MATE PREFERENCE FOR A PHENOTYPICALLY PLASTIC TRAIT IS LEARNED, AND MAY FACILITATE PREFERENCE-PHENOTYPE MATCHING

Erica L. Westerman,[1,2,3] **Napon Chirathivat,**[1] **Elizabeth Schyling,**[1] **and Antónia Monteiro**[1,4]

[1]*Department of Ecology & Evolutionary Biology, Yale University, New Haven, Connecticut 06511*

[2]*Current Address: Ecology & Evolution Department, University of Chicago, Chicago, Illinois 60615*

[3]*E-mail: ewesterman@uchicago.edu*

[4]*Current Address: Department of Biological Sciences, National University of Singapore, Singapore 117543*

Fixed, genetically determined, mate preferences for species whose adult phenotype varies with rearing environment may be maladaptive, as the phenotype that is most fit in the parental environment may be absent in the offspring environment. Mate preference in species with polyphenisms (environmentally dependent alternative phenotypes) should therefore either not focus on polyphenic traits, be polyphenic themselves, or learned each generation. Here, we test these alternative hypotheses by first describing a female-limited seasonal polyphenism in a sexually dimorphic trait in the butterfly *Bicyclus anynana*, dorsal hindwing spot number (DHSN), and then testing whether male and female mate preferences for this trait exist, and whether they are seasonally polyphenic, or learned. Neither naïve males nor naïve females in either seasonal form exhibited mating preferences for DHSN. However, males, but not females, noticed DHSN variation and learned mate preferences for DHSN. These results suggest that individuals may accommodate environmentally dependent variation in morphological traits via learned mate preferences in each generation, and that learned mate preference plasticity can be sexually dimorphic.

KEY WORDS: *Bicyclus anynana*, mate choice, seasonal polyphenism, sexual dimorphism, sexual imprinting.

Many animal species develop discrete adult phenotypes induced by different environmental conditions experienced during development (polyphenisms; reviewed in Nijhout 2003). These polyphenisms are often adaptive because the environmental cues used to induce the alternative phenotypes are predictive of the future selective environment where each phenotype will live as an adult (Levins 1968; Moran 1992). In seasonal polyphenisms, there is a high probability that the phenotypes present in the parental environment will be absent in the offspring environment because successive generations are often influenced by distinct environmental cues. For this reason, innate, genetically determined mate preferences for plastic traits in these species may be maladaptive, as an inherited mate preference would result in offspring searching for mates with an absent or maladaptive phenotype. One way to circumvent this potential mate preference-phenotype mismatch would be for species with polyphenisms to only use nonpolyphenic traits in mate selection. An alternative way to circumvent this potential mate preference-phenotype mismatch would be for mate preferences to vary across seasonal forms to track (or accentuate) the plasticity of adaptive traits. If species use the latter mechanism, then, seasonal variation in mate preferences could potentially occur in one of two ways: either species evolve polyphenic innate mate preferences, or they learn preferences each generation.

Mate preference learning, where a premating social experience influences future mate selection, occurs across animal taxa displaying variable reproductive strategies, such as birds, fish, mammals, and arthropods (reviewed in Verzijden et al. 2012). Learning a mate preference is hypothesized to be advantageous because it allows preferences to change in response to changes in

morphological characteristics at a faster rate than having fixed innate mate preferences (Immelmann 1975; Todd and Miller 1993). To date, the study of the evolutionary importance of mate preference learning has been focused on how learned preferences influence speciation rates and sexual selection (Kalmus and Maynard Smith 1966; Laland 1994; Grant and Grant 1997; Irwin and Price 1999; ten Cate and Vos 1999; Plenge et al. 2000; Aoki et al. 2001; Ihara et al. 2003; Albert 2005; Olofsson et al. 2011; Verzijden et al. 2012), not on how mate preference learning may allow species with polyphenisms to develop mate preferences that track intergenerational phenotypic variation. One reason for this may be that mate choice learning has traditionally been studied in systems with parental care where offspring learn mate preferences for phenotypes similar to their parents (Verzijden et al. 2012). In species with polyphenisms, learned mate preferences for a parental phenotype would be maladaptive because future mates would have phenotypes different from those of the parents. However, many species with polyphenisms are insects that do not have parental care (Shapiro 1976; Brakefield and Larsen 1984; Nijhout 1999), and are therefore unlikely to learn preferences for the parental phenotype. It is more likely that individuals in these systems learn preferences for nonparental sexually mature individuals present in the social environment of sexually immature or sexually inexperienced individuals, as has been observed in spiders, butterflies, damselflies, crickets, and fruitflies (Hebets 2003; Bailey and Zuk 2009; Rutledge et al. 2010; Svensson et al. 2010; Dukas et al. 2012; Westerman et al. 2012). Learned preferences for these nonparental adult phenotypes would result in mate preferences for the phenotypes in the offspring's, but not the parent's, environment, facilitating preference-phenotype matching across generations.

An alternative mechanism for polyphenic species to vary their mate preferences across polyphenic forms is for species to evolve genetically determined but polyphenic mate preferences. To date, polyphenic mate preferences have been examined in one species, the seasonally polyphenic butterfly *Bicyclus anynana*, where females were found to be selective in one seasonal form, and males selective in the other (Prudic et al. 2012). This seasonal selectivity may be influencing seasonal ornamentation, as male ornaments are larger and brighter in the seasonal form where females are choosy, and smaller and duller in the seasonal form where females are not choosy. However, while the mate preference selectivity is seasonally polyphenic (Prudic et al. 2012), the trait that the sexes selected for in this previous study, number of dorsal forewing eyespot centers, is neither seasonally polyphenic nor sexually dimorphic in this species, (Brakefield and Reistma 1991). This leaves virtually unexplored the questions of whether mate preferences for polyphenic traits actually exist, and if they do, whether these are polyphenic or learned.

