

# In the Eyes of the Beholders: Female Choice and Avian Predation Risk Associated with an Exaggerated Male Butterfly Color

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**ABSTRACT:** Color ornaments are often viewed as products of countervailing sexual and natural selection, because more colorful, more attractive individuals may also be more conspicuous to predators. However, while evidence for such countervailing selection exists for vertebrate color ornaments (e.g., Trinidadian guppies), similar studies have yet to be reported in invertebrates. Indeed, evidence for female mate choice based on extant variation in male coloration is limited in invertebrates, and researchers have not explicitly asked whether more attractive males are also more conspicuous to predators. Here we provide evidence that more chromatic male cabbage white butterflies (*Pieris rapae*) are more attractive to females but should also be more conspicuous to predators. Female *P. rapae* preferentially mate with more chromatic males when choosing from populations of males with naturally occurring or commensurate, experimentally induced color variation. Mathematical models of female color vision confirm that females should be able to discriminate color differences between prospective mates. Further, chromatic and luminance contrast scores from female visual system models better predicted male mating success than did measures of male color derived more directly from color spectra. Last, models of avian color vision suggest that preferred males should be more conspicuous to known avian predators.

**Keywords:** mate choice, sexual selection, *Pieris rapae*, color vision modeling, color ornament, avian vision.

## Introduction

Sexually selected traits are often thought to be subject to countervailing natural and sexual selective pressures (Zuk and Kolluru 1998). This concept is particularly intuitive for exaggerated color ornaments, because individuals who are more colorful and thus more attractive to mates may

often be more conspicuous to predators. Such interplay between sexual and natural selection has been considered for colorful vertebrates (e.g., in guppies; Endler 1983), but similar studies have not been reported for invertebrate color ornaments (Haynes and Yeargan 1999). Indeed, clear demonstrations of contemporary female choice for more colorful males are rare in invertebrates, and researchers have yet to ask whether more colorful, preferred males experience higher predation risk.

Invertebrates offer unique opportunities not only to extend the taxonomic scope of empirical work on color evolution but also to deepen our understanding of how and why female choice for bright male colors arises. The diversity of color production mechanisms and visual systems in invertebrates raises distinct questions concerning the coevolution of color signal production and reception (Warrant and Nilsson 2006; Osorio and Vorobyev 2008). Likewise, the diverse life-history strategies found in invertebrates offer new opportunities to understand the material costs and trade-offs related to exaggerated color ornaments and the benefits associated with female color-based preferences. Last, many invertebrates are subject to predation by animals whose visual systems are now well characterized (e.g., birds, reptiles, fish; Kelber et al. 2003). Thus, invertebrates represent a promising but thus far understudied arena for considering how sexual and natural selection interact to shape the evolution of exaggerated color ornaments and associated mate preferences.

Demonstrating that extant male color variation is under both natural and sexual selection is a nontrivial challenge, however, because researchers must first establish that male coloration is under contemporary sexual selection. In the case of sexual selection via female choice, this requires evidence that females (1) perceive and (2) act on extant color differences in prospective mates. As such, the most compelling studies have reported female behavioral responses to both naturally occurring and commensurate,

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experimentally induced variation in male coloration (e.g., in house finches; Hill 1990, 1991). In taxa where sufficient information is available regarding female color vision, researchers have further fortified this approach by employing mathematical models of female color processing, arguing that such models allow for more precise inferences regarding the visual information available to choosy females than do traditional measures of coloration (e.g., hue, chroma, and brightness; Endler 1990). The number of studies employing this robust approach continues to grow, but these studies have been restricted to vertebrates (e.g., bowerbirds, guppies, and peacocks; Grether 2000; Endler and Day 2006; Loyau et al. 2007). Once contemporary sexual selection via female choice has been established, researchers can then begin to ask whether preferred males experience higher predation risk as a result of their exaggerated colors. Again, such efforts have been confined to vertebrate color ornaments (Endler 1983; Kemp et al. 2008; but see Lewis and Cratsley 2008 for mate choice and predation on firefly bioluminescent signals).

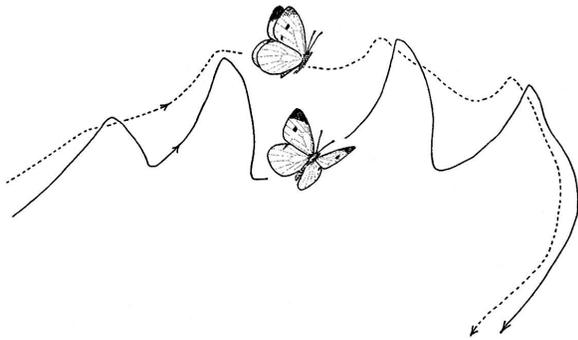
Butterflies represent a promising invertebrate taxon for considering the influence of both female choice and predation risk on male coloration. First, male butterflies are often more brightly colored than their female conspecifics (Rutowski 1997; Kemp et al. 2005), and evidence is mounting that females attend to male coloration during mate assessment (Silberglied and Taylor 1978; Robertson and Monteiro 2005; Kemp 2007, 2008; Papke et al. 2007). Importantly, several recent studies have reported female responses to male coloration within natural bounds (Kemp 2007, 2008; Papke et al. 2007), indicating that female choice can operate within extant levels of male color variation. However, evidence for female responses to both naturally occurring and comparable, manipulated variation in male coloration remains lacking for any single butterfly species. For example, in field populations of the butterfly *Colias eurytheme*, male ultraviolet (UV) coloration is correlated with mating success (Papke et al. 2007), but female preferences for males with brighter UV coloration have not been verified using manipulative studies (although females respond to gross manipulations of male coloration; see Silberglied and Taylor 1978). Similarly, while females of the butterfly *Eurema hecabe* exhibit preferences for males with brighter UV coloration in manipulative studies, comparison of the coloration of mated and unmated males in the field did not provide corroborative evidence for female color-based mating biases (Kemp 2008). Thus, while female choice appears to be operating on male butterfly coloration, more work is needed to clarify its role as a selective agent in contemporary butterfly populations.

Butterflies also present compelling opportunities for probing female visual responses to male color differences

using mathematical modeling (i.e., to verify that females should be able to perceive color differences between prospective mates). Our knowledge of butterfly color vision continues to expand (Stavenga and Arikawa 2006), and in some species we now have sufficient information to construct mathematical models of color processing that are on par with those employed in vertebrates. However, such models of female color processing have yet to be applied to male color variation in butterflies, and thus we lack clear estimates of the visual salience of male color differences to choosy females. This stands in contrast to repeated speculation that butterfly color vision and coloration may have coevolved in the context of mate choice (Bernard and Remington 1991; Stavenga and Arikawa 2006; Osorio and Vorobyev 2008).

Last, while interactions between predators and brightly colored butterflies are now well studied in the context of aposematism (Vane-Wright and Boppré 1993), the importance of predation to male butterfly color exaggeration remains an open question. Birds are thought to be the major predators of many butterfly species (Bowers et al. 1985), and avian color vision is now well described (Endler and Mielke 2005). Therefore, the potential predation risk associated with exaggerated male butterfly coloration can be estimated by modeling the conspicuousness of males to the avian predator community. Indeed, inferences of predation risk derived from avian visual models have been verified empirically in a number of instances, suggesting that visual model estimates of prey conspicuousness may serve as reasonable approximations of predation risk (Stuart-Fox et al. 2003; Husak et al. 2006; Stobbe and Schaefer 2008).

We studied female mate preferences and potential predation risk associated with male coloration in the sexually dichromatic cabbage white butterfly *Pieris rapae*. Extensive research on this butterfly species makes it a compelling and accessible focal organism for employing the integrative approach described above. First, *P. rapae* males are more chromatic on their dorsal wing surfaces than conspecific females and appear to showcase these colors during aerial courtship maneuvers (figs. 1, 2). Second, throughout their adult life span, females control male access to copulation and thus can select among prospective mates. Both virgin and mated females employ behavioral tactics to curtail unwanted male courtship, including ascending flights (e.g., Rutowski 1978) and the characteristic “mate refusal posture” (Obara 1964). Third, research on the visual capabilities of *P. rapae* clearly indicates that the female eye should be responsive to the coloration of courting males, and this research provides sufficient information for constructing visual system models (Shimohigashi and Tomimaga 1991; Qiu et al. 2002; Qiu and Arikawa 2003a, 2003b; Wakakuwa et al. 2004; Arikawa et al. 2005). Fourth, fe-



**Figure 1:** Stereotypical courtship interaction between a male (bottom; solid line) and a female (top; dashed line) *Pieris rapae*. Illustration reproduced with permission from Suzuki et al. (1977).

