Supporting Online Material for

Dynamic Paternity Allocation as a Function of Male Plumage Color in Barn Swallows

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This PDF file includes:

Supporting Online Material

Materials and Methods

Feather Coloration Measurement

Feather collection and color analyses followed previously described methods (*S1*). To evaluate individual changes in plumage color due to our experimental manipulations, we analyzed hue, saturation, and brightness for each of four ventral regions of red-brown plumage: throat, breast, belly, and vent.

Experimental Details and Methods

There were no differences in the tail-streamer lengths ($F_{2, 25} = 0.12$, $P > 0.89$) or ages (Chi-Square = 2.43, df = 2, $P > 0.29$) of males that were randomly assigned to the three treatment groups. Moreover, because the onset of our experiment was highly synchronized, there were no differences in the clutch-initiation dates of the first breeding attempts among the three treatment groups (ANOVA: $F_{2, 29} = 0.14$, $P > 0.87$). The total number of breeding pairs at each site did not differ significantly among males in the three treatments groups (ANOVA: $F_{2, 29} = 0.73$, $P > 0.48$).

90% (27/30) of study pairs initiated replacement clutches with the same mate after their first clutch was experimentally removed. Three pairs did not initiate replacement clutches. Clutch sizes for first and second breeding attempts, and differences in clutch sizes between breeding bouts, did not differ among males in the three treatment groups (ANOVA: all $F < 0.90$, all $P > 0.40$), removing the possibility that the total number of offspring in each breeding bout confounded the results of our study.

We experimentally enhanced ventral plumage color (n = 13) within the range of natural variation for males in our population (Fig. S1) using a non-toxic marker (PrismaColor,

light walnut, item number 3507). To control for marker application, we applied a sham control treatment (a clear permanent marker; PrismaColor clear blender, item number 3533) to 9 males, and we included 8 additional control males whose ventral plumage was not manipulated. The methods used in this study were approved by Cornell University's Institutional Animal Care and Use Committee (protocol # 01-51).

Although, prior to plumage manipulation, males in the control and sham control treatments had slightly more colorful feathers than males in the "enhanced" treatment, none of these differences approached statistical significance (all $F < 3.0$, all $P > 0.05$).

In all four ventral regions, males in the "enhanced" group had lower hue scores (and hence feathers appearing more reddish-brown and less orange in color), and lower brightness (feathers appearing darker; except for the throat region, Paired Sample T-test: $T = 1.09$, $P > 0.29$, $n = 13$) after their feathers were experimentally colored compared to their coloration at the start of the experiment (Paired Sample T-test: all T between 3.94 and 6.36, all $P < 0.005$, $n = 13$). Saturation scores for all four regions did not change significantly between the two samples (Paired Sample T-test: all T between -0.17 and 01.82, all $P > 0.09$, $n = 13$ for all four regions). Males in the sham control group had no differences in the hue, saturation, or brightness of their feathers in four regions, with the exception of a slight increase in brightness of belly coloration (Paired Sample T-test: $T = 3.10$, $P < 0.03$, $n = 7$) between the start of the experiment and after the sham treatment was applied to their feathers (Paired Sample T-test: all other T between -0.50 and -1.32, all $P > 0.16$, $n = 7$ individuals).

Microsatellite Analyses

We used three polymorphic microsatellite loci for genetic parentage analyses HrU6, HrU7 (*S2*) and HrU10 *(S3*). These hyper-variable markers gave us high power to exclude social males in cases of extra-pair paternity (exclusion probability = 0.997; Table S1).

We extracted genomic DNA from embryos using DNeasy^{TM} Tissue Kits (Qiagen Inc.) and from blood samples using Eppendorf gDNA blood extraction kits (Brinkmann).

