Plumage color and reproduction in the red-backed fairy-wren: Why be a dull breeder?

Michael S. Webster,[a] **Claire W. Varian,**[a] **and Jordan Karubian**[b]
[a]School of Biological Sciences and Center for Reproductive Biology, Washington State University, Pullman, WA 99164-4236, USA and [b]Center for Tropical Research, Institute of the Environment, University of California at Los Angeles, La Kretz Hall, Suite 300, Box 951496, Los Angeles, CA 90095, USA

Males of many species can breed in distinct alternative phenotypes; for example, in many birds some males breed in dull plumage while others breed in bright plumage. Because females often appear to prefer brighter males, it is unclear why some males breed in dull plumage. Males in dull plumage might enjoy enhanced within-pair reproductive success if they can gain access to better breeding territories, or they might have relatively high extrapair reproductive success if they are better able to intrude on the territories of other males. To test these possibilities, we examined the reproductive consequences of plumage color in the red-backed fairy-wren (*Malurus melanocephalus*), a species in which males can breed in either bright plumage or dull plumage or serve as nonbreeding auxiliaries. Male plumage color was distributed bimodally and was loosely associated with age, such that some males molted into bright plumage a year or more earlier than others. Both male phenotypes were cuckolded at similar rates, but bright males sired significantly more extrapair young than did dull males, and this effect was independent of age. Thus, 1-year-old males who bred in dull plumage had low seasonal reproductive success compared with same-aged males who bred in bright plumage. These results suggest that males may not reap any fitness benefits by breeding in dull coloration, compared with breeding in bright plumage, but rather may be constrained to breed in suboptimal plumage by the timing of plumage acquisition. *Key words:* alternative breeding strategies, delayed plumage maturation, extrapair paternity, fairy-wren, *Malurus melanocephalus*, plumage coloration. *[Behav Ecol]*

In many taxa, males within a single population exhibit discontinuous variation in reproductive phenotypes, with some males being ornamented and others being more similar to females in appearance (Gross 1982; Oliveira et al. 2005; Emlen et al. 2007). The coexistence of such alternative types is enigmatic because, all else being equal, we expect selection to lead to the fixation of the most-fit phenotype. In some cases, variation might be maintained by balancing selection such that the alternative male types have approximately equal fitness at equilibrium, but examples are rare (Gross and Charnov 1980; Gross 1985; Shuster and Wade 1991). More typically, male sexual phenotypes appear to be context-dependent conditional strategies with unequal fitness. In these cases, the ornamented male phenotype appears to have high mating success and low-quality males (e.g., socially subordinate and/or in poor physiological condition) adopt the less ornamented phenotype as a "best-of-a-bad-job" tactic (Brockmann 2001). However, most studies have focused on the proximal consequences of variation in reproductive types, and it is often unclear how male phenotype affects fitness. Indeed, it is possible that males adopting the alternative phenotype enhance some components of fitness that partially, or even wholly, counterbalance apparent reductions in mating success.

Plumage color in birds has emerged as a model system for studying intrasexual variation in sexual signals (Hill and McGraw 2006), and in many species, males show pronounced differences in nuptial plumage brightness. This plumage variation is often associated with age (delayed plumage maturation), but typically, there is considerable variation in plumage color within an age class (e.g., Greene et al. 2000); in other cases, coloration may not be correlated with age at all (Badyaev and Duckworth 2003). Studies using a variety of approaches have clearly demonstrated 2 proximal consequences of variable male plumage coloration: adult males are typically less aggressive toward dull-colored males (e.g., Rohwer 1978; Flood 1984; Hill 1989; Huhta and Alatalo 1993; Muether et al. 1997), whereas females often show a behavioral preference for more brightly colored males (e.g., Hill 1988, 1990; Sætre et al. 1994; McGraw et al. 2001). However, few studies have examined how these proximal effects translate to male fitness (but see Conover et al. 2000; Greene et al. 2000; Berggren et al. 2004). Thus, for many species, it is unclear whether males gain fitness benefits from dull coloration that may partially or fully compensate for reduced mating attention from females.

The recent discovery of extrapair paternity (EPP) has added a new dimension to this issue by suggesting relatively subtle yet important ways that plumage color might affect male reproductive success within a season. For example, Greene et al. (2000) demonstrated that older male lazuli buntings (*Passerina amoena*) allow relatively dull 1-year-old males to settle near them, but not brighter 1-year-old males. The older males benefit because they can cuckold the young (dull) males, who in turn benefit by obtaining higher quality breeding territories that produce more offspring (see also Morton et al. 1990). Thus, dull coloration may enhance the within-pair reproductive success of some males.

An alternative and relatively unexplored hypothesis is that dull coloration gives males a direct advantage in gaining EPP. If dull coloration makes males more cryptic or in some other way allows males to avoid aggression from conspecific males, then dull-colored males may be better able to gain access to neighboring territories and thereby sire extrapair young (EPY). Recently, Delhey et al. (2007) showed that male blue

tits (*Cyanistes caeruleus*) with less ultraviolet coloration are better able to sire EPY, apparently because they are better able to intrude onto the territories of other males. However, in this case, it was unclear whether the effect was due to plumage color or some other factor (e.g., age), and an experimental approach yielded conflicting results (Delhey et al. 2007). Few other studies of birds have explored the possibility that cryptic coloration enhances extrapair mating success, but female-like appearance has been shown to yield direct reproductive benefits in other taxa (Dominey 1980; Norman et al. 1999; Shine et al. 2001) and may also in birds (Slagsvold and Saetre 1991; Sætre and Slagsvold 1996).

