# Male Red-winged Blackbirds with experimentally dulled epaulets experience no disadvantage in sexual selection

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ABSTRACT. The epaulets of male Red-winged Blackbirds (*Agelaius phoeniceus*) are frequently cited as a sexually selected plumage ornament, but a number of laboratory and field studies provide little evidence that they are currently experiencing sexual selection. We used hair dye to dull epaulets of free-living territorial males prior to pair formation to determine if manipulated males experienced disadvantages in comparison with control males. We found no differences between control males and males with dulled epaulets in territorial behavior (territory size, song rate, trespass rate, and loss of territory), paternal care (time spent on territory and in antipredator sentinel behavior, and response to a model crow to simulate the threat of predation), pairing success (number of social mates), apparent reproductive success (numbers of nesting attempts, eggs/nest, nestlings/egg, and fledglings/nestling), or realized reproductive success (numbers of within-pair, extra-pair, and total fledglings as determined by DNA fingerprinting). We then used a meta-analysis of 11 published studies of Red-winged Blackbirds to determine if there is an overall effect of epaulet color or size on male-male competition, female choice, or reproductive success. Our results show that epaulet size has a small positive effect on male reproductive success, but epaulet color has no effect on male-male competition, female choice, and male reproductive success. One explanation for the seeming contradiction between studies that show that epaulets are necessary for territory defense and those that conclude that epaulets are not currently under selection is that epaulets serve as one of several cues of species recognition, especially among males at close range. An alternative explanation proposes counter-balancing intersexual advantages and intrasexual disadvantages of epaulet expression. Additional studies are needed to test these alternatives.

RESUMEN. **Machos de *Agelaius phoeniceus* con charreteras apagadas experimentalmente no tienen ninguna desventaja en la selección sexual**

Las charreteras de *Agelaius phoeniceus* son frecuentemente citadas como un ornamento que se encuentra bajo la selección sexual, pero los estudios en el laboratorio y en el campo proveen poca evidencia de que estos ornamentos están actualmente bajo la selección sexual. Usamos tinte para el cabello para apagar los colores de las charreteras de machos territoriales silvestres antes de que formaran parejas. El objetivo fue determinar si machos afectados con el tinte experimentarían desventajas en comparación con machos usados como control. No encontramos diferencias entre machos usados como control y machos con charreteras apagadas en su comportamiento de territorialidad (tamaño del territorio, tasa de canto, tasa de entradas al territorio por parte de coespecíficos, y perdida de territorio), cuidado paternal (tiempo en el territorio y el comportamiento de centinela contra depredadores, y la respuesta a un modelo de un cuervo para simular el peligro de depredación), éxito de pareja (numero de hembras que nidifican en el territorio del macho), éxito reproductivo aparente (numero de intentos de nidificación, de huevos/nido, de pichones/huevos, y volantones/pichón), o éxito reproductivo actual (numero de volantones por pareja o producidos extra-parentalmente, y total numero de volantones determinado mediante el ADN). Usamos un meta-análisis de 11 estudios publicados de *A. phoeniceus* para determinar si existe un efecto del color o tamaño de la charretera a la competición entre machos, al tipo de macho escogido por la hembra, o al éxito reproductivo. Nuestros resultados demuestran que el tamaño de la charretera tiene un pequeño efecto positivo sobre éxito reproductivo pero que el color de la charretera no tiene un efecto sobre la competición entre machos, al tipo de macho escogido por la hembra, o al éxito reproductivo del macho. Una explicación para la aparente contradicción entre los estudios que demuestran que las charreteras son necesarias para la defensa del territorio y de los que concluyen que las charreteras no están actualmente bajo la selección es que las charreteras sirven como una de varias señales en el reconocimiento de la especie, especialmente entre machos a corta distancia. Una explicación alternativa propone que existe un balance entre ventajas intersexuales y desventajas intrasexuales en la expresión de las charreteras. Estudios adicionales son necesarios para probar estas alternativas.

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The epaulets of male Red-winged Blackbirds (*Agelaius phoeniceus*) are frequently cited as an example of a plumage ornament with intersexual (mate attraction) and intrasexual (territory defense) functions (e.g., Andersson 1994, Hauser 1997, Hill 2002). These epaulets are melanin- and carotenoid-based plumage badges (McGraw et al. 2004) with high reflectance above 600 nm and little reflectance below 550 nm (Götmark 1996, Yasukawa et al. 2009a). Male Red-winged Blackbirds display their epaulets during territory defense and other aggressive encounters, as well as during courtship and precopulatory behavior, but they do not display their epaulets before they establish territory ownership (Nero 1956a, 1956b, Orians and Christman 1968, Hansen and Rowher 1986). Furthermore, experiments where epaulets were blackened (Peek 1972, Smith 1972, Morris 1975, Røskaft and Rohwer 1987) have shown that "black-winged black-birds" incur more trespassing, are more likely to lose their territories, and may be less likely to attract mates than males with unmanipulated epaulets, even though they are still recognized as conspecifics (Hansen and Rohwer 1986). In addition, Eckert and Weatherhead (1987) found a significant correlation between epaulet color and dominance rank in captive flocks of male Red-winged Blackbirds. These results are consistent with the hypothesis that the epaulets of male Red-winged Blackbirds are adaptations that evolved by sexual selection (Searcy and Yasukawa 1995).