Here, we pursue these questions by first describing a newly discovered sexually dimorphic trait with a female-limited, seasonal polyphenism in the same butterfly species, *B. anynana*, and then testing whether mate preferences for this trait exist, and whether they are polyphenic and/or learned. Eyespot number in *B. anynana* has been assumed monomorphic and environmentally insensitive across all wing surfaces, with two eyespots on the ventral and dorsal forewing surfaces, and seven eyespots on the ventral hindwing surface (Condamin 1973; Windig et al. 1994). The dorsal hindwing surface has received little attention. We therefore set out to describe this previously unexplored trait, dorsal hindwing eyespot number (DHSN), to assess whether it is polymorphic, sexual dimorphic, or seasonally polyphenic, both in the lab stock and in museum specimens caught in the wild. After determining that DHSN is a polymorphic, sexually dimorphic, and seasonally polyphenic trait, we then test whether male and female *B. anynana* have naïve mate preferences for DHSN, whether naïve mating outcomes are seasonally polyphenic for DHSN, and whether mate preferences for this trait are learned.

Materials and Methods
STUDY ORGANISM AND ANIMAL HUSBANDRY
Bicyclus anynana is a subtropical African butterfly that has two seasonal forms, a dry season (DS) and wet season (WS) form, that differ in morphology and behavior (Brakefield and Reistma 1991; Prudic et al. 2012). Changes in rearing temperature alone are sufficient to produce the two different phenotypes in laboratory conditions. Forewing dorsal eyespot number, and in particular, the white, UV-reflective scales at the center of the forewing dorsal eyespots, are important in both female and male mate selection, with DS males and WS females being the choosy sex for this trait, that is, discriminating between individuals with and without the trait, and with different numbers of this trait, in mate choice trials (Robertson and Monteiro 2005; Prudic et al. 2012; Westerman et al. 2012). In addition, WS females learn mate preferences for numbers of dorsal forewing spots, and species-specific male odor has been found to influence whether females learn to prefer or avoid males with specific numbers of spots (Westerman et al. 2012; Westerman and Monteiro 2013).

Bicyclus anynana butterflies have been maintained in the laboratory since 1988. A colony was established in New Haven, Connecticut in 2006 from hundreds of eggs collected from a laboratory colony in Leiden, The Netherlands (originally established from 80 gravid females collected in Malawi in 1988). *Bicyclusanynana* were reared in walk-in climate controlled chambers at 27°C, to produce the WS phenotype, and at 17°C, to produce the DS phenotype. Both forms were reared at 80% humidity, and 12h:12h light:dark photoperiod. Larvae of both seasonal forms were fed on young corn plants and adults on mashed banana.

Individuals were sexed and visually isolated by sex on the morning of emergence to prevent uncontrolled intersexual interactions before the day of the experiment.

DHSN IN FIELD-COLLECTED *B. ANYNANA* BUTTERFLIES

To determine whether there was variation in DHSN in nature, we sampled the on-line photograph collection of *B. anynana* butterflies (nine males and eight females, each sex evenly split between the two seasonal forms, giving us nine DS and eight WS butterflies) at the Yale Peabody Museum of Natural History (www.lepdata.org) and scored DHSN for each individual.

SEASONAL POLYPHENISM AND SEXUAL DIMORPHISM IN DHSN

To determine the effect of rearing temperature on variation in DHSN, we collected eggs from a population cage containing 20–30 egg-laying females reared in WS conditions, and randomly separated the eggs into two groups, one that continued to be reared in WS conditions, the other that was reared in DS conditions. After emergence, we recorded DHSN and sex of every individual. We ran this experiment twice to obtain sufficient individuals, using eggs collected on October 31, 2011 and on February 12, 2012. Differences in DHSN between sexes and rearing temperature, as well as an interaction between sex and rearing temperature were tested using a full factorial general linear model using the pooled data from both collection dates.

BEHAVIORAL ASSAYS

All behavioral assays (choice trials and training events) were conducted under sunlamps and in front of east facing windows at 25–28°C. Behavioral assays were conducted using cylindrical hanging net cages. Trainer butterflies were removed and isolated before noon on the day of eclosion from pupa (day 1), whereas focal butterflies were removed and isolated within an hour of emergence from pupa. After emergence, training individuals were put in sex- and age-specific cages, whereas focal animals were isolated from all other butterflies (males and females) until use in a training event or mate choice trial (on day 3). Behavioral assays for all treatments were conducted from October 2011 to May 2012, and assays from three to five different treatments were conducted each day, to remove any effect of day on treatment outcome.

Mate choice trials of males and females were conducted by giving each sex the choice between two and zero dorsal hindwing spots (DHS) on the wings of the opposite sex. Zero and two DHS are the most common male and female phenotype, respectively, and both phenotypes are found in both sexes in museum collections and in our laboratory population. We controlled for variables that could co-vary with DHSN by only using zero-DHS males and two-DHS females in all the behavior experiments. These butterflies were modified using paint to change the number of eyespots on their wings (Fig. S1). Males were painted with two spots of white, UV-reflective paint (Fish Vision™ white) on their hindwing (two DHS) or on top of their forewing dorsal eyespots (as a control for any effect of paint on male or female behavior; zero DHS). The UV paint used had similar reflectance spectra to that of the natural dorsal spots of *B. anynana* butterflies (Westerman et al. 2012). Females were painted with two spots of black paint that does not reflect in the UV (Testors® enamel gloss black 1147), either directly on top of their two DHS (zero DHS), or next to the two DHS (two DHS).

In all mate choice trials, virgin focal animals were given a choice between two virgin individuals of the opposite sex, matched in age and wing size, with no prior intersexual experience. An individual of each phenotype was introduced into the focal individual's cage, and the phenotype introduced first was randomized. Choice individuals ranged between 2 and 5 days of age, but individuals were age-matched within a trial. Focal individual mating outcome was determined by dusting the individual's abdomen with orange "rodent-tracking" florescent dust (risk reactor fluorescent pigment, PF-33 clownfish orange) that is only transferred to the abdomen of an individual of the opposite sex during copulation (Joron and Brakefield 2003). Choice males/females were checked every morning, and the trial was ended when one choice individual had orange dust on his/her abdomen, at which point his/her phenotype was recorded.

ESTIMATING MALE AND FEMALE PREEXISTING MATING BIASES FOR DHSN

Mate choice trials of naïve males and females of both seasonal forms were conducted by giving each sex the choice between two and zero DHS on the wings of the opposite sex. We tested WS male preexisting mating bias by isolating a single male in a cage within an hour of emergence ($n = 30$ males), keeping these individuals isolated until day 3, and then introducing two WS females varying in DHSN (two vs. zero) but matched in age and size. We tested WS female preexisting mating bias by isolating single females in cages within an hour of emergence ($n = 30$ females), and then on day 3 introducing two WS males varying in DHSN (two vs. zero) but matched in age and size. We performed the exact same set of experiments using DS individuals.