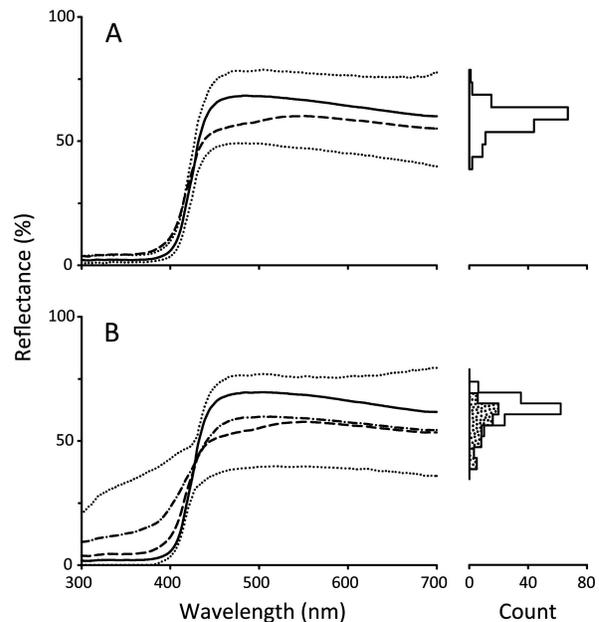
males may gain valuable information by attending to male coloration. Wing coloration in this species is the result of complex optical mechanisms associated with pterin pigments deposited in the wing scales (Stavenga et al. 2004; Morehouse et al. 2007; Luke et al. 2009), such that males who deposit larger amounts of pterins appear both darker in UV wavelengths and brighter in non-UV wavelengths (i.e., are more chromatic). Because pterin pigments are the most nitrogen-rich pigments described from the animal kingdom (Kayser 1985) and *P. rapae* is strongly nitrogen limited during larval development (Morehouse and Rutowski 2010), male wing coloration may be costly to produce and related to a male's success in acquiring a limiting nutrient (i.e., nitrogen) from diet. Finally, members of the avian predator community associated with *P. rapae* are known (e.g., Srygley and Kingsolver 1998; Lytinen et al. 1999), and their visual sensitivities can be inferred from well-established phylogenetic patterns of avian color vision (Ödeen and Håstad 2003).

We adopted an integrative approach to evaluate whether extant color variation in male *P. rapae* is under selection via female choice and, further, whether male color variation might result in differential conspicuousness to known avian predators. First, we tested the hypothesis that female *P. rapae* prefer to mate with more colorful males by recording the mating decisions of virgin females in the context of natural (study 1) and manipulated (study 2) male color variation. We evaluated differences in color phenotype between successful and unsuccessful males in each study, using parameters derived directly from raw reflectance spectra as well as estimates of color and luminance contrast generated by mathematical models of the female color vision system. Because color vision is less well understood in *P. rapae* than in other animals (e.g., birds, bees), we evaluated the utility of our visual system models by comparing the ability of spectral and visual

model parameters to predict observed female preferences. Finally, we tested the hypothesis that exaggeration of male coloration may result in increased predation risk by comparing the salience of preferred versus unpreferred male color phenotypes when viewed through visual system models of known avian predators.

## Material and Methods

We recorded male mating success in two mate choice studies (studies 1 and 2) where females were offered males that varied in color. In the first study, females were able to select mates from a group of wild-caught males that naturally varied in wing coloration as a result of variation in age, larval, and adult history (i.e., history of disease, larval diet quality, encounters with predators, mating history) and genetic makeup (fig. 2A). The second study reproduced this variation in male color by manipulating the coloration of hand-reared males while controlling for age, historical, and genetic differences (fig. 2B). Mate choice protocols corresponded between the two studies, with the exception of



**Figure 2:** Spectral reflectance of male and female *Pieris rapae* participating in study 1 (A) and study 2 (B). Average female phenotype (dashed lines), average male phenotype (solid line; A), and minimum and maximum male values (dotted lines) are displayed. In B, average control male phenotype (solid line) and extracted male phenotype (dashed and dotted line) are plotted separately. Histograms to the right correspond to the distribution of male phenotypes in each study with respect to their reflectance at 700 nm. In the histogram for B, the dotted regions represent extracted males; open regions are control males.

extra precautions taken to avoid and/or detect potential artifacts arising from the color manipulations.

*Study 1: Naturally Occurring Male Color Variation and Mating Success*

*Animals.* Females used in this study were first-generation offspring of gravid females ( $n = 15$ ) collected from a wild population near Page Springs, Arizona ( $34^{\circ}46'03''\text{N}$ ,  $-111^{\circ}53'37''\text{W}$ ), from July to August 2007. We reared all females in a climate-controlled chamber that maintained a 14L : 10D photoperiod and coincident  $30^{\circ}\text{C} : 24^{\circ}\text{C}$  temperature regime at a constant 55% relative humidity. Larvae were fed organically grown kale (*Brassica oleracea* var. *acephala* DC). During adulthood, virgin females were fed a honey-water solution (1 : 4, v:v) ad lib. and used in mate choice assays within 48 h of eclosion. We labeled each female with a unique number written with a black marker on the ventral hindwing. Males participating in mate choice trials were field caught as adults from the same original wild population during September 2007 and were kept at roughly  $4^{\circ}\text{C}$  from capture to use in the study. These males were representative of the demographic composition of their wild population (N. I. Morehouse, unpublished data), with varying ages, individual histories, and genetic backgrounds. We used all males within 48 h of their capture in the field. Males were fed honey-water until satiated on the day before their mate choice trial. We chose not to label individual males to prevent any artifacts from manipulating their appearance.

*Mate Choice Assay.* Cohorts of females ( $n = 5$ ) were released into an outdoor arena ( $3\text{ m} \times 3\text{ m} \times 3\text{ m}$ ) enclosed with fine-mesh screen. We provided flowering plants as nectar sources in the enclosure; both sexes readily nectared during the mate choice assays. After the released females began flying around the enclosure, we released 10 randomly selected males into the arena at 0900 hours. Males could freely court females over the course of a 2-h trial. Females displayed the natural range of courtship behaviors, including courtship solicitations (e.g., Rutowski 1980) and the stereotypical “mate refusal posture” (Obara 1964), but the enclosure prevented them from engaging in ascending flights, which female pierids may use in the field to curtail unwanted male courtship attempts (Rutowski 1978).

A female’s preference for a given male was indicated when she permitted him to copulate with her following courtship. On initiation of copulation, we gently separated the pair and stored the male at  $4^{\circ}\text{C}$  for later phenotypic measurements. Females maintain their sexual receptivity if copulation is not allowed to go to completion (Obara et al. 1975), so we released females back into the arena

along with a replacement male, thus maintaining a constant population of five females and 10 males within the enclosure. Each female was allowed to choose up to three males during a trial, at which point she was removed. We included female identity in statistical models to account for pseudoreplication. If a female did not mate with a male during the first hour of the assay, she was deemed unresponsive and replaced with an alternate female. At the end of the 2-h trial ( $\sim 1100$  hours), all remaining males and females were collected. We considered males that had not mated by this time to be unsuccessful. All individuals were then euthanized by freezing.

*Study 2: Experimentally Manipulated Male Color Variation and Mating Success*

*Animals.* The provenance and protocols for rearing and handling females were the same as those described above for study 1, with all females being lab-reared virgins. Males in study 2, on the other hand, were lab reared from the same stock as females. To circumvent the potential for inbreeding avoidance, we did not place sibling males and females in the same mate choice trials.

*Manipulation of Male Coloration.* To generate variation in male coloration similar to that observed in the field (fig. 2A), we manipulated male coloration by extracting small quantities of pterins from the wings of live males using an aqueous solution of 0.01 M NaOH, which slowly removes pterin pigments from the wing surfaces while leaving other features of wing and wing scale morphology intact (fig. 2B; Morehouse et al. 2007). More specifically, extracted males were held with forceps by the base of their forewings while their wings were first wetted with 70% isopropyl alcohol (IPA) for 10 s, then submerged in 0.01 M NaOH for 10 s, and finally rinsed in IPA to remove NaOH. Preliminary trials indicated that these protocols manipulated male coloration within the natural range of male color variation in the field (fig. 2). Control males were subjected to the same procedure except that their wings were not submerged in 0.01 M NaOH but instead were submerged again in IPA. Males were then held until all IPA had evaporated from their wings. On several occasions in both treatment groups, IPA wicked onto the male bodies and caused damage or death. We therefore tested the flight capability of males after manipulation by having them fly 4 m across a dark room toward a sunlit window. Only males that could continuously fly this distance twice were allowed to participate in mate choice trials. We continued monitoring male behavior and mortality during mate choice trials to ensure that our color manipulations had no unintended effects on either male type.

The resulting dorsal color variation in control and extracted males overlapped, but control male wings were on average more reflective in long wavelengths and less reflective in UV wavelengths (fig. 2B). With the exception of broadened variance in UV reflectance, the experimentally induced variation in male coloration approximated that found in field-caught males (fig. 2A).

