female genotype moot.

Female Mate-Choice Trials

Female mate-choice trials were conducted in specially constructed mate choice chambers measuring $1.5 \times 0.75 \times 0.75$ m. Birds used in female mate choice trials were captured off the study site and individuals trapped within 2 km of each other were never paired in the same trial. A different female ($n = 20$) was used in each trial, and each female was used only once. A total 14 DULL males (six were used in two different trials), 9 BRT-1 males (one was used in two different trials), and 10 BRT-2 males were used as stimulus birds. Six independent observers gathered data in the trials.

Females were allowed three days to acclimate to captivity and then went through two preliminary trials before being used in a mate choice trial. First, each female spent an hour alone in the mate choice apparatus to allow acclimation. Only those females which visited both sides of the apparatus were used in subsequent trials. Second, to ensure that females were making a sexual choice, each female underwent a preliminary trial in which she was provided with a choice between an adult bright male and a female. Only those females which spent more time associating with adult males than with females ($n = 93\%$) were used in the female mate choice trials presented in this paper.

Female mate choice trials lasted 60 min, during which behavior of the female and the two stimulus males was recorded by an observer hidden in a blind 5 m from the choice apparatus. Time spent associating (defined as being in the side chamber of one of the two stimulus males while moving and/or vocalizing at least once every 5 sec) with each stimulus male was recorded, as well as any vocalizations and displays.

TABLE 2. Morphological and plumage variation in male red-backed fairy-wrens by plumage and age. For each morphological parameter, classes of male denoted by * or by ** are statistically similar to each other and statistically different (P value < 0.017 in t-tests, with Bonferroni corrections for multiple tests) from unmarked classes or classes with a different number of asterix. The symbol ψ denotes a class of male that does not differ statistically from other classes.

	Tarsus (mm)			Tail (mm)			Wing (mm)			Mass (gr)			Cloacal protub. (mm³)			Plumage brightness (%)		
	X	SE	n	X	SE	n	X	SE	n	X	SE	n	X	SE	n	X	SE	n
DULL	22.1	0.1	38	52.2*	0.7	38	41.1	0.2	38	7.8*	0.08	37	107.0*	5.83	34	7.8*	1.1	37
All BRT	22.1	0.1	168	46.2	0.18	158	41.4	0.08	168	7.9	0.04	158	129.2	2.95	153	96.0	0.33	184
BRT-2	22.1	0.05	163	46.2	0.18	153	41.4	0.08	163	7.9	0.04	153	129.2	2.95	148	96.4	0.3	177
BRT-1	21.2*	0.25	5	45.7	1.2	5	41.2	0.34	5	7.9ψ	0.2	5	110.9ψ	16.40	5	84.4**	0.8	7

See J. Karubian (in unpubl. data) for further description of choice trial protocol.

Statistical Analyses

All statistical analyses of morphology, behavior, parental care, nesting success, and sexual promiscuity used each male's yearly average as a data point to avoid psuedo-replication. Data are presented as means ± standard error unless otherwise stated. All tests conducted were two tailed, with Bonferroni corrections for multiple tests where appropriate.

RESULTS

General Information

The relative proportion of BRT-1:BRT-2:DULL males in the core study population from 1998–2000 was 4%:68%:28%. These proportions did not vary significantly between 1999 or 2000, but in 1998 there were no known BRT-1 males. Within DULL males, 61.3% of males ($n = 35$) bred in their first year and 39.7% of males ($n = 23$) did not obtain breeding status and remained helpers throughout their entire first year (only breeding DULL males are considered in the analyses below).

Morphology

DULL, BRT-1 and BRT-2 males did not differ in wing length (ANOVA, $F_{2,224} = 0.68$, $P = 0.51$), but differed in other morphological parameters. Regardless of age, males in nuptial plumage had brighter plumage, larger cloacal protuberances, greater mass, and shorter tails than did males in dull plumage (Table 2).

The three classes of male differed significantly in plumage brightness ($F_{2,241} = 9225$, $P < 0.0001$). Specifically, BRT-1 males were duller than BRT-2 males, but still much brighter than DULL males (pairwise t-test P values all < 0.01).

BRT-1 males were also intermediate between DULL males and BRT-2 males (but closer to BRT-2 males) in cloacal protuberance volume ($F_{2,204} = 18.76$, $P < 0.0001$). BRT-2 males had significantly larger cloacal protuberances than did DULL males ($t_{200} = 6.1$, $P < 0.0001$) and BRT-1 males trended toward having larger cloacal protuberances than did DULL males ($t_{58} = 1.8$, $P = 0.08$). There was no statistical difference between BRT-1 and BRT-2 males in cloacal protuberance volume ($t_{151} = 1.14$, $P = 0.26$).