LEARNED MALE AND FEMALE MATE PREFERENCES FOR DHSN

We tested for mate preference learning of DHSN in WS butterflies by giving both males and females a premating experience with an individual of the opposite sex with either zero or two DHS immediately after emergence, recording all behavior during the 3 h premating social encounter, and assessing the effect of

this premating experience on mating outcome, using the same methodology described in Westerman et al. (2012). We exposed newly emerged females to males with either two DHS ($n = 30$ males) or zero DHS ($n = 30$ males) for 3 h immediately following female emergence, during which the male and female were allowed to interact freely. We then removed the trainer male, left the female isolated for 36 h, and then conducted female choice trials using two- and zero-DHS males (as described above). We exposed newly emerged males to females with either two DHS ($n = 30$ females) or zero DHS ($n = 30$ females) for 3 h immediately following male emergence, during which the male and female were allowed to interact freely. We then removed the trainer female, left the male isolated for 36 h, and then conducted male choice trials using two- and zero-DHS females. Neither newly emerged females nor newly emerged males are ready to mate during the 3 h immediately following emergence from their chrysalis, therefore all focal individuals remained virgins throughout the training period, regardless of how aggressively they were pursued by the sexually mature, training male or female (Westerman et al. 2012; Westerman and Monteiro 2013). All learning experiments were conducted on the WS form.

Effect of seasonal form on mating outcome was assessed by conducting a Pearson's χ^2 test on naïve WS versus naïve DS outcomes, whereas effect of premating experience on mating outcome was assessed by conducting a whole experiment (naïve WS, WS exposed to zero-spot trainer, and WS exposed to two-spot trainer) Pearson's χ^2 test, followed by pairwise Pearson's χ^2 tests. Mating preferences in each case were identified if mating outcome differed significantly from random mating (50:50) using a sign test. Data for each sex were analyzed separately. A full-factorial generalized linear model with sex and seasonal form as fixed factors was used to determine whether there was sexual dimorphism in polyphenic naïve mating outcomes. A full-factorial generalized linear model with sex and premating experience (naïve, exposed to zero-spot trainer, exposed to two-spot trainer), on mating outcome was used to determine whether there was sexual dimorphism in mate preference learning.

EFFECT OF BEHAVIOR DURING THE TRAINING PERIOD ON FUTURE MATING OUTCOME

We recorded all behavior for both individuals, using Spectator GO!© data collection software by BIOBSERVE. A principal component analysis (PCA) of behavior for each sex was performed to create variables that were representative of activity levels and activity categories. A logistic regression was used to determine whether quantity of specific behaviors or composite behavior was significantly correlated with likelihood of trainee to mate with trainer phenotype. Analyses of variances (ANOVAs) of the individual behaviors and the composite behavior variables from the

PCA were used to determine whether trainer butterflies with different numbers of spots exhibited similar amounts of behavior during the training period.

Results

DHSN IS POLYMORPHIC, SEASONALLY POLYPHENIC, AND SEXUALLY DIMORPHIC

Examination of museum specimens collected in Kenya and Malawi revealed that DHSN is polymorphic in both sexes. Our lab rearing experiments confirmed the presence of a DHSN polymorphism in both sexes, and found that male DHSN ranges from 0 to 3, and female DHSN ranges from 0 to 5 (Fig. 1). In addition, the lab stock has sexual dimorphism, and sexually dimorphic seasonal plasticity for DHSN (general linear model [GLM] of DHSN with sex, rearing temperature, and sex × rearing temperature as parameters. Total $N = 270$, 144 females, 126 males. Whole model F ratio $= 25.737$, $P < 0.0001$, effect test: sex: F ratio $= 75.120$, $P < 0.0001$; rearing temperature: F ratio $= 1.713$, $P = 0.192$; sex × rearing temperature: F ratio $= 4.556$, $P = 0.034$; Fig. 2A). Females have, on average, more eyespots than males, and DS females have more eyespots than WS females. There was no effect of rearing temperature on male eyespot number.

NEITHER NAÏVE MALES NOR NAÏVE FEMALES EXHIBIT PREEXISTING MATING BIASES FOR DORSAL HINDWING SPOTS

Neither naïve males nor naïve females exhibited significant preexisting mating biases for either zero- or two-DHS individuals (naïve WS males: $n = 30$, Pearson $\chi^2 = 1.200$, $P = 0.2733$; DS males: $n = 29$, Pearson $\chi^2 = 2.7931$, $P = 0.0947$; naïve WS females: $n = 29$, Pearson $\chi^2 = 0.8621$, $P = 0.3532$; naïve DS females: $n = 29$, $\chi^2 = 0.0370$, $P = 0.8474$), and there was no significant sex × rearing season effect on naïve mating outcome (generalized linear model with binomial distribution with sex, rearing temperature, and sex × rearing temperature as parameters. Whole model Akaike information criterion (AICc) = 160.955, $\chi^2 = 5.309$, $P = 0.150$, effect test: sex: $\chi^2 = 0.556$, $P = 0.456$; rearing temperature: $\chi^2 = 3.415$, $P = 0.065$; sex × rearing temperature: $\chi^2 = 1.262$, $P = 0.261$; Fig. 2B).