*Mate Choice Assay.* Protocols for the mate choice assays followed those of study 1, except that the population of 10 males in each trial was composed of five extracted males and five control males. When a female copulated with a male of either type, he was immediately removed, stored, and replaced with another male of his type, thus maintaining a uniform composition of male types in the experimental population. In addition, because we were concerned that our male wing manipulation would introduce unwanted behavioral or mortality differences between extracted and control males, we monitored male mortality, male courtship attempts, and female mate solicitations as often as was feasible during the experiment. For the latter two behaviors (both aerial), we recorded these only when we could unambiguously identify the type of male involved.

#### *Phenotypic Measurements*

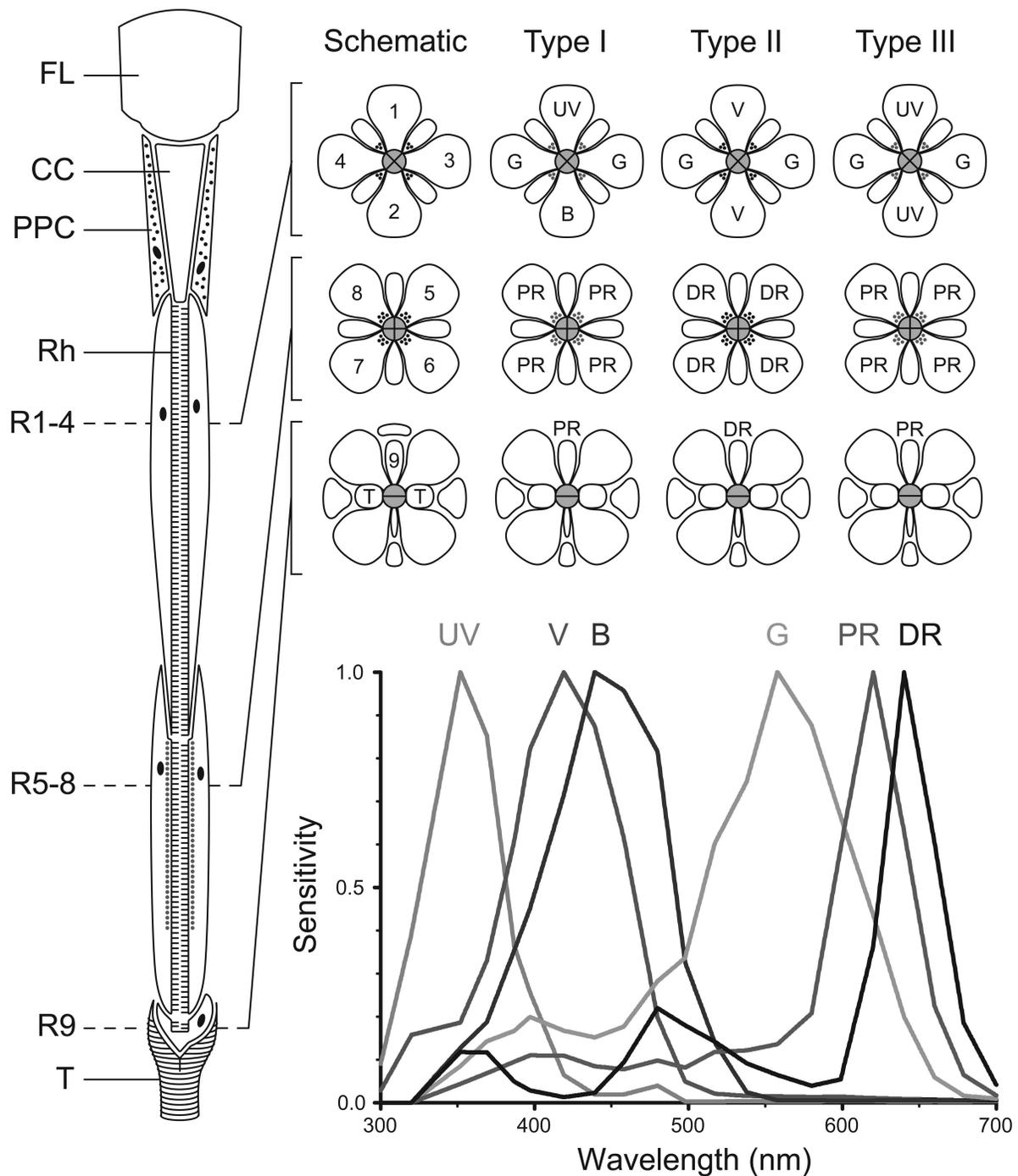
We measured all individuals from studies 1 and 2 for size and color phenotype. Forewing length (FWL), a proxy for body size, was measured as the distance between wing tip and attachment to the thorax of the left forewing using digital calipers (Digimatic Caliper 500, Mitutoyo, Tokyo). We measured color phenotype by recording the spectral reflectance of pterin-based coloration from a circular area (2-mm diameter) on the dorsal left forewing between wing veins CuA1 and 1A using a spectrophotometer (USB2000, Ocean Optics, Dunedin, FL). Wings mounted on matte black cardstock were illuminated with a pulsed xenon light source (PX-2, Ocean Optics) oriented at the zenith above the dorsal wing surface. A collimated fiber optic collector attached to the spectrophotometer was positioned 45° below the zenith toward the proximal wing margin along the distal-proximal axis of the wing. All color measurements were recorded as reflectance from 300–700 nm in relation to a magnesium oxide white standard. Each individual's coloration was measured on two separate occasions to statistically isolate measurement error. Overall repeatability between these two measurements was 99.1%, estimated according to Ludbrook (2002) as the average  $r^2$  from linear relationships fitted using standardized major axis routines run in SMATR 2.0 (Falster et al. 2006) on raw spectral parameters calculated as described below.

#### *Color Quantification and Visual Modeling*

*Quantification of Raw Spectra.* We derived four parameters ( $R_{300-375}$ ,  $R_{450-550}$ ,  $\lambda_{R_{50}}$ , and  $\beta_{R_{50}}$ ; fig. A1 in the online edition of the *American Naturalist*) from raw reflectance spectra that characterize aspects of coloration relevant to both color perception and pterin concentration (Stavenga and Arikawa 2006; Wijnen et al. 2007). Details of these parameters are reported in appendix A in the online edition of the *American Naturalist*; briefly,  $R_{300-375}$  is the average reflectance from 300–375 nm,  $R_{450-550}$  is the average reflectance from 450–550 nm,  $\lambda_{R_{50}}$  is the wavelength of the reflectance midpoint between  $R_{300-375}$  and  $R_{450-550}$ , and  $\beta_{R_{50}}$  is the slope of the spectral curve at that midpoint. A negative correlation between  $R_{300-375}$  and  $R_{450-550}$  occurs in field-caught males (N. I. Morehouse, unpublished data), produced in part by differences in the number of pterin-filled granules in male wing scales (Morehouse et al. 2007). Males with the greatest amount of pterins in their wings exhibit the highest values of  $R_{450-550}$  and lowest values of  $R_{300-375}$ . In addition, Lambert-Beer models of pigment absorption predict that higher pterin concentrations should result in higher values for  $\lambda_{R_{50}}$  and  $\beta_{R_{50}}$  (Hecht 2001; Wijnen et al. 2007). These spectral parameters should also be roughly related to color characteristics as perceived by female butterflies as follows: variation in  $R_{300-375}$  and  $R_{450-550}$  should be related to perceived brightness, variation in  $\lambda_{R_{50}}$  to hue (although how butterflies perceive hue is not fully understood, shifts in this variable should result in shifts in perceived color), and  $\beta_{R_{50}}$  to perceived chroma.

*Modeling the Visual System of Pieris rapae.* In each ommatidium of a butterfly compound eye, a paired facet lens and crystalline cone focus light from a restricted area of space ( $\sim 1^\circ$ ) down the axis of a rhabdom into which nine photoreceptor cells extend ciliated rhabdomeres containing photosensitive pigments (fig. 3; Stavenga and Arikawa 2006). In pierid butterflies, the rhabdomeres of eight of these photoreceptor cells are arranged in two distinct tiers (distal tier, R1–R4; proximal tier, R5–R8), followed proximally by the ninth photoreceptor (R9) and a broadband tapetal reflector (Stavenga and Arikawa 2006). Visual information from each ommatidium is transmitted to the lamina and medulla, where color and luminance information are thought to be processed independently, with color encoded by comparing inputs from different photoreceptor types (i.e., color opponency; Kelber 2001; Osorio and Vorobyev 2005).