Tail length differed among the three classes of male as well ($F_{2,204} = 114$, $P < 0.0001$). BRT-1 and BRT-2 males did not differ in tail length, but both had significantly shorter tails than did DULL males (posthoc, pairwise t-test P values both < 0.01; see Swaddle et al. 2001).

BRT-1 and BRT-2 males had the same mass, but only BRT-2 males were significantly heavier than DULL males, perhaps due to the small sample size of BRT-1 males (BRT-2 vs. DULL males: $t_{214} = 3.0$, $P < 0.001$; BRT-1 vs. DULL males: $t_{66} = 0.8$, $P = 0.4$). Interestingly, the difference in mass between DULL and BRT males could be due entirely to differences in testis and seminal glomera mass (i.e., cloacal protuberance volume).

The tarsi of BRT-1 males bordered on being significantly shorter than those of BRT-2 or DULL males ($F_{2,224} = 4.96$, $P = 0.01$, Bonferroni corrected $\alpha = 0.01$; pairwise t-test P values both < 0.017). This difference in tarsus length may represent physiological costs associated with acquisition of bright plumage at a relatively young age, but more likely is an artifact of small sample size of BRT-1 males ($n = 5$).

I compared nutritional state of males by conducting an ANCOVA on mass with tarsus length as a covariate. There was significant variation among the three classes of male in this measure of condition ($F_{3,215} = 10.97$, $P < 0.0001$). Posthoc, pairwise ANCOVA's revealed that BRT-2 males were in significantly better condition than DULL males ($F_{2,211} = 16.63$, $P < 0.0001$). BRT-1 males were in better condition than BRT-2 males ($F_{2,153} = 6.36$, $P = 0.002$), and bordered on being in statistically significantly better condition than DULL males ($F_{2,65} = 4.8$, $P = 0.011$; Bonferroni corrected $\alpha = 0.017$). It is possible, however, that the extremely short tarsi value for BRT-1 males—most likely an artifact of small sample size—is largely responsible for the differences between BRT-1 males and the other two classes of male.

Behavior

Focal samples ($n = 1263$: 329 samples on 23 DULL males, 108 samples on 8 BRT-1 males, and 824 samples on 60 BRT-2 males) suggest that BRT-2 males devote more time to seeking extrapair copulations than do DULL or BRT-1 males (Fig. 1).

BRT-1, BRT-2, and DULL males varied in the amount of time they spent on their own territories (Fig 1; $F_{2,76} = 5.6$, $P = 0.005$). BRT-2 males spent less time on their territories (93 ± 1.0% of time on territory) than did DULL males (99 ± 0.0%; $t_{71} = 3.0$, $P < 0.004$) or, nonsignificantly, BRT-1 males (99 ± 0.0%; $t_{52} = 1.54$, $P = 0.13$). There was no difference in the amount of time BRT-1 and DULL males spent on territory ($t_{30} = 0.4$, $P = 0.7$).

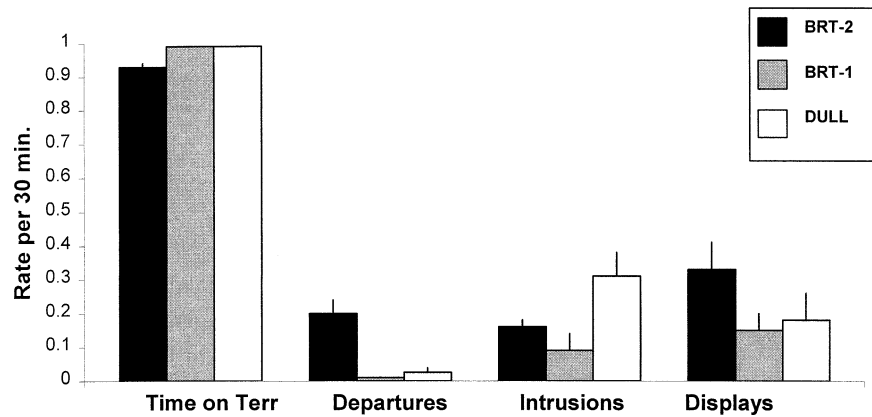There were also differences in the frequency with which

FIG. 1. Behavioral differences between BRT-2 males ($n = 824$ focal samples on 60 males), BRT-1 males ($n = 108$ focal samples on 8 males), and DULL males ($n = 329$ focal samples on 23 males). Shown for each class of male are proportion of time spent on territory (SE $= 0.01, 0.001$, and 0.001, respectively), average rate of departures from territory to intrude on neighboring groups (SE $= 0.02, 0.01$, 0.01, respectively), average rate of intrusions on a male's territory (SE $= 0.02, 0.05$, and 0.07, respectively), and rate of courtship displays (SE $= 0.07, 0.08$, and 0.05, respectively). BRT-2 males differed from DULL males in time on territory, departure rate, and intrusion rate, and from BRT-1 males in time on territory and departure rate (t-tests, $P < 0.05$ in all tests).