The red-backed fairy-wren (*Malurus melanocephalus*) is an Australian passerine in which adult males adopt 1 of 3 alternative phenotypes: "bright breeders" acquire a mate and breed in bright (red and black) nuptial plumage, "dull breeders" also acquire a mate but breed in dull, female-like plumage, and "auxiliaries" have dull plumage but remain as helpers on their natal territory (see Karubian et al. forthcoming). Plumage acquisition in this system is flexible (Karubian 2002), with some males molting into bright plumage a year or more earlier than do other males (see below). Karubian (2002) demonstrated that, compared with dull (younger) breeding males, bright (older) breeding males pair earlier in the breeding season, invest relatively more in mating effort than parental effort, are preferred by females in behavioral choice tests, and are cuckolded at lower rates (but see below). These results suggest that bright males have higher fitness than do dull males, but due to limited sample size, Karubian (2002) was unable to separate the effects of plumage coloration from those of age and also was unable to assign parentage to EPY. Thus, important fitness benefits of dull coloration to breeding males remain unexplored.

Elsewhere (Karubian et al. forthcoming) we demonstrate that dull males receive less aggression from conspecifics than do bright males but that plumage color does not have strong effect on male survival. Reduced aggression from conspecifics, though, might increase the ability of dull males to gain access to neighboring territories to gain EPP. In this paper, we more fully document the relationship between male plumage color and breeding status as well as age and also examine the effects of male plumage color on within-pair and extrapair reproductive success. These analyses, combined with those of Karubian et al. (forthcoming), directly test whether dull males obtain benefits that raise their fitness to near that of bright males or whether males breed in dull plumage as a best-of-a-bad-job strategy.

MATERIALS AND METHODS

Study species and general field methods

The red-backed fairy-wren is a small (ca., 8 g) insectivorous passerine that ranges across northern and eastern Australia (Rowley and Russell 1997). This species inhabits open woodlands and grasslands, with females building domed nests in tall grass (Schodde 1982). Like most other species of fairy-wren (genus *Malurus*), red-backed fairy-wrens breed cooperatively, with sons often staying on their natal territory to assist parents in raising subsequent broods (Rowley and Russell 1997). Previous genetic studies have shown that EPP is very common in this species (Karubian 2002), similar to all other *Malurus* studied to date (Rowe and Pruett-Jones 2006).

We studied a population of red-backed fairy-wrens breeding in open forests surrounding the Herberton Shire Reservoirs on the Atherton Tablelands in Queensland, Australia (145°25′E, 17°22′S). Birds in this population start breeding in the early rainy season (typically early October) and continue breeding until the heavy cyclone rains begin (typically early February). We monitored the breeding of this focal population for every breeding season from 1998 to 2000 and 2003 to 2006 (breeding seasons are designated by the year that breeding ended; i.e., the 1998 season began October 1997 and extended to February 1998). During each breeding season, we captured most adults and marked them with individually specific combinations of colored leg bands and an Australian Bird and Bat Banding Scheme numbered aluminum band. At the time of capture, we measured several morphological traits, including tarsus length, wing length, bill measures, and weight, and also collected a small (ca., 20–50 μl) blood sample from the wing or tarsus vein for genetic analyses. All blood samples were stored in lysis buffer (White and Densmore 1992) at 4 °C.

We scored plumage coloration of captured birds using the system described in Karubian (2002). Briefly, each bird's body was divided visually into 5 parts (head, back, belly, chest, and tail), and each area was scored on a scale of 1–10 for the proportion of that area that was in bright (jet black or crimson red) or dull (brown) plumage. These scores were then summed and multiplied by 2 to produce an overall brightness score ranging from 0 (completely dull) to 100 (completely bright). To verify the consistency of our plumage color score, 2 different observers independently scored a subset of males ($n = 27$), and in these cases, the scores of the 2 observers were strongly correlated ($r = 0.994$, $P < 0.0001$). For most analyses including plumage color, we used the plumage color score, which is a continuous measure, but for some analyses, it was necessary to categorize plumage color. For these analyses, we placed each male into a plumage class based on plumage color score: dull males had brightness scores less than 33, intermediate males had plumage scores between 33 and 66, and bright males had plumage scores greater than 67. Although this categorization is somewhat arbitrary, it is unambiguous and our results would not be affected greatly by using somewhat different cutoffs between categories because few males had intermediate plumages (see below).

We were able to determine social groupings of banded individuals unambiguously through daily observations of behavioral interactions. For groups with more than 1 male, we defined the dominant breeding male as that male who spent the most time with and sang with the group's breeding female; other males in the group were defined as auxiliary helpers. In all cases, these designations were consistent with known pedigree information (e.g., the auxiliary was typically a male offspring from a previous season). We monitored the breeding activity of each group through daily observations of nesting behavior and by searching appropriate areas for nests. Nests were monitored by brief visits once every 3 days. When nestlings were approximately 6 days old, we banded and measured them (weight, tarsus) and collected a blood sample for genetic analyses.

Genetic analysis of paternity

We assessed paternity of all nestlings sampled using a panel of 10 microsatellites isolated from other species of birds (Table 1). DNA was extracted from blood samples using a standard phenol:chloroform protocol (Westneat 1990). To amplify each microsatellite locus for an individual, we added 1 μl of extracted DNA suspended in sterile water (ca., 50 ng genomic DNA) from each individual to a 10-μl polymerase chain reaction (PCR) that contained 0.15 mM deoxynucleoside triphosphate (each), 0.50 μM primers (each, 1 primer being labeled with a fluorescent dye), 3.0 mM MgCl$_2$, 2.5 units *Taq* polymerase, and 1× PCR reaction mix (ABI, Foster City, CA). Following an initial 3 min denaturation at 94 °C, the reaction mix went through 30 cycles of 94 °C for 60 s, X °C for 60 s, and