MALES, BUT NOT FEMALES, LEARN PREFERENCES FOR DHSN, AND THIS LEARNING IS BIASED

After a brief (3 h) exposure to a zero-spot female, virgin WS males mated preferentially with zero-spot females (78%, $n = 29$, Pearson $\chi^2 = 9.965$, $P = 0.002$; Fig. 2C), while males exposed to two-spot females did not mate preferentially with two-spot females (53%, $n = 30$, Pearson $\chi^2 = 0.133$, $P = 0.715$). Males exposed to zero-spot females mated significantly more often with zero-spot females than did socially naïve males, and significantly more often than males exposed to two-spot females (whole
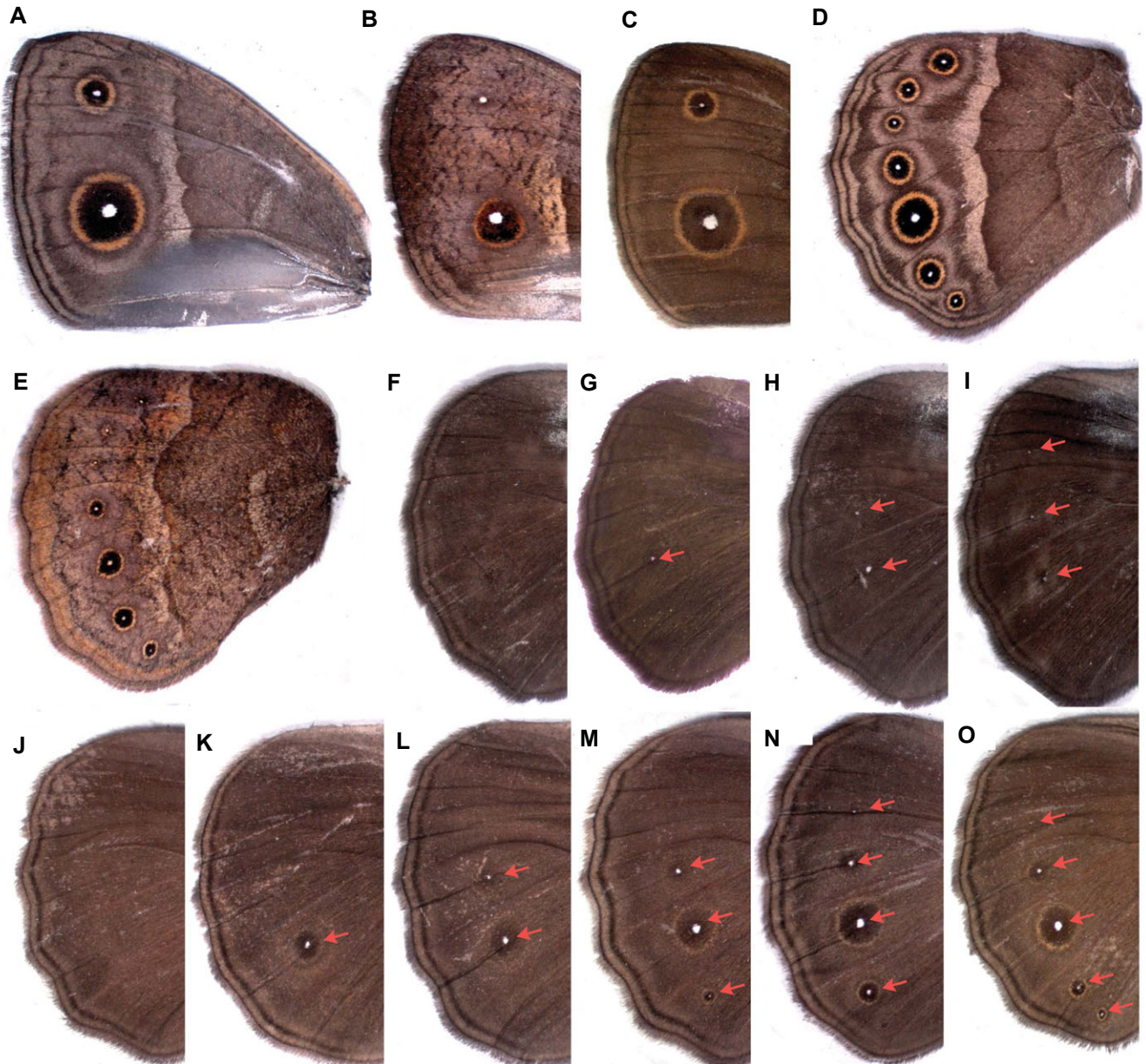
Figure 1. Wing patterns and hindwing spot polymorphism in B. anynana. A) Ventral forewing of WS forms, B) Ventral forewing of DS forms, C) Dorsal forewing of both WS and DS forms. D) Ventral hindwing of WS forms, E) Ventral hindwing of DS forms (males and females look alike on these three wing surfaces in both seasonal forms). (F-I) Dorsal hindwing variation in males, ranging from zero to three UV reflective spots. (J-O) Dorsal hindwing variation in females, ranging from zero to five UV-reflective spots. UV-reflective spots are marked by red arrows.

experiment χ^2 test, df $= 2$, $n = 90$, Pearson $\chi^2 = 11.172$, $P = 0.020$; pairwise comparison exposed zero-spot vs. naïve, 78% vs. 40%, $n = 59$, Pearson $\chi^2 = 9.443$, $P = 0.002$, exposed zero-spot vs. exposed two-spot 78% vs. 53%, $n = 59$, Pearson $\chi^2 = 4.441$, $P = 0.035$). Exposure to two-spot females had no significant effect on male mating outcomes relative to those of naïve males (40% vs. 53% mated with zero-spot females, $n = 61$, Pearson $\chi^2 = 1.346$, $P = 0.246$; Fig. 2C).

Exposure of a naïve WS female to either a two- or a zero-spot male did not influence mating outcome relative to that of naïve females (whole experiment χ^2 test, df $= 2$, $n = 89$, Pearson $\chi^2 = 0.643$, $P = 0.886$; pairwise comparisons: naïve and exposed to two-spot $n = 59$, Pearson $\chi^2 = 0.624$, $P = 0.430$; naïve and exposed to zero-spot, $n = 60$, Pearson $\chi^2 = 0.087$, $P = 0.768$; exposed to two-spot and exposed to one-spot, $n = 59$, Pearson $\chi^2 = 0.151$, $P = 0.698$; Fig. 2C).