We quantified the color and luminance properties of male color phenotypes as viewed through the female *P. rapae* visual system. *Pieris rapae* females, which differ in their spectral sensitivity from male *P. rapae* (Arikawa et al. 2005), have six photoreceptor types distributed in



**Figure 3:** Visual system of female *Pieris rapae*. *Left*, diagram of a single ommatidium, with facet lens (*FL*), crystalline cone (*CC*), primary pigment cells (*PPC*), tiered rhabdom (*Rh*), and basal tapetum (*T*). Photoreceptors (*R*) are arranged in a distal (*R1–R4*) and proximal (*R5–R8*) tier, with a single photoreceptor at the base (*R9*). *Pieris rapae* have three ommatidial types that differ in the arrangement and sensitivity of their photoreceptors. Receptor arrangements for these three types (I, II, and III) are illustrated in the upper right using cross-sectional diagrams that correspond to three points labeled with dashed lines on the ommatidial diagram to the left. The wavelength-specific visual sensitivities of each of the six photoreceptor types are illustrated on the bottom right; data used with permission from Stavenga and Arikawa (2006). We follow the numbering (*R1–R9*) and naming (*UV*, *V*, *B*, *G*, *PR*, *DR*) conventions for photoreceptors and ommatidia type (types I, II, and III) established in the literature on this species (e.g., Qiu et al. 2002; Wakakuwa et al. 2004). A color version of this figure is available in the online edition of the *American Naturalist*.

known combinations across three ommatidia types (fig. 3; Qiu and Arikawa 2003a; Wakakuwa et al. 2004). These photoreceptor types are named according to their peak sensitivity as follows: ultraviolet (UV), violet (V), blue (B), green (G), pale red (PR), and deep red (DR). Ommatidia types are randomly arrayed in the ventral two-thirds of the female eye, where courting males are likely to be viewed (fig. 1), but differ in their relative abundance. We derived estimates of the abundance of each ommatidia (and photoreceptor) type using histological data from *P. rapae* reported by Qiu et al. (2002). The identity of the most proximal receptor (R9) has not been established conclusively but is most likely PR in types I and III ommatidia, and DR in type II ommatidia (fig. 3; Shimohigashi and Tominaga 1991; K. Arikawa, personal communication). We modeled these receptors accordingly. We used spectral sensitivities for each photoreceptor type from intracellular recording data provided by K. Arikawa (fig. 3). These data reflect the actual absorbance profile of the photoreceptors in vivo, including light filtering by the facet lens, crystalline cone, screening pigments, and any photoreceptors in more distal tiers of the rhabdom.

We constructed models of receptor noise-limited color vision for female *P. rapae* by extending methods described by Vorobyev and Osorio (1998). Details of our methods are reported in appendix B in the online edition of the *American Naturalist*. While six photoreceptor types have been described in this species, we do not know whether all six of these are used in color discrimination. Thus, we first developed a hexachromatic visual model including all photoreceptor types. This model, which includes the most photoreceptor types and is therefore the most mathematically complex, is notable for requiring the least arbitrary assumptions regarding the visual system. We then constructed six pentachromatic visual models by sequentially excluding one photoreceptor type from the visual system. Finally, we developed a tetrachromatic visual model by removing type II ommatidia from the color vision system, thus eliminating input from V and DR photoreceptor types and changing the relative abundance of G photoreceptors (by removing the input of G photoreceptors present in type II ommatidia). This final model is compelled by recent evidence from *Papilio xuthus* that indicates these butterflies are tetrachromats because they ignore input from an ommatidia type similar to *P. rapae* type II ommatidia during color discrimination (Koshitaka et al. 2008).

Female color discrimination between average color phenotypes (female, mated male, unmated male) was modeled as the parameter  $\Delta S^t$ , which represents the number of standard deviations of receptor noise between two color stimuli. A  $\Delta S^t$  value  $<1$  is considered to be indiscriminable by a color vision system because the two stimuli fall within 1 SD of receptor noise (Vorobyev and Osorio 1998). This

parameter is critical because females can base behavioral decisions only on perceivable differences in male color (i.e.,  $\Delta S^t$  between male A and male B must be  $>1$ ). We also derived estimates of color contrast ( $\Delta S$ ) and luminance contrast ( $L$ ) with background foliage as independent measures of the salience of individual male color phenotypes to the female visual system. We follow literature precedent for the notation of visual parameters. However, for clarity we remind the reader that  $\Delta S^t$  represents color discriminability between color phenotypes, whereas  $\Delta S$  is color contrast against background foliage.

*Comparison of Visual System Models.* Although this study does not allow us to identify which of the eight visual models most accurately describes the actual capabilities of the female visual system, we evaluated the results provided by the penta- and tetrachromatic models for congruence with the hexachromatic model. The latter “full” model should in principle encode the most information regarding male color variation. Thus, comparison with this model allows us to estimate the amount of information regarding male coloration that would be “lost” if *P. rapae* actually employed a more limited subset of photoreceptors during color discrimination. Using the luminance ( $L$ ) and color contrast ( $\Delta S$ ) scores from study 1, we statistically estimated this loss of information by quantifying the linear relationships between the hexachromatic visual model and each of the reduced models using standardized major axis routines run in SMATR 2.0 (Falster et al. 2006). We interpret decreases in  $r^2$  from these pairwise linear relationships as indications of a loss of information associated with mathematical removal of a specific photoreceptor type (or types).

*Evaluation of Visual System Modeling versus Spectral Parameters.* Visual system modeling has become an increasingly pervasive feature of studies on the evolution of male coloration in vertebrates (e.g., Hudon et al. 2003; Loyau et al. 2007; Amy et al. 2008; Delhey and Peters 2008; Stoddard and Prum 2008; Lenouvel et al. 2009), replacing the use of computationally simpler parameters derived from raw spectra. However, visual system models are simplifications of visual processing phenomena (Lee 2008) and may themselves introduce undesirable artifacts into data analyses. Thus, their utility is largely based in their capacity to better describe observed patterns of visually mediated behavior. Using single- and best-subset logistic regressions, we tested whether the results of our visual system modeling better described patterns of female choice behavior by comparing the power of spectral and visual system parameters to predict male mating success in studies 1 and 2.

*Avian Visual Modeling.* Birds are ubiquitous lepidopteran predators, and a range of bird species have been observed preying on *P. rapae* and other closely related (and similarly colored) pierid butterflies (Ley and Watt 1989; Srygley and Kingsolver 1998; Lyytinen et al. 1999). Importantly, avian predators of *P. rapae* include birds whose visual system is equipped with an ultraviolet-sensitive photoreceptor (UV-type birds, including parids, muscicapids, and icterids such as *Parus major*, *Ficedula hypoleuca*, and *Agelaius phoeniceus*; Srygley and Kingsolver 1998; Lyytinen et al. 1999; Ödeen and Håstad 2003), as well as birds whose visual system includes a violet-sensitive photoreceptor (V-type birds, including corvids such as *Perisoreus canadensis*; Ley and Watt 1989; Ödeen and Håstad 2003). We therefore modeled the luminance and color contrast ( $\Delta S$  and  $L$  against green foliage) of *P. rapae* coloration to both UV-type and V-type avian visual systems, using standard tetrachromatic visual system models (following Vorobyev and Osorio 1998; specific methods further elaborated in app. B). We specifically asked whether the coloration of preferred males in study 1 would be more conspicuous to avian predators (i.e., have higher  $\Delta S$  and/or  $L$ ) than the coloration of unpreferred males and/or females in the same study.

### Statistics

All statistical tests were conducted in SAS (ver. 9.2; SAS Institute, Cary, NC), with the exception of standardized major axis line fitting, conducted in SMATR 2.0 (Falster et al. 2006). Our mate choice protocols introduced a complex covariance structure within the data set. We used statistical methods designed to specifically address the following potential sources of covariance: (1) nonindependence of mated males chosen sequentially by a given female and (2) nonindependence within the population of males in a given trial. For both studies 1 and 2, we tested for the difference between mated and unmated male phenotypes by subtracting the average unmated male phenotype of a given trial from each mated male measurement in that trial ( $FWL$ ,  $R_{300-375}$ ,  $R_{450-550}$ ,  $\lambda_{R_{50}}$ ,  $\beta_{R_{50}}$ ,  $\Delta S$ , and  $L$ ). This procedure accounts for nonindependence within the population of males in each trial and the fact that unmated males were not associated with a particular female. The resulting difference values for each mated male were then analyzed using a generalized least squares model using the PROC MIXED function in SAS. Covariance parameters within the model (e.g., between males selected by a given female) were estimated using restricted maximum likelihood, with degrees of freedom for our hypothesis tests adjusted using the Kenward-Roger method (Kenward and Roger 1997). Female was included as a random effect and trial as a fixed effect. Measurement error in the color var-

iables was estimated by including repeated measures, with individual as a random effect. We then estimated the model's intercept term, which represents the average difference between mated and unmated males for a given variable accounting for the effects of female, trial, and measurement error. We used a  $t$ -test adjusted for the model above to test whether the estimated intercept was significantly different from 0. Significance of this  $t$ -test indicates that mated and unmated male phenotypes are statistically distinguishable from each other.