Table 1

Characterization of microsatellite loci used for parentage analyses

Locus	Annealing Temp (°C)	No. of alleles (x_i)	Heterozygosity Expected (h_e)	Heterozygosity Observed (h_o)	Probability of maternal exclusion	Probability of paternal exclusion	Null allele frequency
$Mcy1$	58	6	0.743	0.716	0.341	0.520	0.019
$Mcy2$	64	6	0.342	0.362	0.062	0.194	−0.035
$Mcy3$	55	18	0.903	0.691[a]	0.671	0.803	0.132
$Mcy4$	55	10	0.860	0.825	0.559	0.720	0.019
$Mcy7$	58	12	0.745	0.709	0.379	0.566	0.024
$Msp4$	65	20	0.870	0.786	0.602	0.752	0.050
$Msp6$	53	9	0.784	0.749	0.403	0.581	0.021
$Ase9$	63	10	0.804	0.770	0.461	0.638	0.021
$Cuu28$	60	18	0.691	0.562[a]	0.322	0.515	0.098
$Phtr3$	55	5	0.573	0.219[a]	0.169	0.288	0.445

Data shown for 2005 analyses (n = 309 individuals); patterns were similar for other years. Loci were characterized using the program CERVUS 2.0 (Marshall et al. 1998). Probability of maternal exclusion is the probability that a randomly selected adult will not match the nestling at a locus (when neither parent is known), and probability of paternal exclusion is the probability of excluding a randomly selected unrelated male as the sire, given the genotype of the mother and nestling. References for microsatellite primers are as follows: All Mcy loci from Double et al. (1997), Msp primers from Webster et al. (2004), $Ase9$ from Richardson et al. (2000), $Cuu28$ from Gibbs et al. (1999), and $Phtr3$ from Fridolfsson et al. (1997).

[a] Significantly different from expected, goodness-of-fit tests, df = 1, $P < 0.05$.

72 °C for 45 s, where X was the optimized annealing temperature (Table 1). After PCR, products from 1 to 4 loci labeled with different dyes (1998–2000: 6FAM, HEX, and TET; 2003–2005: 6FAM, PET, VIC, and NED) were combined with formamide and a size standard (1998–2000: GeneScan-500 TAMRA size standard; 2003–2005: GeneScan-500 LIZ size standard, both from Applied Biosystems) for separation in an ABI 3730 automated sequencer (for the 1998–2000 samples, PCR products were separated on an ABI Prism 377 automated sequencer). Fragment sizes were calculated with GeneScan (1998–2000) or GeneMapper (2003–2005) software (Applied Biosystems) and verified by eye.

All microsatellites used for parentage analyses were highly polymorphic and informative for parentage analyses. For example, for the 2005 samples, we found a mean of 11.4 alleles per locus and a mean expected heterozygosity of 0.732 (Table 1). Allele frequencies did not deviate significantly from Hardy–Weinberg expectations for most loci, but 3 loci ($Mcy3$, $Cuu28$, and $Phtr3$) appeared to have a high frequency of null alleles (Table 1); the possibility of null alleles was taken into account when assessing parentage (see below). The average probability of excluding a randomly chosen female as the mother (i.e., the probability that the female would not possess 1 of the offspring's alleles at the locus in question) was high, with a combined probability of exclusion of 0.9960 for nondams. Similarly, the combined probability of paternal exclusion for these loci (following Jamieson 1994) was 0.9999.

To assign the parentage of each nestling, we assumed that each breeding female was a biological parent of the nestlings in her own nest and assessed the validity of this assumption by examining allele mismatches between females and nestlings. We used CERVUS 2.0 (Marshall et al. 1998) to select the male from the population who, based on genetic evidence, had the highest likelihood of being the sire. CERVUS does this by calculating a log likelihood score (LOD) for each male based on the offspring and maternal genotypes and taking into account scoring errors (e.g., due to null alleles). For each paternity assignment, we used a "total evidence" (Prodöhl et al. 1998) approach to determine whether we felt the CERVUS assignment was reasonable. In most cases, we accepted the CERVUS assignment if the selected male had 0 or 1 mismatch with the nestling, but we rejected the CERVUS assignment if the selected male showed 2 or more mismatches. In addition, we

rejected the CERVUS assignment and assigned paternity to a male with lower LOD score under 3 circumstances: 1) if both males had similar LOD scores but the lower ranked male had fewer mismatches, 2) if both males had a single mismatch but the lower ranked male's mismatch was consistent with the presence of a null allele, and 3) if the males had the same low number of mismatches (0 or 1) and similar LOD scores, but independent evidence suggested that the lower ranked male was a more likely sire. In this last case, we considered whether either male was the social father, whether either male sired other young in the nest, or whether either male's mismatch was likely caused by a scoring error (e.g., mismatched alleles differed in size by only 1 repeat unit). These rules likely improved the accuracy of our assignments, particularly by reducing the influence of null alleles, but are unlikely to have affected our overall patterns because we accepted the CERVUS male in the majority of cases.

We used paternity results to calculate male reproductive success and its component parts for each male in the data set. A male's annual "within-pair reproductive success" was the number of within-pair young (WPY) that he produced—that is the number of young that he sired in the nests of his social mate. We measured cuckoldry as the proportion of all social young that each male sired in his own nests, counting only those young that could be assigned to or excluded from him (note that a high proportion of young sired indicates a low level of cuckoldry). A male's "extrapair reproductive success" was defined as the total number of EPY that he sired in the nests of other males within a year. Finally, the male's "total reproductive success" within a year was the sum of WPY and EPY.

Statistical analysis

We used all male captures during the 2004–2006 breeding seasons to describe general patterns of variation in male plumage coloration. For each male, we included his plumage score as a 1-year-old (if available) and also as an older male; for males captured multiple times at older ages, we randomly selected a single capture. The final data set included capture records for 193 males, 27 of which were captured both as a 1-year-old and as an older male.