BRT-1, BRT-2, and DULL males departed their territories to intrude on neighboring groups (Fig 1; $F_{2,76} = 7.2$, $P = 0.001$). BRT-2 males departed their territories more frequently (0.2 ± 0.02 departures per 30 min) than did DULL males (0.03 ± 0.01 departures per 30 min; $t_{71} = 3.3$, $P < 0.001$) or, nonsignificantly, BRT-1 males (0.01 ± 0.01 departures per 30 min, $t_{52} = 1.9$, $P = 0.07$). Of 537 total intrusions recorded, 518 (96.5%) were by BRT-2 males, 4 were by BRT-1 males (0.7%), and 15 (2.8%) were by DULL males (χ^2 adjusted for the relative proportion of each class of male in the population: $\chi^2_2 = 1025.5$, $P < 0.0001$).

The recipients of these intrusions were mostly groups with sexually receptive females: 82.6% of intrusions were on groups in nest construction (65%) or prenest construction (17.6%) phases of nesting, when females are potentially sexually receptive. Further, across all three classes of male, there was significant variation in male display rate ($F_{2,157} = 6.25$, $P < 0.0001$) and time spent within 5 m of mate ($F_{2,216} = 18.0$, $P < 0.0001$) in relation to nesting stage. Specifically, both display rate and rate within 5 m of mate were dramatically higher during prenest construction (0.7 ± 0.2 and 0.6 ± 0.05 per 30 min, respectively) and nest construction (0.4 ± 0.1 and 0.6 ± 0.03 per 30 min, respectively), when females are potentially fertile, than in incubation (0.08 ± 0.03 and 0.3 ± 0.03 per 30 min, respectively) and feeding of nestlings (0.07 ± 0.06 and 0.3 ± 0.04 per 30 min, respectively), when females are not fertile (posthoc, pairwise t-test values < 0.01). These data suggest that BRT-2 males are focusing their philandering efforts in periods of female fertility, probably in order to maximize EPFs, and that males are mate-guarding during periods of female fertility. There was no difference in the overall rate of departures from territory ($F_{2,221} = 0.3$, $P = 0.9$), presence on territory ($F_{2,225} = 0.6$, $P = 0.7$), or song rate ($F_{2,217} = 1.9$, $P = 0.1$) in relation to nesting stage (i.e., prenest construction, nest construction, incubation, feeding of nestlings). There was no difference in the relative sampling rates of the three classes of male in relation to nesting stage ($F_{2,227} = 1.6$, $P = 0.2$), and all three classes

of male followed the same qualitative pattern presented above.

DULL males tended to be intruded upon more frequently than either BRT-1 or BRT-2 males (0.31 ± 0.07 vs. 0.09 ± 0.05 or 0.16 ± 0.02 intrusions per 30 min), although this difference was not statistically significant after Bonferroni adjustment for multiple tests (Fig 1; $F_{2,88} = 4.35$, $P = 0.016$; Bonferroni corrected $\alpha = 0.008$). The only statistically significant pairwise difference in intrusion rate was between BRT-2 and DULL males ($t_{81} = 2.59$, $P = 0.012$). The very low rate of intrusions recorded for BRT-1 males may be an artifact of sample size and the relative rarity of intrusions. These data suggest that BRT-2 males devote more time to pursuing extrapair fertilizations than do DULL or BRT-1 males, and that DULL males may be the target of their cuckoldry efforts.

An analysis of displays by males ($n = 1,885$ total displays by 67 BRT-2 males, 6 BRT-1 males, and 22 DULL males) further suggests that BRT-2 males are philandering. Although there was no difference in the overall rate of displays by the three classes of male ($F_{2,56} = 1.38$, $P = 0.26$), BRT-2 males' displays tended (nonsignificantly) to have a courtship context (petal presentation, puffbacks directed toward females, and copulatory chases) more often than an aggressive context (aggressive chase, puffbacks directed toward males, and raised crest) compared to displays by BRT-1 and DULL males. For BRT-2 males, an average of $57 \pm 4\%$ of displays had a courtship context, compared to $43 \pm 17\%$ for BRT-1 males and $38 \pm 8\%$ for DULL males ($F_{2,90} = 2.9$, $P = 0.06$). Posthoc pairwise t-test reveal that BRT-2 males bordered on differing significantly from DULL males ($t_{85} = 2.3$, $P = 0.02$; Bonferroni corrected $\alpha = 0.017$), but that BRT-1 males did not differ significantly from either BRT-2 males ($t_{71} = 1.0$, $P = 0.4$) or DULL males ($t_{24} = 0.3$, $P = 0.8$).