The different learning behaviors of males and females described above resulted in a significant sex by premating experience interaction on likelihood of focal butterfly to mate with the familiar (trainer) phenotype (generalized linear model
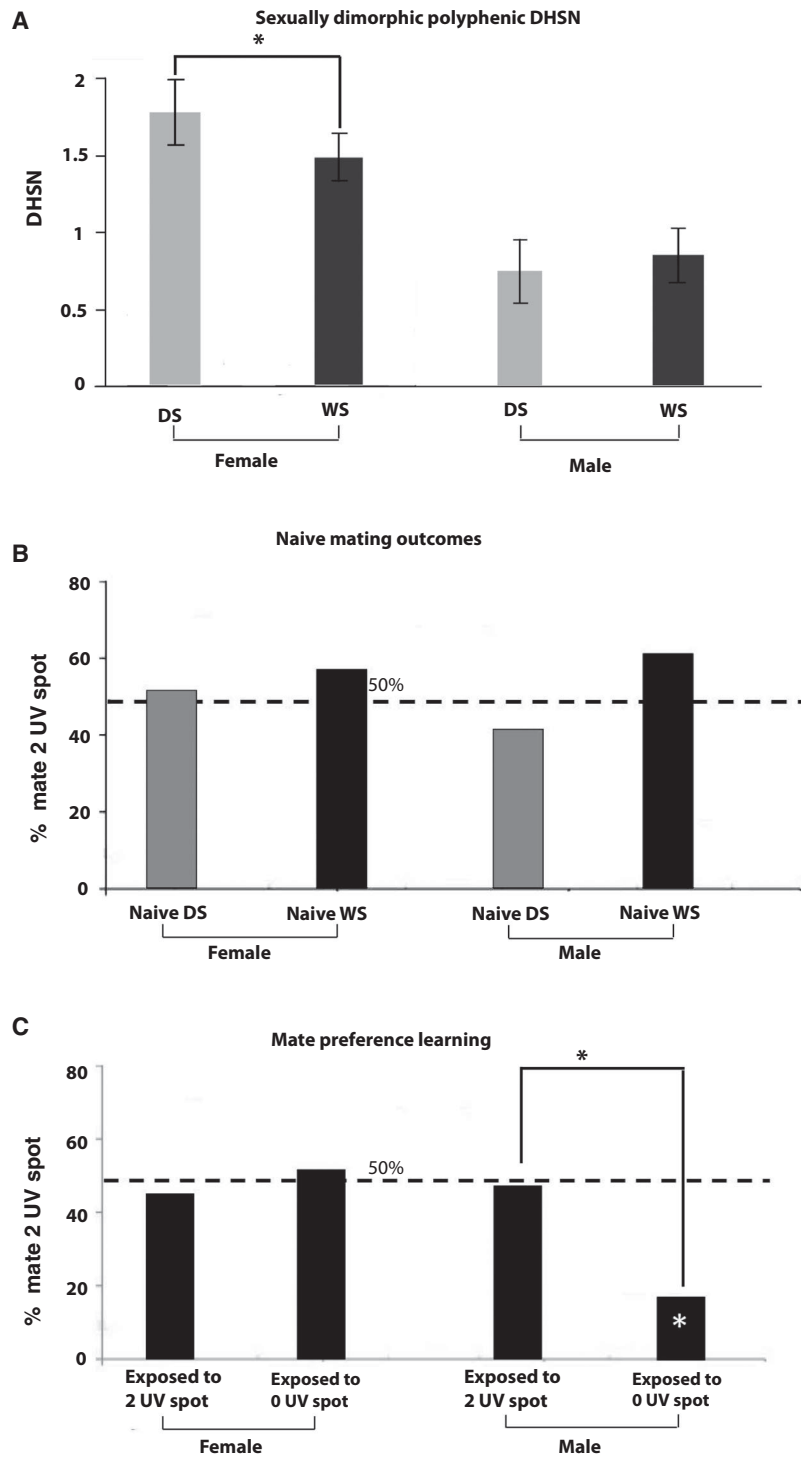
A Sexually dimorphic polyphenic DHSN

B Naive mating outcomes

C Mate preference learning

Figure 2. Sexual dimorphism in DHSN, seasonality of naïve mating outcomes, and mate preference learning. (A) Effect of rearing environment and sex on hindwing spot number. Hindwing spot number is averaged across both wings, resulting in some individuals having noninteger numbers of DHS. (B) Effect of rearing environment on male and female naïve mating outcome. (C) Effect of premating experience on male and female mate preference. All mate choices are between individuals with zero and two DHS. Gray bars represent DS butterflies, black bars represent WS butterflies. Black asterisk represents a significant difference between treatments ($P < 0.05$). White asterisk represents a mating preference significantly different from 50%. Females reared in DS conditions have more spots, on average, than females reared in WS conditions, and females consistently have, on average, more spots than males. Naïve males and females do not exhibit significant pre-existing biases for DHSN, and there is an effect of premating experience on male mate preference but not female mate preference.

with binomial distribution of mate with trainer phenotype, containing sex, premating exposure phenotype (zero or two DHSN), and sex × premating exposure phenotype, whole model test, AICc = 160.002, $\chi^2 = 9.712$, $P = 0.0212$; effect tests: sex: $\chi^2 = 3.323$, $P = 0.068$; premating exposure phenotype: $\chi^2 = 3.323$, $P = 0.068$; sex × premating exposure phenotype: exposure: $\chi^2 = 3.971$, $P = 0.0463$).

In summary, these learning experiments indicate that males notice DHSN variation in females, and that males learn to prefer zero-spot females, but not two-spot females, after exposure to these females. Females, on the other hand, do not notice DHSN variation in males, and do not learn to prefer males with up or down variation in this trait.

ZERO-SPOT FEMALE TRAINERS ARE MORE ACTIVE THAN TWO-SPOT FEMALE TRAINERS

Males may have exhibited mate preference learning of zero-spot females and not two-spot females as a result of a learning bias, where it is easier for males to learn preferences for zero-spot females than to learn preferences for two-spot females, or because zero-spot females behaved differently from two-spot females during the training period. We therefore asked whether zero- and two-spot trainer females behaved differently during the training period.

Females exhibited the following behaviors during the training period: (1) flights, (2) wing flutters, (3) walking on the cage with wings closed (for short or long periods of time), (4) circling males, (5) sitting on the cage with wings open for over half a second (bask), and (6) sitting on the cage near to, and parallel with, the male. A PCA of these six behaviors generated a first principal component (PC1) that explained 40.52% of the variation in the behavioral data, and consisted primarily of equal loadings of flutters, flights, short walks, and basks; and a second principal component (PC2) that explained an additional 27% of the variation in the behavioral data and consisted primarily of equal loadings of parallel sitting and circling the male while in flight (Table S1).