However, Yasukawa et al. (2009a), comparing the responses of male and female Red-winged Blackbirds to male models, found that territorial males approached models with unmanipulated epaulets more closely than models with dulled epaulets, and that females showed no significant preference. Similarly, Westneat (2006) dulled the epaulets of free-living, territorial male Red-winged Blackbirds and found that males with normal and dulled epaulets did not differ in their ability to defend territories, attract mates, or sire offspring. Thus, studies to date indicate that the epaulets of male Red-winged Blackbirds are sexually selected traits and are therefore adaptations (sensu Williams 1966), yet variance in epaulet size and brightness does not influence reproductive success (see also Searcy and Yasukawa 1995). Given this apparent contradiction, our objective was to examine further the possible role of the epaulets of male Red-winged Blackbirds in competition for territories, ability to mate, and reproductive success.

Like Westneat (2006), we dulled the epaulets of free-living, territorial male Red-winged Blackbirds prior to pair formation. If sexual selection currently favors bright epaulets, then males with experimentally dulled epaulets should experience a disadvantage in territory defense and pairing success compared to control males and, as a result, males with dulled epaulets should have lower reproductive success than males with normal epaulets. Because reproductive success depends on territory ownership, pairing success, and nest success, we observed male territorial behavior and antipredator paternal care, and located and tracked nesting attempts on focal territories. We then used multi-locus DNA fingerprints to identify sires of nestlings on focal territories.

In addition, given the many studies of Red-winged Blackbird epaulets, we performed a meta-analysis (Whitlock and Schluter 2009) to determine whether epaulet expression affects male–male competition, female choice, or reproductive success. This analysis provides a quantitative way to interpret the wide range of results from a variety of observational and experimental studies of Red-winged Blackbird epaulets as sexually selected adaptations.

## METHODS

We conducted our study at Newark Road Prairie in south-central Wisconsin (42°32′N, 89°08′W) during the breeding season of 1995. The study area is a wet-mesic prairie and sedge meadow habitat that supports 20–35 Red-winged Blackbird territories and was described in more detail by Yasukawa (1989).

All territorial male Red-winged Blackbirds and all females whose nestlings reached 3 d of age were captured and banded with U.S. Geological Survey numbered aluminum bands

and a unique color combination of plastic wrap-around bands. Males and some females were captured in Potter traps placed on feeding platforms and baited with nonviable sunflower seed. In addition, some females attempting to feed nestlings were captured in Potter traps placed over nests with nestlings. Traps were placed over nests for no longer than 30 min to minimize disturbance. The capture and handling of birds and the presence of bands had no apparent effect on either their behavior or site fidelity.

**Measurements of territorial behavior.** Observations of male Red-winged Blackbird territorial behavior were conducted from sunrise until 1100 CST. During each 10-min observation, we identified the territory owner and noted each song so we could calculate song rates. We identified territory boundaries by plotting the locations of male territory defense displays and territorial encounters (Nero 1956b, Orians and Christman 1968) on maps of the study area. Plotting was aided by the presence of a 20-m grid of marker posts throughout the study area. Territory sizes were measured with NIH Image (National Institutes of Health 1996) using the convex polygons constructed from display locations. We also noted all occurrences of extra-territorial males flying through or trespassing (perching) in focal territories, and all occurrences of territorial challenges (Nero 1956b) by extra-territorial males. In a territorial challenge, both the territory owner and challenger fly upward in a spiral above the defended area, with the territorial male maintaining a position above the challenger. Most challenges lasted from one to several minutes, with the spiral flights being repeated several times in lengthy challenges. We never observed fighting as a direct result of these challenges.

**Paternal care.** We quantified the antipredator behavior of male Red-winged Blackbirds as a measure of paternal care. During the incubation stage of each male's primary (most advanced) nest, we observed territorial males from a blind for 30 min each day for up to 10 d, weather permitting. During these observations, we noted the amount of time the male spent perching at prominent locations and scanning the territory (time on sentinel; Yasukawa et al. 1992) as well as the amount of time the male spent on the territory (males make frequent forays off territory).