There was no difference between BRT-2, BRT-1, and DULL males in the rate of territorial singing (4.8 ± 0.62, 5.6 ± 2.1, and 5.2 ± 0.90 songs per 30 min, respectively; $F_{2,75} = 0.11$, $P = 0.90$), or the percentage of time spent
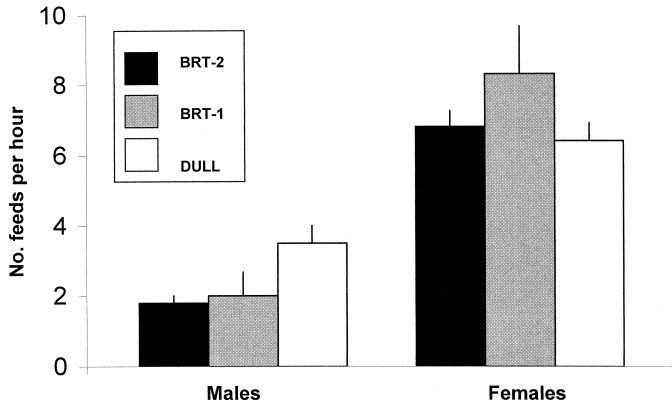
FIG. 2. Parental care at the nest by BRT-2 males and their mates ($n = 52$ pairs, SE = 0.2 and 0.4, respectively), BRT-1 males and their mates ($n = 6$ pairs, SE = 0.7 and 1.4, respectively), and DULL males and their mates ($n = 16$ pairs, SE = 0.5 and 0.5, respectively). DULL males fed at a higher rate than did BRT-2 males ($t_{66} = 4.6$, $P < 0.0001$) or (nonsignificantly) BRT-1 males ($t_{20} = 1.6$, $P = 0.11$). There was no difference in the feeding rates of BRT-1 and BRT-2 males ($t_{56} = 0.6$, $P = 0.6$). There was no difference in feeding rates of females ($F_{2,71} = 1.8$, $P = 0.1$).

within 5 m of mate, ($52 \pm 3\%$, $52 \pm 8\%$, and $47 \pm 4\%$, respectively; $F_{2,75} = 0.57$, $P = 0.57$).

Breeding Behavior and Nesting Success

Average annual feeding rates at the nest were calculated for 74 pairs ($n = 556$ h of observation). There was no difference between the three classes of male in number ($F_{2,71} = 1.87$, $P = 0.2$) or age ($F_{2,71} = 1.63$, $P > 0.2$) of nestlings at nests where data were taken. There was no difference between BRT-2 and DULL males in sampling effort across years ($F_{1,73} = 1.03$, $P > 0.3$), but data were only taken on BRT-1 males in 1999 and 2000 because there were no known BRT-1 males in 1998. However, there was no difference in average annual feeding rate across 1997–2000 ($F_{2,91} = 0.09$, $P > 0.9$).

There was, however, a significant difference in the feeding rates of the three classes of male (Fig. 2; $F_{2,71} = 10.27$, $P < 0.0001$). DULL males fed at a higher rate (3.7 ± 0.5 feeds/ h) than did BRT-2 males (1.73 ± 0.2 feeds/h, $t_{66} = 4.6$, $P < 0.0001$; Bonferroni corrected $\alpha = 0.05/3$ pairwise tests = 0.017) or (nonsignificantly) BRT-1 males (2.05 ± 0.7 feeds/ hr, $t_{20} = 1.6$, $P = 0.11$). There was no difference in the feeding rates of BRT-1 and BRT-2 males ($t_{56} = 0.6$, $P = 0.6$).