We used all offspring sampled from the 1998 through the 2005 breeding seasons for parentage analyses. To characterize

general patterns of within-pair parentage (i.e., rates of EPP) without pseudoreplication, we chose a single brood per group per year; if more than 1 brood had been analyzed for a group, we chose either the brood with the most complete information (e.g., the higher proportion of analyzed young) or the first brood of the season. For other analyses of reproductive success, we used all broods analyzed but excluded males for which we did not have full information on social reproductive success (i.e., number of social young produced within a year). We also excluded males whose reproductive success was truncated due to unusual circumstances within a breeding season; this included males who died during the breeding season ($n = 18$), had their territories burned by wildfire ($n = 7$), were removed for experiments ($n = 4$), or who did not obtain a social mate ($n = 1$). This left a total sample size of 255 male years for 176 males. We had multiple years of data for 65 males (37%). To reduce problems of pseudoreplication, we repeated most analyses using only 1-year-old males (this also helped to separate effects of age from those of plumage color; see below). We examined the relationship between male phenotype (i.e., auxiliary, dull breeder or bright breeder, and intermediate males excluded) and components of reproductive success using categorical nonparametric tests. Where possible, we also examined relationships between male plumage color score and fitness with regression analyses, as these approaches tend to have higher power.

RESULTS

Distribution of male plumage brightness

All auxiliary males ($n = 26$) had dull plumage: 1 auxiliary male had a plumage score of 19.0 and the others all had scores below 5.0. Among breeding males, the distribution of male plumage brightness was strongly bimodal, with most breeding males having very bright or very dull plumage and few males having intermediate plumage color scores (Figure 1). This bimodality was primarily due to male age: the plumage color scores of 1-year-old breeding males were lower than those of older breeding males (Figure 1) and individual males had significantly lower plumage color scores as 1-year olds than when older (21.7 ± 6.8 vs. 78.2 ± 5.7 [mean $\pm$ standard error], $n = 27$ males, paired $t = 8.40$, $P < 0.0001$). However, male plumage coloration was a function of breeding status as well as age, as many 1-year-old breeding males had high plumage color scores (Figure 1), and plumage color scores for 1-year-old breeding males were significantly greater than those for 1-year-old auxiliary males (Mann–Whitney $U = 624.0$, $n = 107$ and 26, $P < 0.0001$).

Analyses of maternity

Females matched well with their offspring at most loci. For example, out of 1647 comparisons between offspring and their presumed mothers in the 2005 breeding season ($n = 171$ offspring and 74 females), we found 164 mismatches (10.0%). Of these mismatches, most (88.4%) occurred at 4 loci (the 3 loci with apparent null alleles and $Msp4$, which appeared to have a relatively high genotyping error rate) and 49.4% of the mismatches were consistent with the presence of a null allele. Moreover, of the females who showed mismatches with their offspring, the vast majority (92.1%) were mismatched at only 1 or 2 loci and only 2 females were mismatched at 4 or more loci. Levels of mismatching between females and their offspring were lower in other years. These results support the assumption that brood parasitism is extremely rare in this population and that these loci accurately reflect parentage.
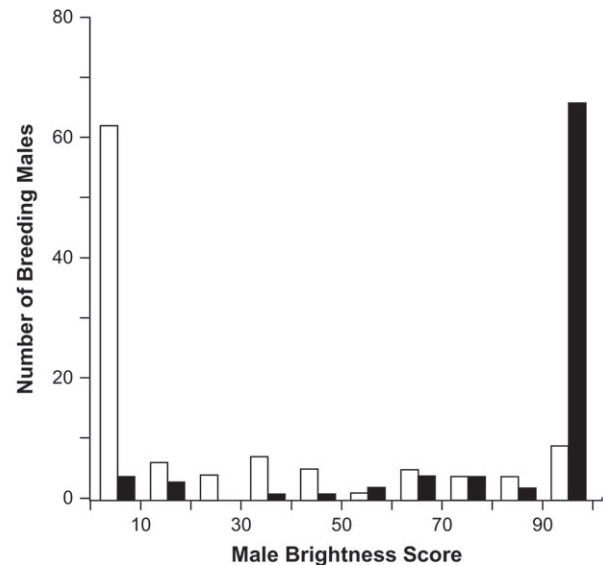

Figure 1
Top panel shows distribution of plumage brightness scores for 1-year-old breeding males (white bars, $n = 107$) and older breeding males (black bars, $n = 87$), which differed significantly (Mann–Whitney $U = 825.0$, $P < 0.0001$). Bottom panel shows two 1-year-old breeding males captured in 2004 differing in plumage brightness: a dull breeder with plumage score = 1 (left) and a bright breeder with plumage score = 98 (right).

Male reproductive success, age, and phenotype

In contrast to comparisons between offspring and their presumed mothers, many of the offspring sampled in this study did not match with their social fathers, confirming a high frequency of EPP. Overall, approximately half of all nestlings were sired by extrapair males, and nearly two-thirds of all nests contained one or more EPY (Table 2).

Total male reproductive success was strongly related to male phenotype (Kruskal–Wallis $H = 37.2$, degrees of freedom [df] = 3, $P < 0.0001$), with older bright breeding males siring the most offspring and auxiliary males siring the fewest (Figure 2a). This result remained strongly significant even after excluding auxiliary males (breeding males only, Kruskal–Wallis $H = 15.4$, df = 2, $P = 0.0005$), indicating that dull breeding males sired fewer offspring than did bright breeding males.

Male relative reproductive success also was weakly associated with age among breeding males (analysis included only breeding males of known age, $n = 112$, $F_{1,110} = 4.18$, $R^2 = 0.028$, $P = 0.0434$). Because male age and plumage color are associated (Figure 1), associations between plumage and reproductive success may be confounded. However, the relationship between male phenotype and reproductive success (Figure 2a) remained significant when analyses were restricted to 1-year-old males (Kruskal–Wallis $H = 7.86$, df = 2, $P = 0.020$). Similarly, for all males of known age, a multiple regression that

Table 2
Patterns of EPP across years

| Year | Broods | | Nestlings | |
	No. analyzed	Containing EPY (% ± 95% CI[a])	No. analyzed	EPY (% ± 95% CI[a])
1998	37	26 (70.3 ± 14.7%)	97	59 (60.8 ± 9.7%)
1999	44	24 (54.6 ± 14.7%)	126	53 (42.1 ± 8.6%)
2000	35	25 (71.4 ± 15.0%)	100	55 (55.0 ± 9.8%)
2003	10	5 (50.0 ± 31.0%)	24	13 (54.2 ± 19.9%)
2004	26	11 (42.3 ± 19.0%)	68	23 (33.8 ± 11.3%)
2005	33	25 (75.8 ± 14.6%)	102	61 (59.8 ± 9.5%)
Total	185	116 (62.7 ± 7.0%)	517	264 (51.1 ± 4.3%)