We also measured the male's response to a simulated predator by placing a crow (*Corvus* sp.) model 20 m from the primary nest for 5 min, then repeating the presentation with the crow 1 m from the same nest. When a focal male was off territory, we chose a direction away from any other active nests on the same territory, used a tape measure to locate the 20-m point, pushed a 2-m-tall pole with the crow model attached into the ground, and retreated to a position off the male's territory at least 50 m away. We began the 5-min observation when the male returned to the territory. At the end of this first presentation, we then moved the pole to 1 m from the nest and conducted a second 5-min observation. During the two presentations, we noted the male's closest approach to the crow model, whether the male struck the crow, and, if so, the number of strikes. Closest approach distances were measured after both presentations were completed. We used closest approach and number of strikes to produce a single response score. Males that approached, but did not strike the crow, were given a response score corresponding to the negative of their measured closest approach (e.g., a male that approached to 25 m was given a response score of −25, whereas a male that approached to 1 m was given a score of −1). Males that struck the crow were given a score corresponding to the number of strikes (e.g., a male that struck once was given a score of 1 and a male that struck 10 times was given a score of 10). Thus, higher scores indicate more responsive males.

**Pairing and reproductive success.** We estimated pairing success by observing territories and females, and by locating all nesting attempts ($N = 74$) on focal territories. Social mates were defined as all females that nested on a male's territory. Using the chronologies for each nesting attempt, we calculated two estimates of pairing success. Harem size was the maximum number of simultaneously active nests on a male's territory (Yasukawa 1981), and the number of females was the total number of individual females nesting on a male's territory over the course of the breeding season (Holm 1973).

Apparent reproductive success with social mates depends on the number of nesting attempts, clutch size, hatching success, and nestling survival. To produce independent estimates of these components of apparent success,

we counted the number of nesting attempts (nests receiving eggs) and calculated the number of eggs/nest, number of nestlings/egg, and number of fledglings/nestling on each control and experimental territory. Loss of territory, failure to attract nesting females, and nest failure during egg and nestling stages resulted in changes in sample sizes from one estimate to the next.

For 62 nestlings from 20 broods at least 3 d old, we used DNA fingerprinting in an attempt to identify sires (Parker et al. 1999), but in the majority of cases, nests failed before we could obtain blood samples. DNA fingerprints were used to estimate the numbers of within-pair and extra-pair fledglings produced by each male.

Two blood samples of approximately 50 µl each were collected from the wing veins of adult and nestling Red-winged Blackbirds and were immediately stored in lysis buffer (0.1 m Tris, 0.1 m EDTA, 0.01 m NaCl, 0.5% SDS; Longmire et al. 1988). The samples were incubated for 8 h at 65°C, after adding 300 µg of proteinase K. DNA was extracted several times in phenol/phenol:chloroform:isoamyl alcohol, then dialyzed against TNE2 (0.01 m Tris, 0.01 m NaCl, 0.02 m EDTA). DNA (4–5 µg) was digested with excess Hae III restriction endonuclease at 37°C. Resulting fragments were separated on a 0.8% agarose gel (22 cm) at 20 V for 65 h until all fragments smaller than 1600 bp were run off, and then transferred to nylon by Southern blot in 10× SSC (1.5 m NaCl, 0.15 m sodium citrate) buffer. The probe (33.15; Jeffreys et al. 1985) was radio-labeled by random primer extension with [32P]dCTP. Hybridizations were run overnight at 62°C in 1.5× SSC, 0.1% SDS, 5× Denhart's solution, and 6% dextran sulfate. Hybridized filters were washed 4 × 30 min at 62°C in 1.5× SSC. 0.1% SDS, and exposed to x-ray film at −20°C.

Samples collected in 1992–1994 were used to develop criteria for assignment. There was sufficient polymorphism in the population to make confident paternal exclusions and assignments on the basis of a single enzyme/probe combination. Initial gels were run as family groups, with putative parents no more than five lanes from offspring to minimize scoring errors resulting from distance between lanes (Piper and Parker Rabenold 1992). We assessed the parentage of each nestling by estimating its genetic similarity to the putative parents as the proportion of fingerprinting bands shared (band-sharing analysis), and by determining whether all bands in an offspring lane could be attributed to the fingerprints of the putative parents (unattributable band analysis). Band-sharing values were calculated as $2N_{AB}/(2N_{AB} + N_A + N_B)$, where $N_{AB}$ is the number of bands shared by individuals A and B, $N_A$ is the number of bands unique to A, and $N_B$ is the number of bands unique to B (Wetton et al. 1987, Lynch 1990). Band-sharing values for all dyads of first-order relatives came from 67 mother-offspring comparisons because there was no evidence of egg dumping in our study population. A set of 64 dyads of unrelated individuals came from comparisons of social mates, with no individual being used in more than two dyads. From the resulting distributions of genetic similarity, the threshold for parental exclusions was set at a band-sharing value of 0.40 (on the basis of the mean band sharing of unrelated birds (0.25) plus 1.96 standard deviations (0.15)). This value represents the upper 95% confidence limit for genetic similarity between nonrelatives.