This difference in parental care did not translate into higher nesting success for DULL males relative to BRT-1 and BRT-2 males ($F_{2,146} = 0.81$, $P = 0.5$). In fact, although the differences are not statistically significant, DULL males ($n = 25$ males) fledged fewer young (1.15 ± 0.3) and had fewer young survive to three weeks out of nest (0.83 ± 0.24) per nesting effort than did BRT-2 males ($n = 119$ males; 1.38 ± 0.10 and 0.96 ± 0.09, respectively; P values for t-tests > 0.1). BRT-1 males ($n = 6$ males) had the lowest nesting success of all, with only 0.88 ± 0.24 young fledging per nesting effort and 0.54 ± 0.25 young surviving to three weeks out of nest per nesting effort. However, there was no statis-
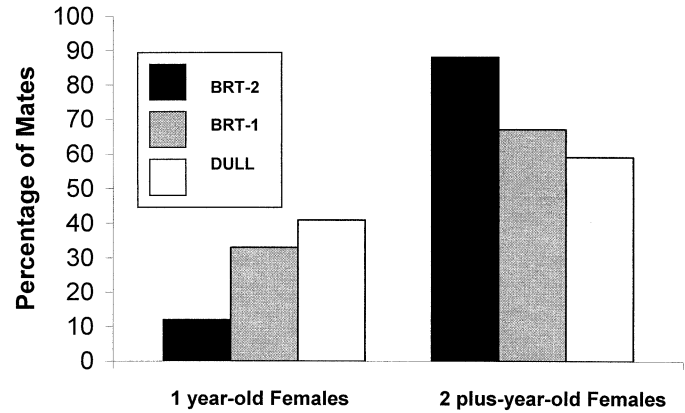

FIG. 3. Percentage of males mated to females one year of age or two or more years of age ($n = 133$ BRT-2 males, 8 BRT-1 males, and 27 DULL males).

tical difference between BRT-1 males and either DULL or BRT-2 males (pairwise t-test P values all > 0.1).

Because nesting success in this species depends in large part on females, I tested for variation among females mated to DULL, BRT-1 and BRT-2 males that might explain the inverse relationship observed between male parental care and nesting success. There was no difference in the feeding rates of females mated to the three classes of male (Fig.2, $F_{2,71} = 1.8$, $P = 0.17$; P value in all posthoc, pairwise t-tests > 0.1). Nor was there any difference in the condition of females mated to the three different classes of males as measured by ANCOVAs comparing female weights with tarsus length as a covariate ($P > 0.02$ in all pairwise comparisons).

There was, however, a difference in ages of females mated to BRT-1, BRT-2 and DULL males (Fig. 3): females mated to BRT-2 males were older (89.5% ≥ two years of age) than females mated to DULL males (59% ≥ two years of age) or to BRT-1 males (67% ≥ two years of age; Fisher Exact test, df = 2, $P < 0.0001$). Posthoc pairwise comparisons reveal that females mated to BRT-2 were significantly older than those mated to DULL males (Fisher Exact test, df = 1, $P < 0.0001$). There was no difference between the ages of females mated to BRT-2 and BRT-1 (Fisher Exact test, df = 1, $P = 0.07$; Bonferroni corrected $\alpha = 0.017$) or between females mated to BRT-1 and DULL males (Fisher Exact test, df = 1, $P = 0.8$).

The difference in ages of females mated to BRT-2, BRT-1 and DULL males is important because older females began nesting earlier in the breeding season when nesting success was significantly higher. On average, egglaying by pairs with BRT-2 males began on 25 October $\pm$ 6.8 days, whereas the average for pairs with BRT-1 males was 06 November $\pm$ 2.6 days, and the average for pairs with DULL males was 23 November $\pm$ 6.1 days ($F_{2,143} = 4.0$, $P = 0.02$). Posthoc, pairwise t-tests reveal that pairs with DULL males nested significantly later than did BRT-2 male pairs ($t_{136} = 2.7$, $P < 0.01$) and, nonsignificantly BRT-1 male pairs ($t_{28} = 2.1$, $P = 0.03$; Bonferroni corrected $\alpha = 0.017$). There was no difference between pairs with BRT-1 and BRT-2 males ($t_{122} = 0.6$, $P = 0.6$).

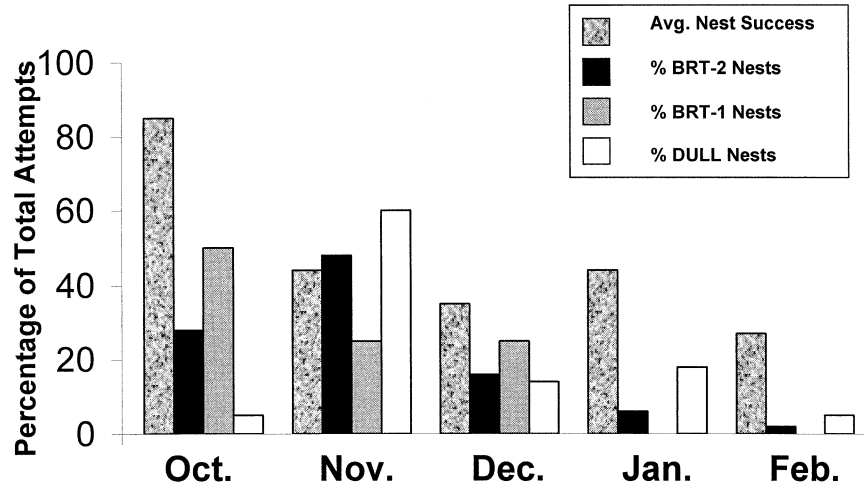There was a steady decline in nesting success over the