[a] Confidence intervals (CIs) (95%) calculated assuming a binomial distribution.

included both male age and plumage color score was significant ($n = 109$, $F_{2,106} = 9.54$, $R^2 = 0.137$, $P = 0.0002$) and post hoc tests showed that relative reproductive success was associated with plumage color ($t = 2.89$, $P = 0.0047$) but not with male age ($t = 0.51$, $P = 0.6143$). Excluding auxiliary males from this analysis also yielded a significant overall model ($n = 88$ breeding males, $F_{2,85} = 4.28$, $R^2 = 0.070$, $P = 0.0170$), and the effect of age remained nonsignificant ($t = 0.589$, $P = 0.5574$), but the relationship between relative reproductive success and male plumage color was marginally nonsignificant ($t = 1.81$, $P = 0.0744$).

Components of male reproductive success

The effects of male phenotype on within-pair reproductive success were relatively weak. Although the number of WPY produced differed among classes of male (Figure 2b), the difference was marginally nonsignificant (breeding males only, Kruskal–Wallis $H = 5.6$, df = 2, $P = 0.0602$). Regression analyses revealed a significant association between male plumage score and relative WPY success ($F_{1,162} = 4.8$, $P = 0.0303$), but plumage score explained very little of the variance in WPY success ($R^2 = 0.023$). Moreover, the latter association was not

the result of brighter males being cuckolded less, as the probability of cuckoldry was not related to plumage score (logistic regression, $n = 124$, $n = 124$, $\chi^2 = 0.591$, $P = 0.4422$). Finally, among breeding 1-year-old males, the number of WPY was not associated with plumage brightness ($n = 59$, $F_{1,57} = 0.015$, $P = 0.9040$).

In contrast, plumage color appeared to have a strong effect on the extrapair success of males, as number of EPY produced was strongly associated with male type (Figure 2c; Kruskal–Wallis $H = 23.6$, df = 3, $P < 0.0001$), even after auxiliary males were excluded (Kruskal–Wallis $H = 19.4$, df = 2, $P < 0.0001$). Among breeding males, male plumage score was associated with relative EPY success ($F_{1,163} = 12.9$, $R^2 = 0.068$, $P = 0.0004$) and the probability of siring one or more EPY (logistic regression, $n = 165$, $\chi^2 = 13.975$, $P = 0.0002$). These analyses might be confounded by male age because most older males were bright breeders (Figure 1); however, restricting the analysis to 1-year-old males showed that relative EPY success was significantly associated with plumage score ($n = 80$, $F_{1,78} = 6.02$, $R^2 = 0.060$, $P = 0.0163$), even after auxiliaries were excluded ($n = 59$, $F_{1,57} = 4.05$, $R^2 = 0.050$, $P = 0.0489$). In sum, 1-year-old males who bred in dull plumage sired fewer EPY than did same-aged males who bred in bright plumage.
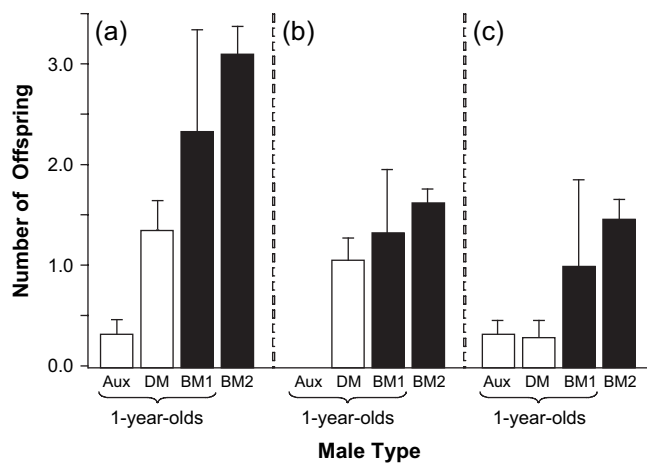
DISCUSSION

Reproduction and male phenotype

The question of why males adopt alternative breeding phenotypes has long puzzled behavioral and evolutionary ecologists. An early and popular hypothesis was that such alternatives are maintained within a population by frequency-dependent selection and have approximately equal fitness at equilibrium, but the paucity of examples suggests that this may be an unlikely explanation in most cases (but see Miles et al. 2007). A more likely explanation appears to be that some males adopt an unornamented phenotype as a best-of-a-bad-job conditional strategy that has not only lower fitness benefits but also lower costs. In support of this context-dependent hypothesis, variation in male ornamentation is typically associated with age, condition, or social context in several taxa (Emlen 1994; Gross 1996) including birds (Galeotti et al. 2003; Price 2006). Relatively few studies, though, have examined the ultimate fitness consequences of reduced ornamentation, particularly for plumage coloration in birds, and there are 2 distinct possibilities: dull plumage coloration may enhance male survival, for example, through reduced social costs (Conover et al. 2000; Berggren et al. 2004) and/or reduced risk of predation (Götmark and Hohlfält 1995; Huhta et al. 2003), or it may enhance some components of male reproductive success (Greene et al. 2000; Delhey et al. 2007).

Figure 2
Reproductive success of auxiliary males (Aux, $n = 28$), dull breeding males (DM, $n = 50$), bright breeding 1-year-old males (BM1, $n = 12$), and bright breeding older males (BM2, $n = 124$). Male reproductive success is shown as (a) total reproductive success (number of young sired) and its component parts: (b) number of WPY sired and (c) number of EPY sired. Shading of bars indicates plumage color (white = dull coloration, black = bright coloration, and males with intermediate coloration excluded). All males except BM2 are 1-year olds.