The distribution of unattributable bands allows calculation of the rate of mutation (i.e., the rate of appearance of bands unattributable to the assigned parental dyads; Jeffreys et al. 1988, Parker Rabenold et al. 1990, Westneat 1990), and calculation of a criterion number of unattributable bands for exclusion of a dyad as parental. Cases where there were two unattributable bands were considered ambiguous and were run again with Hae III or Hind III to allow scoring of more bands. The mutation rate, estimated as the proportion of the total offspring bands scored that were novel, was 0.0011. Assuming that mutation events are independent and occur randomly across nestlings, the Poisson probability that the true genetic parents would produce an offspring having one or more bands unattributable to either parent is 0.012. The probability that the true genetic parents would produce an offspring having two or more novel bands is 0.001.

**Epaulet manipulation.** All males used in the experiment were captured prior to the arrival of females and removed from traps as quickly as possible. The first captured male was randomly assigned to either the experimental or control group and the second male was assigned to the other group; this assignment procedure was then repeated for all subsequently captured males. Because the epaulets of male Red-winged

Blackbirds exhibit little reflectance in the UV (Götmark 1996, Yasukawa et al. 2009a), our manipulations were designed to reduce reflectance above 500 nm. The epaulets of all males were swabbed with alcohol and air-dried. Epaulets of experimental males were then dulled using Nyanzol D black dye (Smith 1972), and rinsed with water as the red epaulet feathers began to darken. Control males were treated similarly, but with water rather than dye. Each male was then held briefly in a covered cage until epaulets were dry.

Prior to manipulation, epaulets had a red hue (7.5–10R) with value (brightness) of 4–9 and chroma (colorfulness) of 14–16 on the Munsell scale (X-Rite Inc., Grand Rapids, MI). Dying did not alter the hue (7.5–10R), but reduced value (4–5) significantly (Wilcoxon matched-pairs $z = -10.5$, $N = 13$, $P = 0.031$) and chroma (10–16) slightly but not significantly (Wilcoxon matched-pairs $z = 9.5$, $P = 0.063$, $N = 13$). Dulled epaulets were thus around the low end of natural variation in value and chroma for males in the definitive breeding plumage. Epaulets remained altered until the experimental males molted after the breeding season ended.

**Meta-analysis.**    We followed the procedure outlined in Whitlock and Schluter (2009) to perform a weighted, parametric, mixed-model meta-analysis as recommended by Gurevitch and Hedges (1999). We identified nine studies of epaulet color and eight of epaulet size ($N = 11$ studies total) that provided sufficient information to calculate effect sizes on aspects of male–male competition, female choice of mate, or reproductive success. Because correlation analyses were used in most studies, all other test statistics were converted to correlation coefficients as our measure of effect size. We then computed weighted mean effect sizes and 95% confidence intervals to determine if either epaulet color or size affected the reproductive success of male Red-winged Blackbirds.

**Statistical methods.**    All statistical analyses were conducted using JMP 6.0 (SAS Institute 2006). When assumptions of normality were met, we used *t*-tests to perform two-group comparisons of control and experimental males; we used Wilcoxon nonparametric tests otherwise. We also used $\chi^2$ tests of independence to compare controls and experimentals. Effects of epaulet manipulation were tested with Wilcoxon matched-pairs (before and after) tests. Statistical significance was accepted at $\alpha = 0.05$, and values were presented as means $\pm$ SE.

### RESULTS

**Epaulet manipulation.**    We captured 26 male Red-winged Blackbirds; we assigned 13 to the control group and 13 to the experimental group.

**Trespass rate, loss of territory, and territory defense.**    Trespassing rates (number/h) in the territories of control and experimental males were similar during premanipulation (control: $2.23 \pm 0.64$; dull: $1.38 \pm 0.36$; $z = 0.5$, $P = 0.62$) and postmanipulation (control: $1.53 \pm 0.37$; dull: $1.15 \pm 0.25$; $z = 0.2$, $P = 0.82$) observations. Two males, one control, and one experimental, lost their territories following capture and handling.