FIG. 4. Average nest success for each month of the breeding season and timing of nesting efforts by each class of male.

course of the breeding season (logistic regression with success as dependent variable and Julian day as explanatory variable, $\chi^2_1 = 13.3$, $P = 0.003$), and nesting success for months with fledging dates in the first month of each field season (15 October–15 November, $n = 38$ nests) had more than double the success rate (85%) than nests in any other month of the breeding season (Fig. 4; $n = 109$ nests; $\chi^2_1 = 17.6$, $P < 0.0001$).

Female Mate Choice

Female mate choice trials suggest that females found BRT-1 and BRT-2 males more attractive than DULL males. It is important to note that in these trials, females may be choosing a social mate or a sexual mate (i.e., a male they would prefer to copulate with but not to form a social pair with), or both. In the trials ($n = 20$), females spent more time associating with BRT-1 or BRT-2 males (935 ± 121 sec per trial) than with DULL males (449 ± 87 sec per trial; $t_{18} = 3.1$, $P < 0.001$).

In a subset of trials in which females chose between BRT-1 and DULL males ($n = 10$ trials), females demonstrated a statistically significant preference for BRT-1 over DULL males (918.4 ± 118 vs. 386.6 ± 74 sec associating per trial, $t_{18} = 2.6$, $P = 0.02$). Similarly, females also demonstrated a (nonsignificant) trend toward preferring BRT-2 males over DULL males (950.8 ± 147.6 vs. 510.6 ± 139.5 sec associating per trial, $t_{18} = 1.9$, $P = 0.07$). There was no difference in displays or vocalizations by or directed toward the three classes of male, perhaps because of small sample sizes.

Patterns of mate acquisition from the field further suggest that both BRT-2 and BRT-1 males were more attractive to females than were DULL males. In each of three breeding seasons, all BRT-1 males and more than 98% of BRT-2 males obtained mates by the beginning of the breeding season. In contrast, only 36% of DULL males had a mate at the onset of each breeding season (the remainder stayed on their natal territories as adult helpers). As the breeding season progressed, a growing proportion of DULL males obtained mates due to female immigration onto the study site and creation of breeding vacancies due to mortality among BRT-1 or BRT-2 males. However, nearly 40% of DULL males never obtained a mate during their first breeding season (Fig. 5).

Genetic Analyses

The overall rate of sexual promiscuity in the study population was high (Table 3): 56 ± 6.0% of young ($n = 144$) were the result of an extrapair fertilization (EPF), and 74.5%
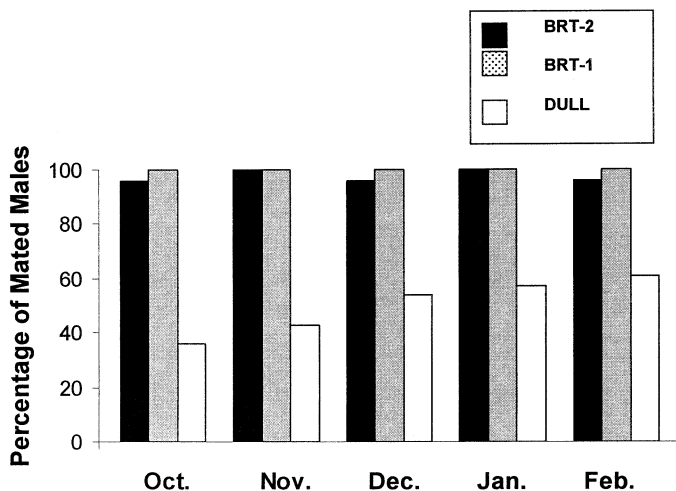

FIG. 5. Mate acquisition in relation to male age and plumage, averaged over three breeding seasons.

TABLE 3. Rates of sexual promiscuity for red-backed fairy-wrens. Shown for each class of male are percentage of extra pair young (with SE and n of nestlings analyzed) and percentage of broods with one or more extra pair young (with n of broods analyzed).