ANOVAs performed on the individual female behaviors or PC1 compound behavioral scores showed that zero-spot trainer females were more active than two-spot trainer females, with greater numbers of flutters, flights, and short walks, as well as greater PC1 compound behavioral scores than two-spot trainer females (Table S2). These results suggest that males may have learned a preference for zero-spot females merely because these females were more active. To test this hypothesis, we looked at whether male learning increased significantly with increases in trainer female activity levels in the two exposure treatments. We found that increased rates of female trainer activity did not increase the likelihood that males would mate with trainer phenotype, irrespective of trainer phenotype (Table S5). In ad-

dition, PC1 scores were not significantly associated with increased likelihood to mate with trainer phenotype for either males exposed to zero-spot female or males exposed to two-spot females.

We conclude that a learning bias toward zero-spot females, and not biased learning for any wing pattern associated with more active females, was the likely factor for the observed mating outcomes in the male learning trial. This result notwithstanding, however, we also examined whether trainer males in the female learning trials exhibited phenotype-specific activity rates, and whether female trainers were, on average, more active than male trainers. In addition, we assessed whether any particular trainer behaviors (of males and females) were associated with learning (or lack of learning) of the focal individual in each treatment.

TRAINER MALE BEHAVIOR DOES NOT CHANGE WITH PHENOTYPE, AND DOES NOT INFLUENCE FEMALE LEARNING

Males exhibited the following behaviors: (1) a stereotypic courting behavior (Nieberding et al. 2008), (2) flights, (3) wing flutters, (4) walking on the cage with wings closed (for short or long periods of time), (5) areal circling of females, (6) sitting on the cage with wings open for over half a second (bask), (7) sitting on the cage (with wings closed) near to, and parallel with, the female. A PCA containing these six behaviors generated a PC1 that explained 40.47% of the variation in the data, and consisted primarily of equal loadings of flutters, flights, short walks, and long walks (Table S1). PC2 explained an additional 19.48% of the variation in the data and consisted primarily of equal loading of courting and areal circling of females.

ANOVAs performed on the individual male behaviors or on PC1 compound behavioral scores showed that zero- and two-spot trainer males displayed similar activity levels (Table S3). In addition, trainer males and trainer females had similar average levels of individual behaviors (Table S4). There was also no effect of rate of male trainer activity on female likelihood to mate with males with trainer phenotype, irrespective of trainer phenotype.

In summary, while neither WS males nor females exhibit preexisting mating biases for zero or two DHS, WS males readily learn a mate preference for zero-, but not for two-spot females (see Table S6 for effect sizes). Zero-spot trainer females were more active than two-spot trainer females, however trainer female activity was not positively correlated with trainee male likelihood to mate with trainer phenotype. In contrast, WS females do not change their mating outcomes after exposure to either male type, and there was no effect of male phenotype on male activity levels. These data show that WS males exhibit a mate preference learning bias in the same direction as the WS female DHSN polyphenism:

WS males are better at learning preferences for zero DHS than for two DHS, and WS females have fewer spots than DS females.

Discussion
MATE PREFERENCE-PHENOTYPE MATCHING FOR POLYPHENIC TRAITS

Here, we describe a trait, DHSN, with female-limited plasticity where males, but not females, notice this trait and learn mate preferences for variation in the trait. Our results suggest that this developmentally plastic trait may be used as a sexual signal, and highlight the importance of exploring whether mate preference-phenotype matching is occurring in species with polyphenic ornamentation.

We tested two hypothesized mechanisms of mate preference-phenotype matching for polyphenic traits in the butterfly *B. anynana*: polyphenic naïve mate preferences and mate preference learning. As plasticity in DHSN was sex specific, we also had the opportunity to compare mate preference plasticity between the sexes. We found no significant naïve mate preferences for DHSN in either sex, and no significant sex by environment effect on naïve mating outcome plasticity. This finding, therefore, did not fit our hypothesis that plasticity in naïve mate preference was likely to be associated with plastic, sexually dimorphic, traits. However, we did find support for the second mechanism of preference plasticity, mate preference learning, in male, but not female butterflies. This latter mechanism of preference plasticity supports the hypothesis of preference-phenotype matching in *B. anynana*, as the sexual dimorphism in DHS mate preference learning matched the sexual dimorphism in DHSN plasticity. In addition, the mate preference learning bias for reduced DHSN exhibited by the WS males corresponds to the female DHSN polyphenism of fewer spots in WS versus DS females. We found, thus, that males do not exhibit significant naïve preferences for hindwing spots, but do exhibit significant learned preferences for number of hindwing spots, as well as a learning bias in the same direction as female trait plasticity, which suggests that male learning may be more important than male naïve preference plasticity for preference-phenotype matching in *B. anynana*. Future experiments, looking at learned preferences of DS males, would be able to test whether selection by these males also contributes to the observed pattern of female eyespot number plasticity.

This male learning mechanism is likely to be relevant in nature because male WS *B. anynana* butterflies have the opportunity to observe and learn preferences for female wing patterns before engaging in mating activities, as they have been observed to engage in social interactions in natural conditions (Brakefield and Reistma 1991), and newly emerged males require ∼2 days to start producing levels of sex pheromones that females find attractive (peak attractiveness is ∼day 14; Nieberding et al. 2012).

Male *B. anynana* mate preference learning is particularly interesting when compared with that of previous studies of the effect of social experience on male mate choice in polymorphic, but not polyphenic, populations, where individuals become less choosy after social experience with a nonpreferred phenotype (Miller and Fincke 1999; Dukas 2005; Bailey and Zuk 2008). Unlike these studies, male *B. anynana* became more selective after a social experience, and became selective in a biased way: learning to prefer females with no spots but not females with two spots. This learned and biased choosiness facilitates the formation of mate preferences for phenotypes more common in their social environment than in that of their parents.