Neither premanipulation (control: $1998 \pm 482$ m²; dull: $2651 \pm 491$ m²; $t_{24} = 1.1$, $P = 0.29$) nor postmanipulation (control: $2055 \pm 299$ m²; dull: $2750 \pm 603$ m²; $t_{22} = 1.0$, $P = 0.32$) territory sizes differed for control and experimental males. In addition, the singing rates (number/min) of control and dulled-epaulet male Red-winged Blackbirds did not differ either before (control: $4.1 \pm 0.2$; dull: $2.5 \pm 0.2$; $t_{24} = 1.7$, $P = 0.11$) or after (control: $4.6 \pm 0.2$; dull: $3.1 \pm 0.2$; $t_{24} = 2.0$, $P = 0.054$) epaulet manipulation.

**Paternal care.**    We found no differences in the antipredator behavior of control and dulled-epaulet male Red-winged Blackbirds. Percent time on sentinel (control: $64.9 \pm 5.0\%$; dull: $55.5 \pm 4.2\%$; $z = 1.5$, $P = 0.13$, $N_C = 12$, $N_D = 11$), percent time on territory (control: $90.1 \pm 2.5\%$; dull: $86.5 \pm 2.9\%$; $z = 1.0$, $P = 0.31$, $N_C = 12$, $N_D = 11$), and response to a crow at 20 m (control: $-18.35 \pm 5.9$; dull: $-13.0 \pm 4.5$; $z = 0.4$, $P = 0.69$, $N_C = 12$, $N_D = 11$) and 1 m (control: $14.8 \pm 4.9$; dull: $27.9 \pm 12.3$; $z = 0.9$, $P = 0.39$, $N_C = 12$, $N_D = 11$) did not differ.

**Pairing and reproductive success.**    Although males with dulled epaulets attracted fewer females than control males (Fig. 1A), neither difference was significant (harem size: $z = 0.9$, $P = 0.37$, $N_C = N_D = 12$; number of females: $z = 1.2$, $P = 0.25$, $N_C = N_D = 12$).

We found no significant differences in apparent reproductive success of control and dulled-epaulet males (Fig. 1B). Control and
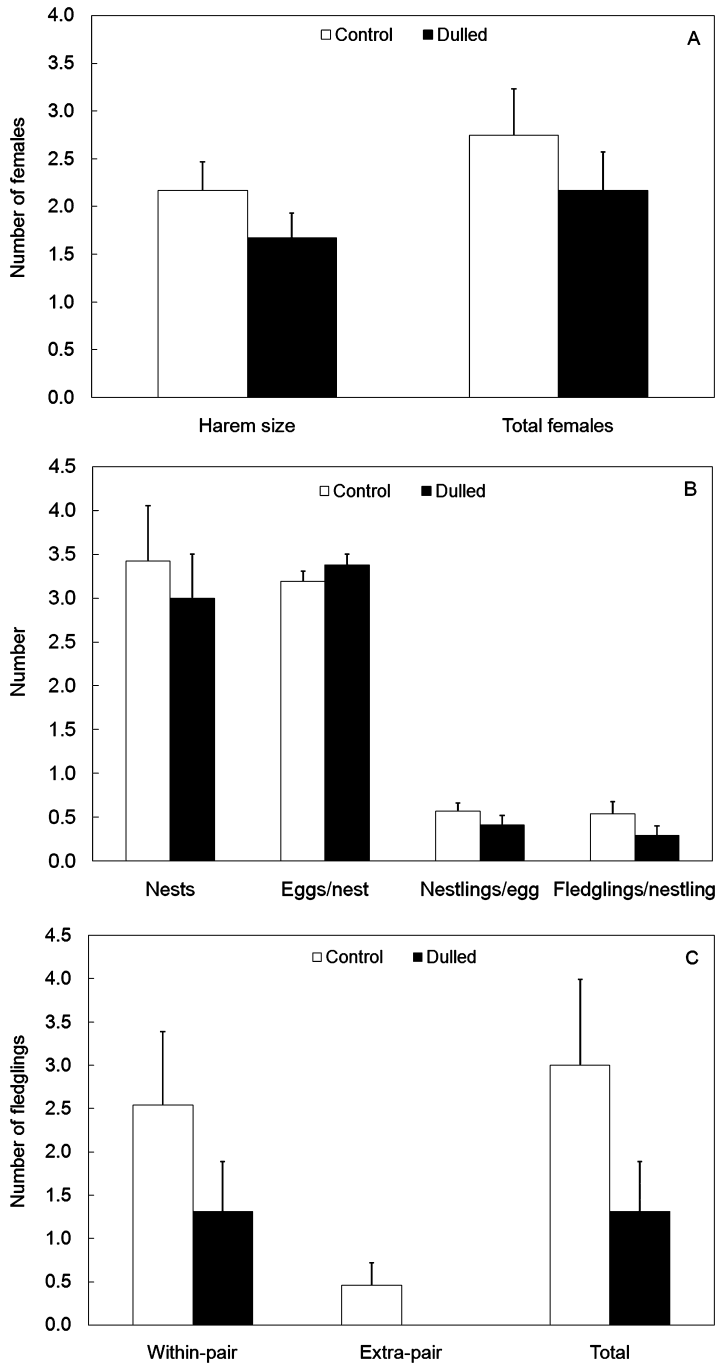
Fig. 1. Male Red-winged Blackbirds with control and dulled epaulets did not differ in (A) pairing success, (B) apparent reproductive success, and (C) reproductive success. Harem size is the maximum number of simultaneously active nests on a territory. Number of females is the total number of females that nested on a territory. Apparent reproductive success assumes that social mates sire all nestlings. Reproductive success estimates use genetic analysis to identify sires. The total number of nestlings produced with both social mates (within pair) and extra-pair females is the realized reproductive success.