In this study, we examined the hypothesis that some males may be able to increase components of their seasonal reproductive success by retaining dull coloration. One possibility is that dull-colored males have relatively high extrapair reproductive success, as suggested by studies of other taxa showing that less conspicuous coloration increases the ability of some males to gain "sneak" copulations (e.g., Dominey 1980; Norman et al. 1999; Shine et al. 2001). Our previous experiments with red-backed fairy-wrens (Karubian et al. forthcoming) have shown that free-flying males are much less aggressive toward dull intruding males than they are toward bright intruding males, and this reduced aggression may improve the ability of dull males to intrude and thereby sire EPY on neighboring territories (Delhey et al. 2007).

The results presented in this paper, however, strongly contradict this hypothesis, as dull males were significantly less likely to sire EPY than were same-aged bright males (Figure 2). This result agrees with behavioral studies showing that captive females preferentially associate with bright males over dull (Karubian 2002). Similarly, in the congeneric superb fairy-wren (*Malurus cyaneus*), all males breed in bright plumage but those who acquire their plumage earlier have higher extrapair mating success than those who molt later (Mulder and Magrath 1994; Dunn and Cockburn 1999). We were unable to measure molt date in this study, but observations suggest that it is also highly variable in red-backed wrens and might have an important effect on male extrapair success. Overall, the timing and/or acquisition of bright plumage may be an important determinant of extrapair success in other malurids, most of which show very high rates of EPP (Rowe and Pruett-Jones 2006). Indeed, low aggression toward dull male red-backed wrens is likely a direct consequence of the low extrapair mating success of these males, as other breeding males may not see them as a reproductive threat.

This tolerance of dull males by older bright males suggests another possible benefit of dull coloration: subordinate breeding males may be able to enhance their within-pair reproductive success by honestly signaling their social status. In their study of lazuli buntings, Greene et al. (2000) found that older males allowed dull-colored young males, but not bright-colored young males, to settle on neighboring territories; the older males benefited because they could cuckold the younger dull males (see also Morton et al. 1990), and the dull males benefited because they were allowed to settle on higher quality breeding habitat. However, this mechanism is unlikely to apply to red-backed fairy-wrens because dull breeding males typically acquire their breeding territories relatively late in the season (Karubian 2002, 2008), often by filling breeding vacancies on neighboring territories that appear after breeding commences (Webster MS, unpublished data; see also Pruett-Jones and Lewis 1990). Moreover, in this study, plumage coloration had little effect on number of WPY produced (Figure 2), particularly among 1-year-old males, and dull males were not more likely to be cuckolded than were bright males. Thus, there seems little scope for male–male competition of the sort driving the mechanism described by Greene et al. (2000).

Our results contrast somewhat with those of Karubian (2002), who reported an association between plumage color and cuckoldry rates in this same study population. However, Karubian's previous study was based on a much smaller sample size and used fewer microsatellite loci to identify EPY, and these differences likely account for the discrepancy in our results. If females prefer bright males, it is somewhat surprising that dull males are not more likely to be cuckolded than are bright males. One possible explanation is that dull males guard their mates more closely than do bright males (Karubian 2002), which may constrain a female's ability to copulate with preferred extrapair males (Komdeur et al. 1999; Chuang-Dobbs et al. 2001).

Combined with results showing that male coloration has little effect on male survival (Karubian et al. forthcoming), our results show that, relative to bright plumage coloration, breeding in dull coloration is a best-of-a-bad-job strategy with relatively low fitness benefits to males, at least within the first year of life. One important caveat is that males who breed in dull plumage as 1-year olds may have enhanced reproductive success as 2-year olds (e.g., through higher quality plumage signals), relative to males who bred in bright plumage coloration as 1-year olds. We are currently examining this possibility; however, given the magnitude of the fitness difference between bright and dull breeding males (Figure 2c), it seems unlikely that such carryover effects will fully balance reproductive output of the 2 male phenotypes. Our conclusion that breeding in dull coloration is a best-of-a-bad-job strategy also is consistent with our finding that this plumage coloration is adopted most often by males who are relatively young (Figure 1) and in poor body condition (Webster MS, unpublished data).

Why be a dull breeder?

Our results have demonstrated that the reproductive success of dull breeder males is higher than that of nonbreeding auxiliaries but lower than that of bright breeding males, even after controlling for the effects of age. This begs the question of why males would breed in dull, rather than bright, plumage. One possible explanation is that breeding in dull plumage is a low payoff conditional strategy adopted by breeding males in relatively poor condition. Other studies of malurid wrens have indicated that bright plumage (and/or timing of molt into bright plumage) is condition dependent (Mulder and Magrath 1994; Peters 2000), but a second key prediction is that breeding males who adopt dull coloration have higher fitness than they would have had if they had adopted bright coloration. Such context-dependent selection has been demonstrated in some systems (e.g., Emlen 1997), but this is often difficult to do because the very conditions that lead a male to adopt the less ornamented phenotype will likely obscure the fitness benefits of that phenotype. For example, males in poor condition may develop a reduced ornament that has low fitness costs, but because they are in poor condition, the overall fitness of these males may be comparable with that of ornamented males who are in better condition but who also bear the costs of the ornament. Our results (e.g., Figure 2) suggest that fitness benefits to low-quality males of breeding in dull coloration are likely to be slight. Nevertheless, convincing tests of this prediction require detailed analyses of male lifetime reproductive success that control for male quality in some way and/or experiments that manipulate male plumage coloration, both of which are beyond the scope of the present study.