	% Extra pair young			% Broods w/≥1 extra pair young	
	%	SE	n	%	n
DULL males	77	0.08	54	94	19
All BRT males	43	0.08	90	62	34
BRT-2 males	34	0.09	53	50	21
BRT-1 males	69	0.17	37	86	13

of broods analyzed had $\geq$ one extrapair young ($n = 53$ broods by 43 males).

There was significant variation between the three classes of male in the rate at which they were cuckolded (Table 3; $F_{2,40} = 6.34$, $P = 0.004$). Both DULL males ($n = 19$ broods by 16 males) and BRT-1 males ($n = 13$ broods by 7 males) were cuckolded at much higher rates than were BRT-2 males ($n = 21$ broods by 20 males). DULL males, for example, were cuckolded more than twice the rate of BRT-2 males: $77 \pm 8.0\%$ of DULL males' young were the result of an EPF, compared to $34 \pm 9.0\%$ of BRT-2 males' young ($t_{34} = 6.34$, $P < 0.001$). For BRT-1 males, $68.5 \pm 17\%$ of young were the result of an EPF, a rate of similar to that of DULL males ($t_{21} = 0.53$, $P = 0.6$), but (nonsignificantly) lower than that of BRT-2 males ($t_{31} = 1.94$, $P = 0.06$; Bonferroni corrected $\alpha = 0.017$). In a limited number of cases where samples were obtained for individuals which bred as DULL males in one year and BRT-2 males in a subsequent season ($n = 5$ individuals), cuckoldry rates were higher for DULL males in all cases ($P = 0.03$).

There was also significant variation among the three classes of male in the proportion of broods with at least one extrapair young (Table 3; Fisher Exact test, df = 2, $P < 0.001$). Again, BRT-2 males had a lower proportion of broods with one or more extra-pair young (50%) than did DULL males (94%; Fisher Exact test, df = 1, $P < 0.01$) or (nonsignificantly) BRT-1 males (86%; Fisher Exact test, df = 1, $P = 0.11$). There was no difference between DULL and BRT-1 males in the proportion of broods with one or more extra-pair young (Fisher Exact test, df = 1, $P = 0.5$).

DISCUSSION

A fundamental difference between BRT-2, BRT-1 and DULL male red-backed fairy-wrens is that BRT-2 males actively seek to increase their reproductive success via extrapair fertilizations (EPFs), while DULL and BRT-1 males do not. Most traits in which BRT-2 males differ from DULL or BRT-1 males, particularly in morphology (e.g., larger cloacal protuberance volume) and behavior (e.g., decreased time on territory, increased departure rate to intrude on neighboring groups with fertile females, and increased courtship displays) can be seen as causes or consequences of this difference in reproductive strategy.

Although a measure of inclusive male reproductive success (i.e., intra- and extrapair paternity) was not available, all indications are that the differences exhibited by BRT-2 males do in fact result in higher reproductive success. BRT-2 males had reduced rates of cuckoldry within their own broods and also had higher pairing success with females in mate choice trials and in the field than did DULL males. Females mated to BRT-2 males were older than females mated to DULL males (Fig. 3) and began nesting earlier in the breeding season, when nesting success was higher. Thus, despite providing less parental care and spending less time on their territories, BRT-2 males had higher within-pair paternity and were mated to higher quality females than were DULL males.

These findings emphasize the importance of using molecular analyses of paternity to quantify reproductive success because although the red-backed fairy-wren is a socially mo-

nogamous species, most of the traits studied in this paper appear to be adaptations related to sexual promiscuity. While *Malurus* species are well known for high levels of sexual promiscuity (Brooker et al. 1990; Mulder et al. 1994), the red-backed fairy-wren is the first species outside of the "blue wren" clade for which data on promiscuity have been published (Rowley and Russell 1997). Levels of cuckoldry reported here reveal a level of promiscuity comparable to but slightly lower than that of superb fairy-wrens, *Malurus cyaneus*, in which 76% of young were the result of an EPF (Mulder et. al 1994). Females of other *Malurus* species have nearly complete control over paternity (Double and Cockburn 2000; Pruett-Jones, unpubl. data), and this is likely to be the case for red-backed fairy-wrens as well. In over 3000 h of observation, my field assistants and I witnessed only two copulations, suggesting that female red-backed fairy-wrens may be copulating with extragroup males in predawn forays, as has been reported for *M. cyaenus* (Double and Cockburn 2000).