For HrU6 and HrU7, each 10µl PCR reaction contained 10ng of DNA, 20mM Tris-HCl (pH 8.4), 50mM KCl, 1.5mM $MgCl_2$(1mM $MgCl_2$ for HrU10), 0.2µM dNTPs (Invitrogen), 1.2pmol of each primer and 5 Units of Taq DNA polymerase (Invitrogen). The PCR cycling profile consisted of an initial step at 95^{o}C (HrU6 and HrU7)/ 94^{o}C (HrU10) for 2 minutes followed by 34 cycles at 95^{o}C (HrU6 and 7)/ 94^{o}C (HrU10) for 50 seconds, 62^{o}C(HrU6)/60^{o}C(HrU7)/ 56^{o}C(HrU10) for 1 min and 72^{o}C for 1 min (HrU6 and 7) or 40 seconds (HrU10). After the final cycle an extension step at 72^{o}C for 45 minutes was included for all three markers. Samples were genotyped on an ABI 3100 (Applied Biosystems) and fragments sizes were determined using Genemapper. We repeated the electrophoresis with HrU10 primers for individuals found to be homozygous during electrophoresis with the shorter LIZ-500 ladder, using the longer MapMarker 1000 ladder to ensure that we detected alleles longer than 500 base pairs.

Paternity Exclusion Analyses

We used Genemapper version 3.0, (Applied Biosystems) to determine allele sizes and assign genotypes for each individual at the three loci. We used CERVUS version 2.0 to calculate the exclusion probabilities for assessing parentage, and to test for the presence of null alleles (*S4*).

Statistical Analyses

All analyses were conducted using SAS version 9.1.

To analyze the results of our experiment, we made four comparisons. First, we analyzed the proportion of a male's paternity and the number of young he sired in his own nest at the start of the experiment. Next, we analyzed the differences in these two variables

between the two successive breeding attempts to directly assess the consequences of the phenotypic manipulation.

We used Anderson-Darling Normality Tests and Levene's Tests to check the two primary assumptions of using ANOVAs: normality and equal variances, respectively. Because the proportion data (paternity of males across each treatment group at the start of the experiment (Fig. 1A in) and after the manipulation), were not normally distributed, we applied a logistic model using the binomial independent variable (number of own young in clutch/total number of young in clutch) and a logit-link function to analyze differences among treatment groups.

Differences in proportions and number of young between the two breeding attempts, and the number of a male's own young in each breeding attempt (before and after the manipulation), were all normally distributed, with statistically equivalent variances, within each treatment group. Accordingly, we were able to use F-tests (one-way ANOVAs) to describe the results of our experiment with respect to these three response variables, as well as the other baseline analyses presented throughout the text (e.g., that demonstrate no differences among males in each treatment with respect to streamer length, the onset of breeding of the first clutch, first clutch size, second clutch size, and group size at the breeding site). When the statistical significance of the model was $P < 0.05$, we applied Tukey's pairwise difference tests (individual error rate = 0.05) to analyze differences among treatment groups.

Figure S1. Distributions of male color across four different ventral regions, using three standard axes of color: hue, saturation, and brightness, indicate that after the experimental manipulation, males in the enhanced treatment group had coloration that was within the range of natural coloration of a sample of 190 males from the study population, including males in the control treatment groups. These box plots represent the median plus the 25th and 75th quartiles and extreme values. Individuals with lower hue values, higher saturation values, and lower brightness values are redder and darker in appearance.