*Red-winged Blackbird Epaulets*

Table 1. Effect of epaulet color in male Red-winged Blackbirds.

| Reference | Type of study | Test statistic | Statistic value | Effect size | $n$ | $P$ |
|---|---|---|---|---|---|---|
| | **Male-male competition** | | | | | |
| Searcy 1979b | Color vs dominance rank | $r_S$ | 0.289 | 0.289 | 13 | 0.34 |
| Eckert and Weatherhead 1987 | Color vs dominance rank | $r_S$ | 0.594 | 0.594 | 7 | 0.14 |
| | | $r_S$ | −0.241 | −0.241 | 9 | 0.51 |
| | | $r_S$ | 0.376 | 0.376 | 9 | 0.31 |
| Yasukawa et al. 2009a | Dulling vs male approach | $z$ | −0.22 | −0.031 | 46 | 0.83 |
| | **Female choice** | | | | | |
| Searcy 1979a | Color vs harem size | $r_S$ | 0.084 | 0.084 | 41 | 0.61 |
| | | $r_S$ | −0.308 | −0.308 | 33 | 0.080 |
| | | $r_S$ | 0.263 | 0.263 | 40 | 0.098 |
| Westneat 2006 | Dulling vs harem size | $t$ | 1.1 | 0.087 | 26 | 0.29 |
| Yasukawa et al. 2009a | Dulling vs female preference | $z$ | 2.05 | 0.377 | 27 | 0.057 |
| | **Reproductive success** | | | | | |
| Weatherhead and Boag 1995 | Color vs total fledglings | $r_S$ | −0.13 | −0.13 | 65 | 0.29 |
| Westneat 2006 | Dulling vs WP paternity | $t$ | 3.3 | 0.291 | 17 | 0.07 |
| | Dulling vs EP fledglings | $t$ | 0.03 | 0.002 | 26 | 0.97 |
| | Dulling vs total fledglings | $t$ | 1.61 | 0.126 | 26 | 0.12 |
| Yasukawa et al. 2009b | Reddening vs total fledglings | $z$ | −0.9 | −0.177 | 26 | 0.36 |
| This study | Dulling vs total fledglings | $z$ | 1.34 | 0.270 | 24 | 0.18 |

experimental males did not differ in total number of nesting attempts ($z = 0.4$, $P = 0.73$, $N_C = 12$, $N_D = 11$), number of eggs/nest ($z = 1.0$, $P = 0.32$, $N_C = 12$, $N_D = 11$), number of nestlings/egg ($z = 1.0$, $P = 0.34$, $N_C = 12$, $N_D = 11$), or number of fledglings per nestling ($z = 1.1$, $P = 0.26$, $N_C = 11$, $N_D = 7$). Although dulled-epaulet male Red-winged Blackbirds were less successful in producing fledglings than control males, none of the differences in reproductive success were significant (within-pair: $z = 1.1$, $P = 0.27$, $N_C = N_D = 13$; extra-pair: $z = 1.8$, $P = 0.079$, $N_C = N_D = 13$; realized reproductive success: $z = 1.3$, $P = 0.18$, $N_C = N_D = 13$; Fig. 1C).

**Meta-analysis.** We identified nine studies of Red-winged Blackbird epaulet color with 16 effect-size estimates (Table 1). Three studies of male–male competition produced a weighted mean effect size of 0.09 (95% CL = −0.24 and 0.42). Three studies of female choice yielded a weighted mean effect size of 0.10 (95% CL = −0.22 and 0.41). Four studies of reproductive success resulted in a weighted mean effect size of 0.01 (95% CL = −0.19 and 0.21). The overall weighted mean effect size for all 16 effect-size estimates was 0.06 (95% CL = −0.06 and 0.18). These small effect sizes thus do not appear to differ significantly from 0.