Here, we provide an empirical example of how mate preference learning may be associated with a polyphenic trait. Theoreticians have suggested that alternative phenotypes should evolve when organisms live in environments that fluctuate in a predictable way (Levins 1968; Moran 1992). These are also the conditions theorized to select for learning over innate behaviors, particularly when the environmental fluctuation occurs between generations and offspring experience different environments from that of their parents (Bergman and Feldman 1995; Borenstein et al. 2008). This theory concerning the evolution of learning was originally developed in the context of changes in an organism's physical environment that would result in intergenerational changes in habitat, such as foraging locations and possible nesting sites. However, the theory can easily also be applied to changes in an organism's social environment, that is, changes in the appearance of conspecifics. In such cases, the theory might predict that species that display phenotypic plasticity have learned, but not preexisting, mating biases. *Bicyclus anynana* is the first species in which the existence of learned preferences for a polyphenic trait has been tested. Future research in other species with polyphenic traits is needed to determine the prevalence of this association and the potential role of preference learning as a mechanism that enables species to recognize or attract conspecifics despite the fluctuating alternative phenotypes displayed by the opposite sex.

SEXUALLY DIMORPHIC PREFERENCE LEARNING AND POTENTIAL CONSEQUENCES ON SEXUAL DIMORPHISM IN ORNAMENTATION

In this study, we found no significant innate mate preferences for variation in DHSN for either sex or seasonal form, however, we found sexually dimorphic variation in learned preferences in WS butterflies. Previously, we showed that WS *B. anynana* females have a learning bias for males with extra forewing dorsal eyespots (Westerman et al. 2012); here, we show that WS males have a learning bias for females with fewer hindwing dorsal eyespots (Fig. 2C). In addition, males learn a preference for zero-spots (on the hindwing), and distinguish between zero- and two spots,

while females do not, regardless of whether the spots are on the forewing (Westerman et al. 2012) or on the hindwing (this study). Males and females, therefore, appear to be paying attention to different traits during the learning period in *B. anynana*, not just to different parental models, as previously reported in species with parental care (ten Cate 1985; Owens et al. 1999; Slagsvold et al. 2002; Verzijden et al. 2008). Male and female *B. anynana* also appear to be learning preferences for traits that may be under different selective pressures. Males learn preferences for a trait that is polyphenic in females (DHSN), whereas females learn preferences for a trait that is currently monomorphic in males (dorsal forewing spot number), but which has recently been enhanced via the gain of an extra eyespot from a lesser spotted *Bicyclus* ancestral lineage (Oliver et al. 2009). Sexual dimorphism in the traits that are learned and in perceptual learning biases could therefore initiate the evolution of sexually dimorphic traits. Furthermore, sexual dimorphism in the type of trait that is learned may enhance the evolution of sexual dimorphism in ornaments, but not necessarily in amount of ornamentation. Males having a strong learned preference for females without ornaments exemplify this in this study.

One still unsolved question is why sexual dimorphism in learning exists in the first place. One possibility is that cognitive characteristics may be sexually dimorphic for reasons unrelated to mate choice (Ryan and Cummings 2013). Males and females often experience different selective pressures with a single sex needing to be good at identifying potential mates, or finding an egg laying site, or both sexes seeking different habitats or food sources (reviewed in Lande 1980; Hedrick and Temeles 1989). Future research examining the neurobiology of perception and learning in the two sexes is needed to determine whether sexual dimorphisms in preference learning are the result of cognitive differences in the social learning and perception abilities of the two sexes.

Conclusions

Here, we demonstrate sexual dimorphism in the ability of males and females to learn mate preferences for a morphological trait with sex-limited plasticity. Naïve mating outcomes for DHSN are modified by social experience in males, while they are not significantly modified by social experience in females. These findings support the hypothesis that mate preferences for polyphenic traits may be learned to facilitate phenotype-preference matching, and illustrate the potential for sexual dimorphism in learning ability to influence the evolution of sexually dimorphic ornamentation.

LITERATURE CITED

Albert, A. Y. K. 2005. Mate, choice, sexual imprinting, and speciation: a test of a one-allele isolating mechanism in sympatric sticklebacks. Evolution 59:927–931.

Aoki, K., M. W. Feldman, and B. Kerr. 2001. Models of sexual selection on a quantitative genetic trait when preference is acquired by sexual imprinting. Evolution 55:25–32.

Bailey, N. W., and M. Zuk. 2008. Acoustic experience shapes female mate choice in field crickets. Proc. R. Soc. Lond. B 275:2645–2650.

———. 2009. Field crickets change mating preferences using remembered social information. Biol. Lett. 5:449–451.

Bergman, A., and M. Feldman. 1995. On the evolution of learning: representation of a stochastic environment. Theor. Popul. Biol. 48:251–276.

Borenstein, E., M. W. Feldman, and K. Aoki. 2008. Evolution of learning in fluctuating environments: when selection favors both social and exploratory individual learning. Evolution 62:586–602.

Brakefield, P. M., and T. Larsen. 1984. The evolutionary significance of dry and wet season forms in some tropical butterflies. Biol. J. Linn. Soc. 22:1–12.

Brakefield, P. M., and N. Reistma. 1991. Phenotypic plasticity, seasonal climate and the population biology of Bicyclus butterflies (Satyridae) in Malawi. Ecol. Entomol. 16:291–303.

Condamin, M. 1973. Monographie du genre Bicyclus (Lepidoptera Satyridae). Ph.D. thesis, Memoires de l'Institut fondamental d'Afrique noire no. 88, IFAN, Dakar.

Dukas, R. 2005. Learning affects mate choice in female fruit flies. Behav. Ecol. 16:800–804.

Dukas, R., Z. Durisko, and L. Dukas. 2012. Learning in the context of sexual behavior and danger in female and male *Drosophila pseudoobscura*. Anim. Behav. 83:95–101.

Grant, P. R., and B. R. Grant. 1997. Hybridization, sexual imprinting, and mate choice. Am. Nat. 149:1–28.

Hebets, E. A. 2003. Subadult experience influences adult mate choice in an arthropod: exposed female wolf spiders prefer males of a familiar phenotype. Proc. Natl. Acad. Sci. USA 100:13390–13395.

Hedrick, A. V., and E. J. Temeles. 1989. The evolution of sexual dimorphism in animals: hypotheses and tests. Trends Ecol. Evol. 4:136–138.

Ihara, Y., K. Aoki, and M. W. Feldman. 2003. Runaway sexual selection with paternal transmission of the male trait and gene-culture determination of the female preference. Theor. Popul. Biol. 63:53–62.