In study 2, variation in male coloration was generated via experimental manipulation. While the resulting male color phenotypes varied continuously and overlapped between extracted and control males, we were also able to track the relative mating success of each male type. We tested whether the probability of mating differed between extracted and control males with a correlated binary logistic regression using PROC LOGISTIC in SAS. We initially included the effects of female and trial, but trial was not a significant predictor of relative mating success (Wald  $\chi^2 = 6.33$ ,  $P = .39$ ), and its removal increased model fit (i.e., decreased Akaike's information criterion). We therefore report results from a model containing only female and an intercept. Overdispersion arising from unbalanced and correlated mate choice decisions within female was accounted for using a Williams (1982) scaling factor. Odds ratio estimates were derived using Wald intercept estimates.

To evaluate whether visual modeling parameters better described female mating behaviors, we ran a series of logistic regressions using PROC LOGISTIC, where we evaluated the power of visual system and spectral parameters to predict male mating success in studies 1 and 2. We first ran logistic regressions with single color parameters as predictor variables, evaluating the predictive power of each variable using the size of the likelihood score ( $\chi^2$ ) statistic. We then included all visual and spectral parameters in a multiple logistic regression. Using the best-subsets selection method (Furnival and Wilson 1974), we identified the two best models (i.e., those that maximized the  $\chi^2$  statistic with the least number of predictors).

Last, we analyzed differences between female, mated male, and unmated male color phenotypes from study 1 as viewed through avian color vision models ( $\Delta S$  and  $L$ ) with a one-way ANOVA using PROC GLM in SAS. This simpler approach was appropriate because the statistical complexities associated with our mate choice design are not relevant to questions regarding predator perception of color phenotypes. Results of multiple comparisons were corrected using the Tukey-Kramer method to maintain an experiment-wide  $\alpha$  of 0.05.

**Table 1:** ANOVA results from comparison of mated and unmated male phenotypes in studies 1 and 2

	Estimated mean difference $\pm$ SE <sup>a</sup>	Trial <sup>b</sup>
Study 1:		
Forewing length (mm)	.11 $\pm$ .11	1.58
$\Delta S$	1.83 $\pm$ .22**	14.6**
<i>L</i>	.07 $\pm$ .01**	7.50**
Study 2:		
Forewing length (mm)	.33 $\pm$ .11*	1.54
$\Delta S$	7.32 $\pm$ 2.08*	2.01
<i>L</i>	-.01 $\pm$ .01	7.57**

Note: See "Material and Methods" for definitions of forewing length,  $\Delta S$ , and *L* and description of the ANOVA models used.

<sup>a</sup> Mean difference = mated males - unmated males, estimated when controlling for effects of trial, female, and measurement error ( $\Delta S$  and *L*). Statistical significance indicates that the mean in question is different from 0 in the reported direction.

<sup>b</sup> *F* values for the main effect of trial. Statistical significance indicates that the phenotypes of males differed between trial cohorts.

\* *P* < .01.

\*\* *P* < .001.

## Results

### *Study 1: Naturally Occurring Male Color Variation and Mating Success*

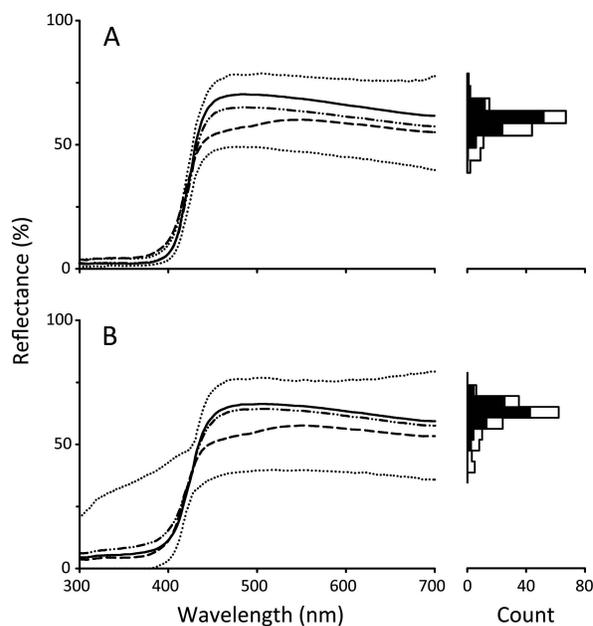
In total, seven mate choice trials were conducted during study 1, involving 35 females and 151 males. Two females were replaced because of unreceptivity. Of the remaining females, one never mated, two mated with only one male, six mated with two males, and 24 mated with three males; in total, we observed 86 matings in this study. Mated males did not differ from unmated males in size (FWL; table 1).

**Quantification of Raw Spectra.** On average, dorsal wing coloration of field-caught males differed from that of females, characterized by higher reflectance in longer wavelengths and lower reflectance in UV wavelengths (fig. 2A). Interindividual variation in male pterin-based coloration followed a normal distribution, in some instances overlapping with the female color phenotype (fig. 2A).

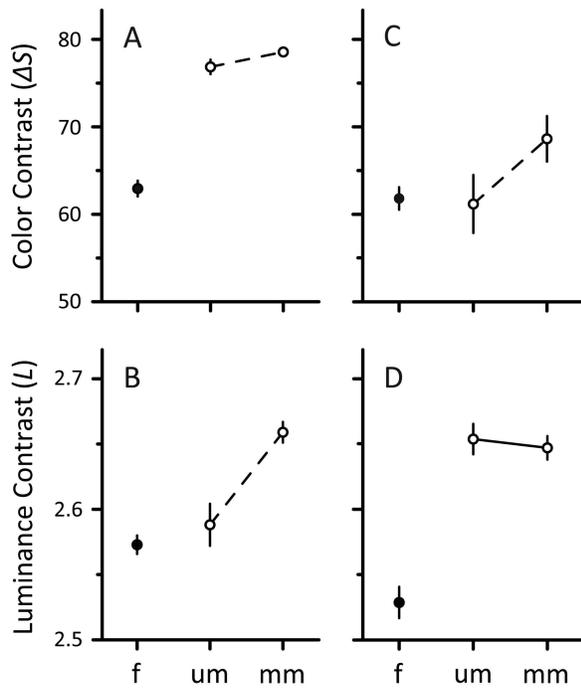
When allowed to choose freely among this population of males, females preferentially mated with males whose color phenotype was brighter in long wavelengths (higher  $R_{450-550}$ ) and darker in UV wavelengths (lower  $R_{300-375}$ ) and had a transition between these two wavelength regions, with a steeper slope occurring at a longer wavelength (higher  $\beta_{R_{50}}$  and  $\lambda_{R_{50}}$ ; table A1 in the online edition of the *American Naturalist*). These differences are all consistent with a female preference for more chromatic males (fig. 4A), whose coloration involves larger quantities of pterin pigments (Morehouse et al. 2007).

**Female *Pieris rapae* Visual Modeling.** When viewed through the visual system of *Pieris rapae* females, mated males exhibited wing coloration with both higher color contrast ( $\Delta S$ ; fig. 5A; table 1) and luminance contrast (*L*; fig. 5B; table 1) against green foliage. This result was robust across all visual models we constructed, with one exception. The removal of UV photoreceptors from female color and luminance vision led to a reversal of the perceived color contrast differences between mated males and unmated males (e.g., unmated males would appear to have higher color contrast against green foliage), although differences in luminance contrast between male types remained consistent and statistically significant (table B1 in the online edition of the *American Naturalist*). In general, our results therefore indicate that females prefer to mate with males whose wing coloration should be more salient against green foliage in both color and luminance.

In addition, results from our estimation of color discriminability ( $\Delta S'$ ) reveal that females should be able to distinguish between conspecific females, mated males, and unmated males on the basis of color differences (fig. 6A). This finding suggests that females should be capable of visually discriminating between males on the basis of naturally occurring variation in their coloration, further sup-



**Figure 4:** Spectral reflectance of male and female *Pieris rapae* in study 1 (A) and study 2 (B), as in figure 2. Average female phenotype (dashed lines), average mated male phenotype (solid lines), average unmated male phenotype (dashed and dotted lines), and minimum and maximum male values (dotted lines) are displayed. Histograms to the right are for all males, with the proportion of mated males (solid region) and unmated males (open region) indicated in each bin.



**Figure 5:** Color contrast ( $\Delta S$ ) and luminance contrast ( $L$ ) with background of female ( $f$ ), unmated male ( $um$ ), and mated male ( $mm$ ) coloration from study 1 (A, B) and study 2 (C, D), as viewed through the hexachromatic model of female *Pieris rapae* vision. Male phenotypes connected by dashed lines are statistically different and those connected by solid lines statistically similar when controlling for the effects of trial, female, and measurement error. Female phenotypes are included for reference. Error bars represent 95% confidence intervals and, when not visible, are smaller than the radius of the data point.

porting the idea that females are capable of implementing color-based mate preferences in this species. Again, this result was robust across all modeled visual systems except for the visual system lacking UV photoreceptors, in which case discriminability between mated and unmated males is lost and discriminability between the sexes is greatly reduced (fig. B1 in the online edition of the *American Naturalist*).