We identified eight studies of Red-winged Blackbird epaulet size with 21 effect-size estimates (Table 2). Four studies of male–male competition produced a weighted mean effect size of 0.09 (95% CL = −0.22 and 0.40). Two studies of female choice yielded a weighted mean effect size of 0.06 (95% CL = −0.07 and 0.18). Two studies of reproductive success resulted in a weighted mean effect size of 0.11 (95% CL = 0.01 and 0.22). The overall weighted mean effect size for all 21 effect-size estimates was 0.08 (95% CL = −0.002 and 0.16). Thus, there is some evidence that epaulet size has a small positive effect on reproductive success, but the other small effect sizes do not appear to differ significantly from 0.

## DISCUSSION

Neither our dulling experiment nor our meta-analysis produced evidence that dull epaulets confer a selective disadvantage for male Red-winged Blackbirds, even though epaulets are

Table 2.   Effect of epaulet size in male Red-winged Blackbirds.

| Reference | Type of study | Test statistic | Statistic value | Effect size | $n$ | $P$ |
|---|---|---|---|---|---|---|
| | **Male-male competition** | | | | | |
| Searcy 1979b | Length of red vs dominance rank | $r_S$ | 0.444 | 0.444 | 14 | 0.12 |
| | Length of yellow vs dominance rank | $r_S$ | −0.101 | −0.101 | 14 | 0.75 |
| Hansen and Rohwer 1986 | Enlarged vs normal # hits | $t$ | −1.64[*] | −0.135 | 49 | 0.11 |
| | Normal vs reduced # hits | $t$ | −1.56[*] | −0.111 | 36 | 0.13 |
| Eckert and Weatherhead 1987 | Epaulet length vs dominance rank | $r_S$ | 0.589 | 0.589 | 8 | 0.15 |
| | | $r_S$ | 0.665 | 0.665 | 10 | 0.048 |
| Røskaft and Rohwer 1987 | Normal vs enlarged floater closest approach | $U$ | 46.5 | 0.491 | 16 | 0.046 |
| | **Female choice** | | | | | |
| Searcy 1979a | Length of red vs harem size | $r_S$ | −0.153 | −0.153 | 42 | 0.35 |
| | | $r_S$ | 0.197 | 0.197 | 34 | 0.28 |
| | | $r_S$ | 0.122 | 0.122 | 41 | 0.46 |
| | Length of yellow vs harem size | $r_S$ | 0.112 | 0.112 | 42 | 0.48 |
| | | $r_S$ | −0.092 | −0.092 | 34 | 0.53 |
| | | $r_S$ | 0.194 | 0.194 | 42 | 0.23 |
| Westneat 2006 | Reduction vs harem size | $t$ | 0.36 | 0.030 | 42 | 0.7 |
| | **Survival** | | | | | |
| Weatherhead et al. 1993 | Returners vs nonreturners | | | | | |
| | length of red | $t$ | −0.45 | −0.036 | 31 | 0.66 |
| | total length | $t$ | −1.68 | −0.132 | 31 | 0.10 |
| | **Reproductive success** | | | | | |
| Weatherhead and Boag 1995 | Length of red vs total fledglings | $r_S$ | 0.05 | 0.05 | 69 | 0.69 |
| | Length of yellow vs total fledglings | $r_S$ | 0.18 | 0.18 | 68 | 0.15 |
| Westneat 2006 | Reduction vs WP paternity | $t$ | 2.6 | 0.266 | 25 | 0.11 |
| | Reduction vs EP fledglings | $t$ | 0.9 | 0.074 | 42 | 0.38 |
| | Reduction vs total fledglings | $t$ | 0.62 | 0.051 | 42 | 0.54 |

[*]Calculated from means and standard deviations in Table II of Hansen and Rohwer (1986).

sexually dichromatic coverable badges that function in territory defense and mate attraction (Searcy and Yasukawa 1983, 1995, Beletsky 1996). Similarly, neither Searcy and Yasukawa (1995) nor Westneat (2006) found evidence of current sexual selection and, in addition, Yasukawa et al. (2009b), using path analysis and a reddening experiment, found no evidence of current sexual selection on epaulet color.

There remains, therefore, the seeming contradiction between results of studies that show that epaulets are necessary for territory defense (Peek 1972, Smith 1972, Morris 1975, Røskaft and Rohwer 1987) and those that conclude that epaulets are not currently under selection. Studies where the effects of natural variation in epaulet color or size have been examined often reveal no correlation between epaulet expression and measures of reproductive success (Searcy 1979a, 1979b, Weatherhead et al. 1993, Weatherhead and Boag 1995; but see Eckert and

Weatherhead 1987). The results of studies where the epaulets of Red-winged Blackbirds have been manipulated suggest that manipulations within the natural range of color or size seem to produce little effect (Westneat 2006, Yasukawa et al. 2009a, this study), but manipulations outside the natural range often produce detrimental effects. When epaulets are removed by blackening (Peek 1972, Smith 1972, Morris 1975, Røskaft and Rohwer 1987), for example, experimental males experience disadvantages such as elevated trespassing rates, more challenging of territory ownership, and loss of territory.