Immelmann, K. 1975. Ecological significance of imprinting and early learning. Annu. Rev. Ecol. Syst. 6:15–37.

Irwin, D. E., and T. Price. 1999. Sexual imprinting, learning and speciation. Heredity 82:347–354.

Joron, M., and P. M. Brakefield. 2003. Captivity masks inbreeding effects on male mating success in butterflies. Nature 424:191–194.

Kalmus, H., and S. Maynard Smith. 1966. Some evolutionary consequences of pegmatypic mating systems (imprinting). Am. Nat. 100:619–635.

Laland, K. N. 1994. On the evolutionary consequences of sexual imprinting. Evolution 48:477–489.

Lande, R. 1980. Sexual dimorphism, sexual selection, and adaptation in polygenic characters. Evolution 34:292–305.

Levins, R. 1968. Evolution in changing environments: some theoretical explorations. Princeton Univ. Press, Princeton, NJ.

Miller, M. N., and O. M. Fincke. 1999. Cues for mate recognition and effect of prior experience on mate recognition in Enallagma damselflies. J. Insect Behav. 12:801–814.

Moran, N. A. 1992. The evolutionary maintenance of alternative phenotypes. Am. Nat. 139:971–989.

Nieberding, C. M., H. de Vos, M. V. Schneider, J. Lassance, N. Estramil, J. Andersson, J. Bang, E. Hedenstrom, C. Lofstedt, and P. M. Brakefield. 2008. The male sex pheromone of the butterfly *Bicyclus anynana*: towards an evolutionary analysis. PLoS One 3:e2751.

Nieberding, C. M., K. Fischer, M. Saastamoinen, C. E. Allen, E. A. Wallin, E. Hedenstrom, and P. M. Brakefield. 2012. Cracking the olfactory code of a butterfly: the scent of ageing. Ecol. Lett. 15:415–424.

Nijhout, H. F. 1999. Control mechanisms of polyphenic development in insects. BioScience 49:181–192.

———. 2003. Development and evolution of adaptive polyphenisms. Evol. Dev. 5:9–18.

Oliver, J. C., K. A. Robertson, and A. Monteiro. 2009. Accommodating natural and sexual selection in butterfly wing pattern evolution. Proc. R. Soc. Lond. B 276:2369–2375.

Olofsson, H., A. M. Frame, and M. R. Servedio. 2011. Can reinforcement occur with a learned trait? Evolution 65:1992–2003.

Owens, I. P. F., C. Rowe, and A. L. R. Thomas. 1999. Sexual selection, speciation and imprinting: separating the sheep from the goats. Trends Ecol. Evol. 14:131–132.

Plenge, M., E. Curio, and K. Witte. 2000. Sexual imprinting supports the evolution of novel male traits by transference of a preference for the colour red. Behaviour 137:741–758.

Prudic, K. L., C. Jeon, H. Cao, and A. Monteiro. 2012. Developmental plasticity in sexual roles of butterfly species drives mutual sexual ornamentation. Science 331:73–75.

Robertson, K. A., and A. Monteiro. 2005. Female *Bicyclus anynana* butterflies choose males on the basis of their dorsal UV-reflectve eyespot pupils. Proc. R. Soc. Lond. B 272:1541–1546.

Rutledge, J. M., A. Miller, and G. W. Uetz. 2010. Exposure to multiple sensory cues as a juvenile affects adult female mate preferences in wolf spiders. Anim. Behav. 80:419–426.

Ryan, M. J., and M. E. Cummings. 2013. Perceptual biases and mate choice. Ann. Rev. Ecol. Evol. Syst. 44:437–459.

Shapiro, A. M. 1976. Seasonal Polyphenism. Evol. Biol. 9:259–333.

Slagsvold, T., B. T. Hansen, L. E. Johannessen, and J. T. Lifjeld. 2002. Mate choice and imprinting in birds studied by cross-fostering in the wild. Proc. R. Soc. Lond. B 269:1449–1455.

Svensson, E., F. Eroukhmanoff, K. Karlsson, A. Runemark, and A. Brodin. 2010. A role for learning in population divergence of mating preferences. Evolution 64:3101–3113.

ten Cate, C. 1985. On sex differences in sexual imprinting. Anim. Behav. 33:1310–1317.

ten Cate, C., and D. R. Vos. 1999. Sexual imprinting and evolutionary processes in birds: a reassessment. Adv. Study Behav. 28:1–31.

Todd, P. M., and G. F. Miller. 1993. Parental guidance suggested: how parental imprinting evolves through sexual selection as an adaptive learning mechanism. Adapt. Behav. 2:5–47.

Verzijden, M. N., R. E. M. Korthof, and C. ten Cate. 2008. Females learn from mothers and males learn from others. The effect of mother and siblings on the development of female mate preferences and male aggression biases in Lake Vicotria cichlids, genus Mbipia. Behav. Ecol. Sociobiol. 62:1359–1368.

Verzijden, M. N., C. ten Cate, M. R. Servedio, G. M. Kozak, J. W. Boughman, and E. I. Svensson. 2012. The impact of learning on sexual selection. Trends Ecol. Evol. 27:511–519.

Westerman, E., and A. Monteiro. 2013. Odour influences whether females learn to prefer or to avoid wing patterns of male butterflies. Anim. Behav. 86:1139–1145.

Westerman, E., A. Hodgins-Davis, A. Dinwiddie, and A. Monteiro. 2012. Biased learning affects mate choice in a butterfly. Proc. Natl. Acad. Sci. USA 109:10948–10953.

Windig, J. J., P. M. Brakefield, N. Reitsma, and J. G. M. Wilson. 1994. Seasonal polyphenism in the wild: survey of wing patterns in five species of *Bicyclus* butterflies in Malawi. Ecol. Entomol. 19:285–298.

Associate Editor: A. Maklakov

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Table S1. Principal component analysis loadings for male and female behavior.
Table S2. Zero-spot trainer females were more active than two-spot trainer females.
Table S3. Male DHSN did not influence trainer male behavior.
Table S4. Sex did not significantly influence trainer behavior.
Table S5. Female activity levels did not significantly influence male preference learning.
Table S6. Power analysis to calculate effect sizes for detecting statistically significant effect of premating experience.
Figure S1. Wing manipulations performed in this study.