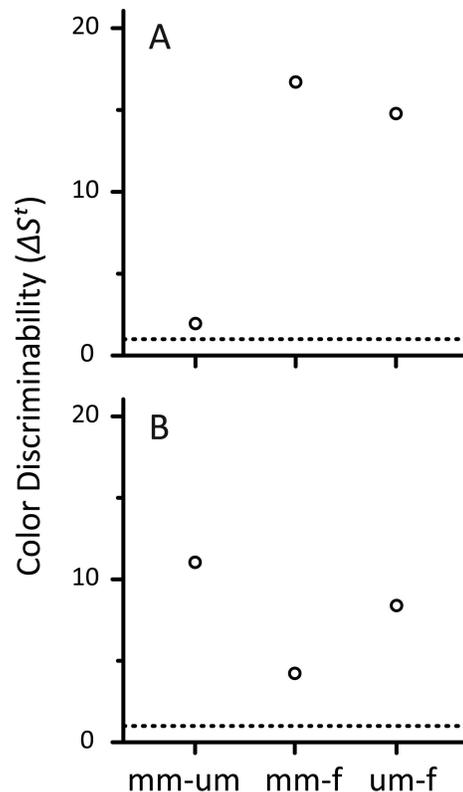
#### Study 2: Experimentally Manipulated Male Color Variation and Mating Success

We conducted seven mate choice trials during study 2, involving 41 females and 153 males. Six females were replaced because of unreceptivity. Of the remaining 35 females, four did not mate, four mated with two males, and 27 mated with three males; in total, we observed 89 matings in this study.

On average, mated males were marginally larger in size than unmated males (FWL; table 1), exhibiting a 0.33-

mm difference in FWL or approximately a 1% difference in size. However, body size was not related to male coloration when FWL was compared with parameters from raw spectra using standardized major axis regression (all  $r^2 < 0.02$ , all  $P > .05$ ). In addition, control and extracted males did not differ from each other in body size ( $t = 1.61$ ,  $P = .11$ ). Thus, if a female mating bias for larger males exists, it appears to be independent of female preferences for male coloration.

*Quantification of Raw Spectra.* Manipulation of male wings resulted in a range of dorsal color phenotypes that overlapped with those observed in field-caught males (fig. 2). Extracted males were less reflectant in long wavelengths ( $R_{450-550}$  means  $\pm$  SE; extracted:  $59.07 \pm 0.94$ ; control:  $69.28 \pm 0.32$ ;  $t = 10.31$ ,  $P < .001$ ) and more reflectant in short wavelengths ( $R_{300-375}$  means  $\pm$  SE; extracted:  $11.91 \pm 0.87$ ; control:  $2.17 \pm 0.04$ ;  $t = 11.24$ ,  $P < .001$ ). Nevertheless, there was considerable phenotypic overlap



**Figure 6:** Color discriminability ( $\Delta S^\dagger$ ) of average female ( $f$ ), mated male ( $mm$ ), and unmated male ( $um$ ) coloration from study 1 (A) and study 2 (B), as viewed through a hexachromatic model of female *Pieris rapae* color vision. The threshold below which two stimuli are indiscriminable is represented by the dotted horizontal line ( $y = 1$ ).

between extracted, control, and wild-caught males in long wavelength ( $R_{450-550}$  range; extracted: 39%–72%; control: 60%–76%; wild caught: 49%–78%) and short wavelength ( $R_{300-375}$  range; extracted: 2%–30%; control: 2%–4%; wild caught: 1%–4%) reflectance. Our color manipulations did broaden variation in male UV reflectance beyond natural levels. This may have been due to unintended removal of wing scales or added reflection from solvent residues remaining on the wings after manipulation. However, male coloration in this study still overlapped with naturally occurring variation in male coloration in the field (fig. 2A). As in study 1, females in study 2 preferentially mated with males whose coloration was brighter in long wavelengths (higher  $R_{450-550}$ ) and darker in UV wavelengths (lower  $R_{300-375}$ ) and had a transition between these two wavelength regions with a steeper slope occurring at a longer wavelength (higher  $\beta_{R_{50}}$  and  $\lambda_{R_{50}}$ ; table A1).

*Female Pieris rapae Visual Modeling.* To the visual system of *P. rapae* females, extracted males should have appeared on average less chromatic than control males ( $\Delta S$  means  $\pm$  SE; extracted:  $46.89 \pm 1.57$ ; control:  $79.34 \pm 0.25$ ;  $t = 20.43$ ,  $P < .001$ ), although the two male types did not differ in luminance contrast ( $L$  means  $\pm$  SE; extracted:  $2.66 \pm 0.01$ ; control:  $2.65 \pm 0.01$ ;  $t = 0.51$ ,  $P = .61$ ). The range of phenotypes in both male treatments overlapped with those recorded for wild-caught males for both color contrast ( $\Delta S$  ranges; extracted: 32.8–79.1; control: 68.5–82.6; wild caught: 64.0–84.3) and luminance contrast ( $L$  ranges; extracted: 2.4–2.8; control: 2.5–2.8; wild caught: 2.4–2.8).

When viewing male mating success through the eyes of females, the color phenotypes of mated males should have higher color contrast with typical backgrounds ( $\Delta S$ ; fig. 5C; table 1) than that of unmated males. However, mated and unmated males do not differ in luminance contrast when viewed using hexachromatic vision ( $L$ ; fig. 5D; table 1). The former result is consistent across all visual models except the visual model lacking UV photoreceptors, in which case mated males should appear to have lower color contrast than unmated males (table B1). In addition, several visual models reported statistical differences in luminance contrast (table B1), but these differences are very small and exhibit no discernible pattern across visual models. Results from estimation of color discriminability ( $\Delta S'$ ) indicate that females should be able to distinguish between conspecific females, mated males, and unmated males on the basis of color differences (fig. 6B). This result was robust across all visual system models (table B1).

*Logistic Regression Results.* While extracted and control males did not differ in their mortality ( $\chi^2 = 0.00$ ,  $P = 1.00$ ) or frequency of courtship attempts ( $\chi^2 = 0.13$ ,

$P = .72$ ), the relative mating success of control males was significantly higher than that of extracted males (Wald  $\chi^2 = 5.21$ ,  $P = .02$ ). Control males were nearly twice as likely to mate compared with extracted males (odds ratio = 1.77). In addition, females solicited matings from control males more than twice as much (nine solicitations for control, four for extracted), although this result is statistically nonsignificant because of the rarity of this behavior in virgin females ( $\chi^2 = 1.92$ ,  $P = .16$ ). Comparison of these latter two results suggests that control males experienced higher mating success as a result of female preference behaviors.

### Comparison of Visual System Models

Qualitative comparison of the results provided by our eight visual system models indicates that females should be able to perceive relevant color differences among potential mates using four, five, or all six photoreceptor types described from their visual system. The one exception to this pattern comes from our pentachromatic visual model lacking inputs from the UV photoreceptor. Exclusion of this photoreceptor type results in a reversal of (and reduction in) the estimated color contrast of successful and unsuccessful male phenotypes (table B1), leading to a loss of color discriminability between male phenotypes (fig. B1).

Quantitative evaluation of the information lost as a result of mathematical exclusion of specific photoreceptors supports this broad pattern. With the exception of the visual model lacking the UV photoreceptor, estimates of color and luminance contrast from all visual models are strongly correlated with those derived from the hexachromatic visual model (table 2). Further, the exclusion of the V and DR photoreceptors, either singly or together, results

**Table 2:** Statistical results from comparison of pentachromatic (5) and tetrachromatic (4) visual models to the hexachromatic visual model

Visual model	$\Delta S$ ( $r^2$ )	$L$ ( $r^2$ )
5:		
No UV	.003	.963**
No V	.999**	.999**
No B	.990**	.995**
No G	1.000**	.987**
No PR	.998**	.995**
No DR	.999**	.999**
4:		
No type II	1.000**	.996**

Note: See "Material and Methods" for definitions of  $\Delta S$  and  $L$ . Comparisons were made by fitting linear relationships between the focal visual model and the hexachromatic visual model using data from study 1.

\*\*  $P < .001$ .

**Table 3:** Results from logistic regression comparisons of the predictive value of spectral and visual model parameters

Models	Study 1 ( $\chi^2$ score)	Study 2 ( $\chi^2$ score)
Single predictor:		
$\Delta S$	9.123	6.093
$L$	32.466	.462
$R_{300-375}$	7.022	4.471
$R_{450-550}$	32.096	2.786
$\lambda_{R_{50}}$	2.062	.323
$\beta_{R_{50}}$	.805	.270
Best subsets:		
$\Delta S, L$	33.207	6.477
$\Delta S, R_{450-550}$	34.368	6.435

Note: See “Material and Methods” and appendix A in the online edition of the *American Naturalist* for definitions of parameters and statistical methods used.

in negligible loss of color information compared with the hexachromatic visual model, indicating that these photoreceptors provide little additional information regarding male color variation.