Our results also suggest an alternative possibility: that breeding in dull plumage coloration is a maladaptive strategy, relative to breeding in bright plumage, resulting from the timing of signal acquisition (see also Rohwer and Butcher 1988). Because males must acquire their plumage signals during a molt that occurs before breeding, they must assess their prospects for independent breeding based on information available at the time, for example, from prebreeding social interactions with conspecifics. We hypothesize that males who are unlikely to obtain a social mate during the breeding season (e.g., because they are in relatively subordinate or because there are few available females in the population) will molt into dull plumage prior to the breeding season to become auxiliaries, whereas males who have good breeding prospects will molt into bright coloration. An auxiliary male likely benefits from dull coloration through reduced aggression from the dominant breeding male, allowing the auxiliary to remain on its natal territory and perhaps increase

likelihood of survival (see discussion in Karubian et al. forthcoming; see also Conover et al. 2000). Once breeding commences, new breeding opportunities often arise through death of breeding males and immigration of new breeding females and auxiliary males quickly fill these vacancies (Webster MS, personal observation), most likely because independent breeding yields higher fitness than does acting as an auxiliary (Figure 2) and the kin-selected benefits of helping appear to be low (Webster MS, unpublished data). However, these auxiliaries turned breeders have already molted and are therefore constrained to breed in dull coloration. Under this hypothesis, dull plumage coloration would be adaptive for auxiliary males but not to those males who molt into dull plumage and then become dull breeders later in the season.

This maladaptive constraints hypothesis is consistent with our results suggesting a lack of fitness benefits to breeding in dull coloration. It is also supported by the observation that dull breeders typically acquire their social mates midseason well after most males have begun breeding (Karubian 2002), typically by filling a breeding vacancy that arises when a neighboring male dies or a new female moves into the area (Webster MS, unpublished data). This hypothesis requires further examination in the red-backed fairy-wren system but also may apply to other species where acquisition of an ornamental signal is separated in time from the use of that signal during breeding.

FUNDING

REFERENCES

Badyaev AV, Duckworth RA. 2003. Context-dependent sexual advertisement: plasticity in development of sexual ornamentation throughout the lifetime of a passerine bird. J Evol Biol. 16: 1065–1075.

Berggren Å, Armstrong DP, Lewis RM. 2004. Delayed plumage maturation increases overwinter survival in North Island robins. Proc R Soc Lond B Biol Sci. 271:2123–2130.

Brockmann HJ. 2001. The evolution of alternative strategies and tactics. Adv Study Behav. 30:1–51.

Chuang-Dobbs HC, Webster MS, Holmes RT. 2001. The effectiveness of mate guarding by male black-throated blue warblers. Behav Ecol. 12:541–546.

Conover MR, Reese JG, Brown AD. 2000. Costs and benefits of subadult plumage in mute swans: testing hypotheses for the evolution of delayed plumage maturation. Am Nat. 156:193–200.

Delhey K, Peters A, Johnsen A, Kempenaers B. 2007. Fertilization success and UV ornamentation in blue tits Cyanistes caeruleus: correlational and experimental evidence. Behav Ecol. 18:399–409.

Dominey WJ. 1980. Female mimicry in male bluegill sunfish—a genetic polymorphism? Nature. 284:546–548.

Double MC, Dawson D, Burke T, Cockburn A. 1997. Finding the fathers in the least faithful bird: a microsatellite-based genotyping system for the superb fairy-wren Malurus cyaneus. Mol Ecol. 6:691–693.

Dunn PO, Cockburn A. 1999. Extrapair mate choice and honest signaling in cooperatively breeding superb fairy-wrens. Evolution. 53:938–946.

Emlen DJ. 1994. Environmental control of horn length dimorphism in the beetle Onthophagus acuminatus (Coleoptera: Scarabaeidae). Proc R Soc Lond B Biol Sci. 256:131–136.

Emlen DJ. 1997. Alternative reproductive tactics and male-dimorphism in the horned beetle Onthophagus acuminatus (Coleoptera: Scarabaeidae). Behav Ecol and Sociobiol. 41:335–341.

Emlen DJ, Corley Lavine L, Ewen-Campen B. 2007. On the origin and evolutionary diversification of beetle horns. Proc Natl Acad Sci USA. 104:8661–8668.

Flood NJ. 1984. Adaptive significance of delayed plumage maturation in male northern orioles. Evolution. 38:267–279.

Fridolfsson A, Gyllensten UF, Jakobsson S. 1997. Microsatellite markers for paternity testing in the Willow warbler Phylloscopus trochilus: high frequency of extra-pair young in an island population. Hereditas. 126:127–132.

Galeotti P, Rubolini D, Dunn PO, Fasola M. 2003. Colour polymorphism in birds: causes and functions. J Evol Biol. 16:635–646.

Gibbs HL, Tabak LM, Hobson KA. 1999. Characterization of microsatellite DNA loci for a neotropical migrant songbird, the Swainson's thrush (Catharus ustulatus). Mol Ecol. 8:1551–1552.

Götmark F, Hohlfält A. 1995. Bright male plumage and predation risk in passerine birds: are males easier to detect than females? Oikos. 74:475–484.

Greene E, Lyon BE, Muehter VR, Ratcliffe L, Oliver SJ, Boag PT. 2000. Disruptive sexual selection for plumage coloration in a passerine bird. Nature. 407:1000–1003.

Gross MR. 1982. Sneakers, satellites and parentals: polymorphic mating strategies in North American sunfishes. Z Tierpsychol. 60:1–26.

Gross MR. 1985. Disruptive selection for alternative life histories in salmon. Nature. 313:47–48.

Gross MR. 1996. Alternative reproductive strategies and tactics: diversity within sexes. Trends Ecol Evol. 11:92–97.

Gross MR, Charnov EL. 1980. Alternative male life histories in bluegill sunfish. Proc Natl Acad Sci USA. 77:6937–6940.

Hill GE. 1988. Age, plumage brightness, territory quality, and reproductive success in the black-headed grosbeak. Condor. 90:379–388.

Hill GE. 1989. Late spring arrival and dull nuptial plumage—aggression avoidance by yearling males. Anim Behav. 37:665–673.

Hill GE. 1990. Female house finches prefer colourful males: sexual selection for a condition-dependent trait. Anim Behav. 40:563–572.

Hill GE, McGraw KJ. 2006. Bird coloration. Volume II. Function and evolution. Cambridge (MA): Harvard University Press.