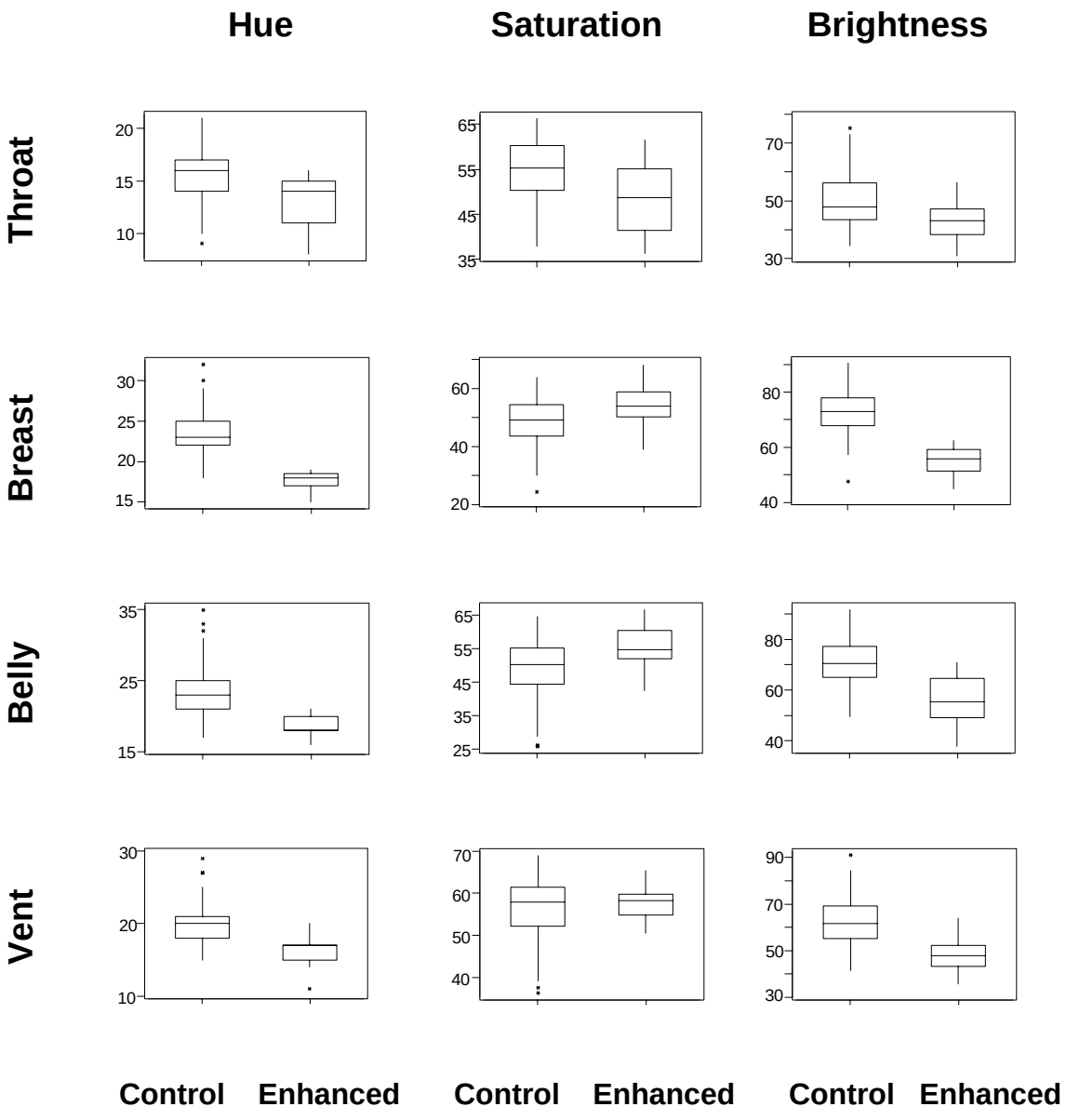

Table S1. Variability of the three microsatellite loci based on a sample of 526 individuals (59 complete families). P_e is the probability of exclusion based on variability at a single locus; h_e is the expected heterozygosity and h_o is the observed heterozygosity. The total probability of paternal exclusion using all three loci is 0.997.

Locus	**No. alleles**	**P_e**	**h_e**	**h_o**	**Null Frequency**
HrU6	70	.96	.96	.92	.005
HrU7	3	.26	.50	.47	.02
HrU10	95	.92	.98	.95	.03

References

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