#### Evaluation of Visual System Modeling versus Spectral Parameters

We evaluated whether parameters derived from the hexachromatic visual system better predict female mate choice patterns than parameters derived from raw reflectance spectra. We find that visual system parameters serve as better predictors of male mating success than spectral parameters in nearly all cases (table 3). In addition, the most predictive multiple logistic regression models selected via best-subsets methods consistently included color contrast ( $\Delta S$ ) and luminance contrast ( $L$ ) rather than the spectral parameters. When comparing these results between studies 1 and 2,  $\Delta S$  has consistently high predictive power, whereas  $L$  is highly predictive in study 1 but much less predictive in study 2, where the spectral parameter  $R_{450-550}$  appears more highly predictive. This latter result is not surprising, given the lack of differences in  $L$  between successful and unsuccessful males in study 2.

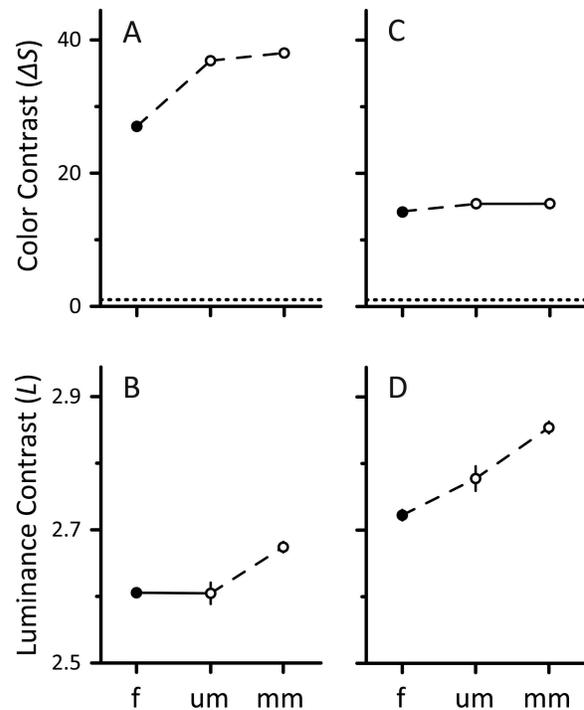
#### Avian Visual Modeling

We modeled the response of both UV-type and V-type avian visual systems to the coloration of females, mated, and unmated males from study 1 because these butterflies are more representative of the color variation viewed by avian predators in the field. To both avian visual systems, female dorsal coloration appears less conspicuous against green foliage than does male dorsal coloration, with lower color contrast to UV-type birds (fig. 7A) and lower color and luminance contrast to V-type birds (fig. 7C, 7D). In

addition, mated males appear more visually salient than unmated males to both avian visual systems, with significant increases in color and luminance contrast to UV-type birds (fig. 7A, 7B) and increased luminance contrast to V-type birds (fig. 7D). Interestingly, our model predicts that V-type birds should be unable to discriminate between mated and unmated males on the basis of color contrast (fig. 7C). This result is consistent with the alteration of color contrast discrimination when UV photoreceptors were ignored during *P. rapae* visual modeling, suggesting again that UV sensitivity is important for the perception of color contrast differences among males.

#### Discussion

Here, we provide evidence that female *Pieris rapae* select among potential mates on the basis of their dorsal wing coloration and that recorded differences in male coloration should be discriminable by the female visual system. Fur-



**Figure 7:** Color contrast ( $\Delta S$ ) and luminance contrast ( $L$ ) with background of female (f), unmated male (um), and mated male (mm) coloration from study 1, as viewed through the visual system of avian predators with an ultraviolet photoreceptor (A, B) or a violet photoreceptor (C, D). Threshold color contrast with the background is represented by the dotted horizontal line (A, C;  $y = 1$ ). Color phenotypes connected by dashed lines are statistically different, and those connected by solid lines are statistically similar. Error bars represent 95% confidence intervals and when not visible are smaller than the radius of the data point.

ther, results from our modeling of avian vision suggest that the more chromatic males preferred by females should be more conspicuous to known avian predators and thus may experience higher predation risk. Our results therefore provide a number of key advances in our understanding of color evolution in male invertebrates. However, while some of these findings parallel those derived from colorful vertebrates, we suggest that our results also motivate new avenues for research that may expand and deepen our understanding of how and why bright colors evolve.

#### *Female Color-Based Mate Choice*

Our work indicates that female *P. rapae* preferentially mate with males that are more chromatic in coloration than those they reject. We find clear evidence for this preference in female responses to wild-caught males (study 1) and, importantly, also in female responses to commensurate, manipulated variation in male coloration (study 2). Further, when compared with males whose coloration had been artificially reduced, the more chromatic control males in study 2 were nearly twice as likely to mate and were approached by females almost twice as often. These findings add to a small but growing number of studies that indicate that female choice in butterflies may act on contemporary variation in male coloration (Papke et al. 2007; Kemp 2008), providing support for Darwin's (1871) contention that bright male coloration in butterflies can evolve via sexual selection. However, translation of our results to population dynamics in the field awaits characterization of differential male mating success in free-flying populations (e.g., Kemp 2008). Further, quantifying the genetic variance (e.g., heritability) underlying male color variation will also be critical for understanding the capacity for female preferences to drive male color exaggeration in existing populations.

We also report the first evidence from butterflies that the female visual system should be able to discern color differences between prospective mates within extant male color variation. Our mathematical models of female visual acuity predict that the color phenotypes of preferred males should be distinguishable from those of unpreferred male suitors as a result of differences in luminance and color contrast. However, females in study 2 appear to have discriminated between males solely on the basis of color contrast, suggesting that differences in male brightness may be less important than variation in male chromaticity in this context. In previous studies, the ability for female butterflies to perceive male color variation has been assumed but untested. Our results provide support for this assumption in *P. rapae*, but more work is needed to verify that female butterflies in other species are similarly able to detect relevant male color differences.

While female *P. rapae* prefer to mate with more chromatic males, the evolution of this preference remains an open question. We highlight a potentially fruitful avenue for future research suggested by our results. In this study, the direction of observed female color preferences is consistent with female choice for males who deposit larger amounts of pterins in their wings. Differences in the spectral characteristics of preferred and unpreferred males follow patterns predicted from known optical mechanisms of pterin-based color production in pierid butterflies (Morehouse et al. 2007) and suggest that the wing colors of mated males were more pterin rich. Because pterins are extremely nitrogen rich (Kayser 1985) and *P. rapae* is strongly nitrogen limited during larval development (Morehouse and Rutowski 2010), the capacity for males to invest in pterin coloration may be constrained by the amount of nitrogen that males are able to acquire from diet. Therefore, females attending to male coloration may gain information regarding a given male's success in acquiring nitrogen, a resource that is likely to be strongly linked to fitness in both sexes (Morehouse and Rutowski 2010).

#### *Evolution of Male Coloration*

Our study suggests that the exaggeration of male pterin-based coloration in this species may have arisen as a result of directional selection imposed by choosy females. However, bright color traits, because of their conspicuousness to both conspecifics and heterospecific predators, are thought to be subject to a balance of sexual and natural selective pressures (Lythgoe 1979). Our results present a preliminary step in this direction, suggesting that male color exaggeration in a butterfly may come at the cost of increased predation risk. Mathematical modeling of avian color vision indicates that the color phenotype of preferred males should contrast more strongly with background foliage and hence be more conspicuous than the color phenotypes of unpreferred males or females. For UV-type birds, these more colorful males should be more visually salient as a result of increases in both color and luminance contrast (fig. 7A, 7B). Interestingly, because of the lack of a UV photoreceptor, V-type birds should not perceive an increase in color contrast with increasing male ornamentation, although increased luminance contrast should still make these males more apparent (fig. 7C, 7D). Experimental evidence suggests that *P. rapae* is likely to be subject to predation pressure from birds equipped with both types of visual systems (Ley and Watt 1989; Srygley and Kingsolver 1998; Lyytinen et al. 1999). However, the predominant visual capabilities of the avian predator community may vary geographically, which could in principle lead to differences in the nature of this selective pressure (e.g., a

shift from selection on color contrast to luminance contrast in predominantly V-type avian communities). Quantification of realized predation pressure on male *P. rapae* and how this might vary on the basis of the predator community is a promising avenue for future effort.