Huhta E, Alatalo RV. 1993. Plumage color and male–male interactions in the pied flycatcher. Anim Behav. 45:511–518.

Huhta E, Rytkonen S, Solonen T. 2003. Plumage brightness of prey increases predation risk: an among-species comparison. Ecology. 84:1793–1799.

Jamieson A. 1994. The effectiveness of using co-dominant polymorphic allelic series for (1) checking pedigrees and (2) distinguishing full-sib pair members. Anim Genet. 25(supplement 1):37–44.

Karubian J. 2002. Costs and benefits of variable breeding plumage in the red-backed fairy-wren. Evolution. 56:1673–1682.

Karubian J. 2008. Changes in breeding status are associated with rapid bill darkening in male red-backed fairy-wrens Malurus melanocephalus. J Avian Biol. 39(1):81–86.

Karubian J, Sillett TS, Webster MS. Forthcoming. The effects of plumage color on aggression and survival in male red-backed fairy-wrens. Behav Ecol. doi:10.1093/beheco/arm159.

Komdeur J, Kraaijeveld-Smit F, Kraaijeveld K, Edelaar P. 1999. Explicit experimental evidence for the role of mate guarding in minimizing loss of paternity in the Seychelles warbler. Proc R Soc Lond B Biol Sci. 266:2075–2081.

Marshall TC, Slate J, Kruuk LEB, Pemberton JM. 1998. Statistical confidence for likelihood-based paternity inference in natural populations. Mol Ecol. 7:639–655.

McGraw KJ, Stoehr AM, Nolan PM, Hill GE. 2001. Plumage redness predicts breeding onset and reproductive success in the House Finch: a validation of Darwin's theory. J Avian Biol. 32:90–94.

Miles DB, Sinervo B, Hazard LC, Svensson EI, Costa D. 2007. Relating endocrinology, physiology and behaviour using species with alternative mating strategies. Funct Ecol. 21:653–665.

Morton ES, Forman L, Braun M. 1990. Extrapair fertilizations and the evolution of colonial breeding in purple martins. Auk. 107:275–283.

Muether VR, Greene E, Ratcliffe L. 1997. Delayed plumage maturation in lazuli buntings: tests of the female mimicry and status signaling hypotheses. Behav Ecol Sociobiol. 41:281–290.

Mulder RA, Magrath MJL. 1994. Timing of prenuptial molt as a sexually selected indicator of male quality in superb fairy-wrens (*Malurus cyaneus*). Behav Ecol. 5:393–400.

Norman MD, Finn J, Tregenza T. 1999. Female impersonation as an alternative reproductive strategy in giant cuttlefish. Proc R Soc Lond B Biol Sci. 266:1347–1349.

Oliveira RF, Ros AFH, Goncalves DM. 2005. Intra-sexual variation in male reproduction in teleost fish: a comparative approach. Horm Behav. 48:430–439.

Peters A. 2000. Testosterone treatment is immunosuppressive in superb fairy-wrens, yet free-living males with high testosterone are more immunocompetent. Proc R Soc Lond B Biol Sci. 267: 883–889.

Price TD. 2006. Phenotypic plasticity, sexual selection and the evolution of colour patterns. J Exp Biol. 209:2368–2376.

Prodöhl PA, Loughry WJ, McDonough CM, Nelson WS, Thompson EA, Avise JC. 1998. Genetic maternity and paternity in a local population of armadillos assessed by microsatellite DNA markers and field data. Am Nat. 151:7–19.

Pruett-Jones SG, Lewis MJ. 1990. Sex ratio and habitat limitation promote delayed dispersal in superb fairy-wrens. Nature. 348:541–542.

Richardson DS, Jury FL, Dawson DA, Salgueiro P, Komdeur J, Burke T. 2000. Fifty Seychelles warbler (*Acrocephalus sechellensis*) microsatellite loci polymorphic in Sylviidae species and their cross-species amplification in other passerine birds. Mol Ecol. 9:2225–2230.

Rohwer S. 1978. Passerine sub-adult plumages and deceptive acquisition of resources: test of a critical assumption. Condor. 80: 173–179.

Rohwer S, Butcher GS. 1988. Winter versus summer explanations of delayed plumage maturation in temperate passerine birds. Am Nat. 131:556–572.

Rowe M, Pruett-Jones S. 2006. Reproductive biology and sperm competition in Australian fairy-wrens. Avian Poult Biol Rev. 17:21–37.

Rowley I, Russell E. 1997. Fairy-wrens and grasswrens, Maluridae. Oxford: Oxford University Press.

Sætre G-P, Dale S, Slagsvold T. 1994. Female pied flycatchers prefer brightly colored males. Anim Behav. 48:1407–1416.

Sætre G-P, Slagsvold T. 1996. The significance of female mimicry in male contests. Am Nat. 147:981–995.

Schodde R. 1982. The Fairy-wrens: a monograph of the Maluridae. Melbourne (Australia): Lansdowne.

Shine R, Phillips B, Wayne H, LeMaster M, Mason RT. 2001. Benefits of female mimicry in snakes. Nature. 414:267.

Shuster SM, Wade MJ. 1991. Equal mating success among male reproductive strategies in a marine isopod. Nature. 350:608–610.

Slagsvold T, Saetre GP. 1991. Evolution of plumage color in male pied flycatchers (Ficedula hypoleuca): evidence for female mimicry. Evolution. 45:910–917.

Webster MS, Tarvin KA, Tuttle EM, Pruett-Jones S. 2004. Reproductive promiscuity in the splendid fairy-wren: effects of group size and reproduction by auxiliaries. Behav Ecol. 15:907–915.

Westneat DF. 1990. Genetic parentage in the indigo bunting: a study using DNA fingerprinting. Behav Ecol Sociobiol. 27:67–76.

White PS, Densmore LD. 1992. Mitochondrial DNA isolation. In: Hoelzel AR, editor. Molecular genetic analysis of populations. Oxford: Oxford University Press. p. 29–58.