One explanation for such results is that epaulets serve as one of several cues of species recognition, especially among males and at close range. Although Hansen and Rohwer (1986) concluded that epaulets were not necessary for species recognition because they found significantly stronger responses to a blackened-epaulet Red-winged Blackbird model than to

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a Brewer's Blackbird (*Euphagus cyanocephalus*) model, a species recognition hypothesis might still explain the results of epaulet blackening experiments. Red-winged Blackbird territories are relatively small and in open habitats (Yasukawa and Searcy 1995), enabling neighboring males to monitor each other visually. If blackening experiments eliminate an important species recognition signal that is otherwise nearly continuously available, black-winged blackbirds would experience elevated rates of trespassing. Black-winged blackbirds still possess other vocal and visual cues of species identification and territory defense, however, so trespassing males still recognize them as conspecifics, but males with blackened epaulets may be less effective at defending their territories because trespassers view them as inferior or submissive. Thus, epaulets in the normal range of variation are needed for territory acquisition and defense.

We are still left to explain why normal variation in epaulet expression does not covary with reproductive success. Based on a comparative analysis, Møller and Pomiankowski (1993) concluded that male ornaments are often holdovers from past sexual selection and suggested that ornaments might be maintained because females use multiple independent signals of different aspects of quality or multiple redundant signals of quality or because the signal is not costly to produce. The epaulets of male Red-winged Blackbirds may be such holdovers of past selection. With the evolution of a resource-defense, polygynous mating system and the resulting reduction in parental care by male Red-winged Blackbirds, territory and nest-site quality became more important for females than male quality (e.g., Searcy et al. 1999). If any male with a suitable territory and with epaulets within the normal range, was acceptable as a social mate, then epaulet expression or quality would no longer be correlated with reproductive success. This process would also decouple epaulet expression from territory quality (i.e., territory acquisition as a lottery system [Searcy and Yasukawa 1995]), and the result would be no covariance between epaulet expression and reproductive success.

Yasukawa et al. (2009a, 2009b) offered an alternative explanation that relies on counterbalancing intersexual advantages and intrasexual disadvantages. Epaulets within the normal range of variation are necessary for territory acquisi-

tion and defense, as in the species recognition hypothesis. However, when the signal is exaggerated by enlarging (Hansen and Rohwer 1986, Røskaft and Rohwer 1987) or reddening the epaulets (Yasukawa et al. 2009a, 2009b), adding red color bands (Metz and Weatherhead 1991, 1993), or removing the scapular feathers used to cover the epaulets (Metz and Weatherhead 1992), manipulated males experience elevated trespassing, frequent territorial challenge, and a high rate of territory loss, as well as an inability to enter neighboring territories and acquire extra-pair fertilizations. Such intrasexual disadvantages appear to place a limit on epaulet size and color. In contrast, Yasukawa et al. (2009a) found that captive females preferred males with reddened epaulets to those with normal epaulets. In addition, our meta-analysis seems to indicate that epaulet size has a small positive effect on male reproductive success and we found that the difference between control and dulled-epaulet males in extra-pair success approached significance ($P = 0.079$), suggesting that females may use epaulet expression to choose copulation partners one they have selected a nesting territory. Such results suggest that intersexual selection, in contrast to intrasexual selection, may favor males with larger and redder epaulets. Thus, as suggested by the counter-balancing sexual selection hypothesis, the lack of covariance between normal levels of epaulet expression and reproductive success occurs because the two forms of sexual selection cancel each other.

The conclusion that Red-winged Blackbird epaulet expression does not covary with reproductive success seems robust given the number of studies supporting that conclusion and the results of our meta-analysis. We have discussed two possible explanations for these results and believe that the counter-balancing selection hypothesis is better supported at this point. Further tests of this hypothesis could involve additional manipulations of epaulet color or size to determine their effects on territory defense and acquisition of social and extra-pair mates. We would predict, for example, that half-blackened epaulets would be disadvantageous in both territory defense and mate attraction, and that reddening the yellow portion of the epaulet to match the rest of the epaulet, thereby slightly enlarging it, would be intrasexually advantageous, but intersexually disadvantageous. Thus, although

many investigators have attempted to untangle the relationships between intersexual selection, intrasexual selection, and epaulet size and color, additional studies would still be informative.

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