The increased reproductive success of BRT-2 males relative to DULL and BRT-1 males depends on two, interrelated factors: plumage and age. The relationship between plumage and age is a complicated one, but the comparison of BRT-1 and DULL males (both of which are the same age, one year old, but differ in plumage) and BRT-1 and BRT-2 males (both of which have the same plumage, but differ in age) provides some tentative insights into the relative importance of the two. BRT-1 males share morphological traits in common with BRT-2 males, yet in terms of reproductive strategy BRT-1 males appear to be more similar to DULL males. Like DULL males, BRT-1 appeared not to actively pursue EPFs and were cuckolded at much higher rates than were BRT-2 males. Thus, the actual reproductive success of BRT-1 males may be only marginally higher than that of DULL males.

In Karubian (unpubl. ms.), I demonstrate that bright plumage carries a cost of increased levels of aggression relative to dull plumage, as well as probable costs associated with maintenance of nuptial plumage (Peters 2000; Peters et al. 2000). These costs to bright plumage, coupled with low levels of paternity by BRT-1 males, raise an obvious question: What are the benefits to first year males of attaining bright plumage (Greene et al. 2000)?

Female choice trials and pairing success in the field provide a partial answer to this question. Although BRT-1 males do not invest in EPFs and suffer much higher rates of cuckoldry than do BRT-2 males, they are still more attractive to females than are DULL males. As a result, BRT-1 males are more likely to obtain a mate than are DULL males (Fig 5), and may also obtain higher quality mates. Relative to females mated to DULL males, the mates of BRT-1 males were older and nested earlier in the breeding season when nesting success was higher. Because red-backed fairy-wrens form lifelong social bonds and "divorce" is rare, an important benefit of developing bright plumage in the first breeding season might be the acquisition of an older, higher quality mate who will produce more young in subsequent years, when within-brood paternity increases. Long-term monitoring of the population and documenting inclusive male fitness (i.e., within-pair and extrapair reproductive success) will be an important

next step in our understanding of fitness consequences of different plumage types.

In the superb fairy-wren (*M. cyaenus*), male reproductive success is also positively associated with age but the most important determinant of male's reproductive success is molt date (Dunn and Cockburn 1999, Green et al. 1995). Male *M. cyaenus* that begin to molt into nuptial plumage earlier have higher within-pair and extrapair reproductive success, and molt is seen as an honest, sexually selected signal of male quality (Dunn and Cockburn 1999, Green et al. 2000, Mulder and Magrath 1994). Unfortunately, I arrived at my study site after most males had initiated their molt into nuptial plumage and thus could not analyze the effects of initiation of molt on reproductive success in the red-backed fairy-wren. However, I did record molt during the breeding season. During the breeding season, BRT-1 males continued to molt into bright plumage long after most BRT-2 males had completed their molt (Karubian, unpubl. data), suggesting that the extent of male plumage, and perhaps molt date, is an important sexually selected trait in red-backed fairy-wrens.

The causal relationship between acquisition of bright plumage and mating status in one-year-old males is unclear. It could be the case that one-year-old males develop bright plumage because they have obtained a mate prior to the pre-breeding season molt, whereas DULL males retain dull plumage because they fail to obtain a mate. Alternatively, it could be the case that BRT-1 males obtain a mate because they have developed bright plumage in the prebreeding season molt, whereas DULL males are less successful in obtaining mates because they have not obtained bright plumage.

The first of these scenarios raises the possibility that plumage color may be socially constrained in one-year-old males (Reyer et al. 1986). In this scenario, one-year-old males which remain as subordinate males (i.e., helpers) in their natal groups retain dull plumage, whereas those which become dominant males in their own group by obtaining a mate develop bright plumage. The pattern of mate acquisition in relation to plumage type (Fig. 5) lends some support to this idea. Nearly all breeding DULL males underwent their pre-season molt while they were subordinate helpers and only later obtained breeding status via female immigration or mortality among established breeding males. Because they had already molted, DULL males were constrained to retain dull plumage for the remainder of the breeding season.

In all cases in which one year old males developed bright breeding plumage, however ($n = 8$), the males had already obtained mates and breeding status before I arrived at the onset of the breeding season. This pattern suggests that social status (subordinate helper in a group or dominant breeder in a pair) may influence the development of bright plumage in one-year-old males. In *M. cyaenus*, where all males develop bright plumage in their first potential breeding season but vary in timing of molt, there was no evidence for social suppression of molt date (Mulder and Magrath 1994). In the same species, however, testosterone is necessary and sufficient for the production of nuptial plumage, suggesting a mechanism by which nuptial plumage could be under social control (Peters et al. 2000) The ideal test to determine the relationship between plumage acquisition and mating status is an experiment in which mating status (paired or unpaired) is manipulated for a group of yearling males and plumage acquisition is recorded.

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