#### *Female Color Vision and Visual Modeling*

Comparison of our eight visual models, while not designed to test underlying color vision mechanisms, does provide clues regarding the nature of color vision in *P. rapae*. Our hexachromatic visual model best predicted male mating success on the basis of coloration. However, females may not necessarily use all of the six or more photoreceptor types in their eyes to discriminate color. Recent work on color vision in *Papilio xuthus* revealed that these butterflies, despite being endowed with eight photoreceptor types, are functional tetrachromats because input from photoreceptors unique to one ommatidial type are not incorporated into color processing (Koshitaka et al. 2008). Interestingly, comparison of our hexachromatic and tetrachromatic visual system models indicates that excluding input from the photoreceptors in type II ommatidia results in negligible information loss regarding male coloration. At face value, this result hints that type II ommatidia may not participate in color discrimination, analogous to the results from *Papilio xuthus*. However, demands on the visual system of female *P. rapae* are clearly not limited to discriminating among potential mates, and thus more careful evaluation of the importance of type II ommatidia to color perception in *P. rapae* will be useful.

Our visual system modeling indicates that UV sensitivity plays a pivotal role in the perception of color differences between males. Exclusion of UV photoreceptor inputs qualitatively changed the response of visual system models to male coloration, resulting in a reversal of perceived color contrast (table B1) and an inability to predict the color features of attractive males (fig. B1). However, given that male mate-location behaviors are dependent on the presence of UV light (Obara et al. 2008) and that females appear able to select mates on the basis of color contrast alone (study 2), we infer that female color vision includes input from their UV photoreceptors.

In terms of relating male coloration to male attractiveness, our work shows that mathematical models of female color vision are a significant improvement over previous methods of color quantification. In studies 1 and 2, we find that color contrast consistently outperforms spectral parameters in predicting male mating success (table 3). Luminance contrast also provides higher predictive power than most spectral parameters, although its predictive power appears comparable to the average spectral reflectance between 450 and 550 nm in study 1. In study 2,

luminance contrast, although still included in best-subsets models, appears less predictive than reflectance at long wavelengths, a result that makes intuitive sense, given that mated and unmated males did not differ in luminance contrast ( $L$ ; table 1) but did differ in reflectance at long wavelengths ( $R_{450-550}$ ; table A1).

Further support for the use of visual model parameters comes from our result that regression models including only luminance and color contrast were identified as one of the two most predictive models in both studies (table 3). This indicates that the female eye successfully extracts relevant variation in male color phenotypes using a combination of luminance sensitivity and color perception via opponency mechanisms (on which our models are based). Our results therefore support more extensive use of visual models in studies of color signals and color-mediated behavior in butterflies and other animals. However, we encourage additional efforts to test the assumption that current visual modeling methods provide accurate representations of visual processing of color traits.

#### *Conclusion*

In summary, we find that sexual dichromatism in *P. rapae* has likely evolved under sexual selection from female color-based mate choice and that avian predation pressure may represent a source of countervailing natural selection. We also find that mathematical modeling of invertebrate color vision corroborates our behavioral data by confirming that females should be able to discriminate males on the basis of perceivable color differences and, further, that such modeling efforts represent an improvement over more traditional measures of color quantification. In the light of these results, we encourage more work to understand the diversity and evolution of color ornaments in invertebrates, particularly efforts to combine manipulative and correlative mate choice studies with inferences from visual system modeling. Butterflies present a compelling taxon for such efforts as a result of the depth of visual system information, but similar information is available in other invertebrate systems, including stomatopod shrimp (Marshall et al. 2007) and hymenopteran insects (Peitsch et al. 1992). Comparison of insights from such invertebrates with those derived from vertebrates should significantly advance our understanding of unifying features and mechanistic particularities guiding the evolution of colorful communication.

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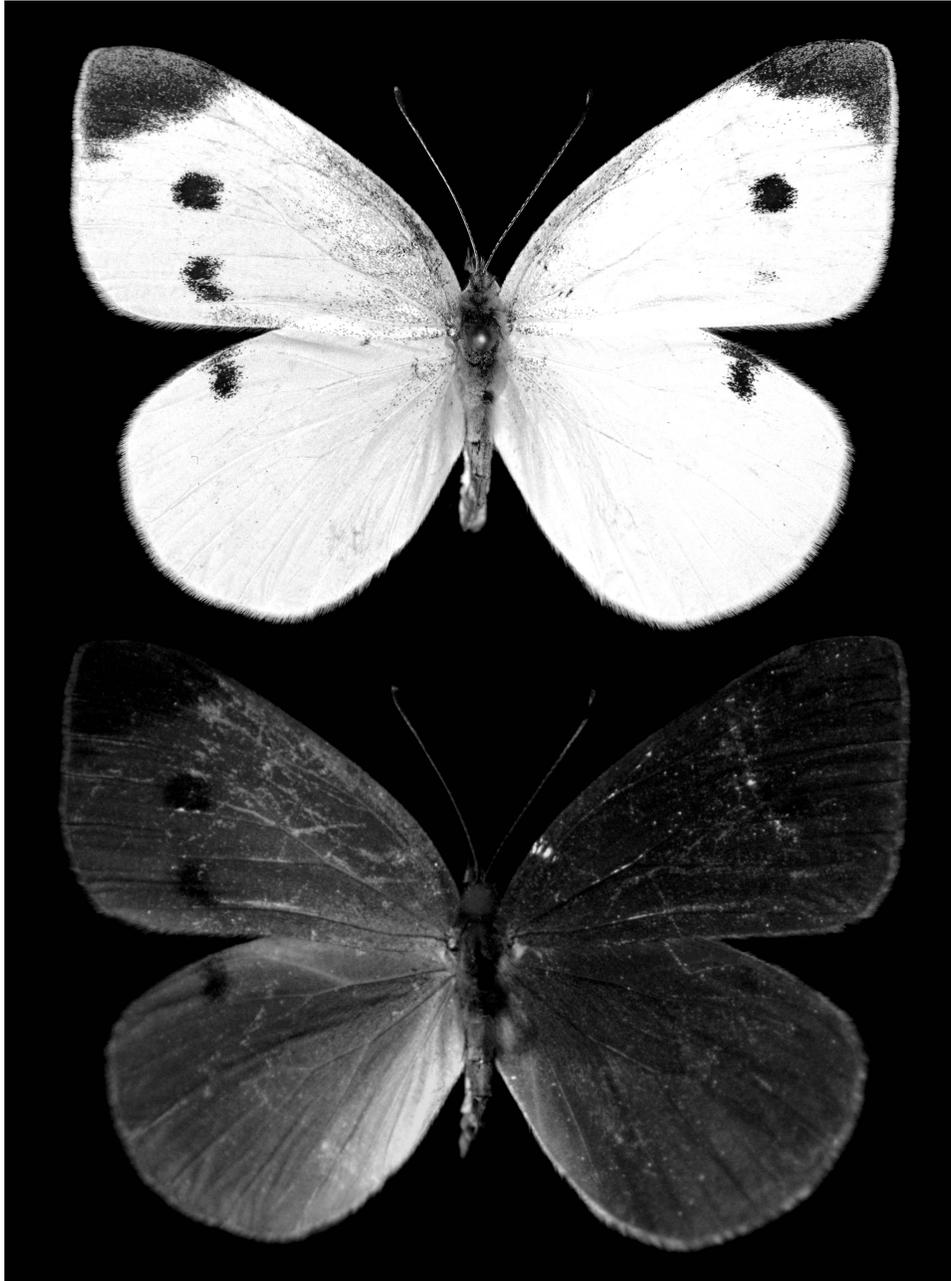
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*Pieris rapae* bilateral gynandromorph, with typical female coloration on the left side and male coloration on the right. *Top*, image produced using wavelengths visible to the human eye. *Bottom*, image produced using only ultraviolet wavelengths. Photographs by N. Morehouse. A color version of this figure is available in the online edition of the *American Naturalist*.

**Appendix A from N. I. Morehouse and R. L. Rutowski, “In the Eyes of the Beholders: Female Choice and Avian Predation Risk Associated with an Exaggerated Male Butterfly Color”**  
**(Am. Nat., vol. 176, no. 6, p. 768)**

**Definitions and Results from Spectral Analyses**

**Table A1**

ANOVA results from comparison of mated and unmated male coloration in studies 1 and 2 using parameters derived from raw spectra

	Estimated mean difference $\pm$ SE <sup>a</sup>	Trial <sup>b</sup>
Study 1:		
R <sub>300-375</sub>	-.20 $\pm$ .03**	6.00**
R <sub>450-550</sub>	5.17 $\pm$ .37**	7.58**
$\lambda_{R_{50}}$	.29 $\pm$ .11*	7.60**
$\beta_{R_{50}}$	.14 $\pm$ .01**	8.13**
Study 2:		
R <sub>300-375</sub>	-2.40 $\pm$ .68**	2.06
R <sub>450-550</sub>	1.91 $\pm$ .77*	2.80*
$\lambda_{R_{50}}$	1.69 $\pm$ .76*	1.80
$\beta_{R_{50}}$	.24 $\pm$ .07**	2.26

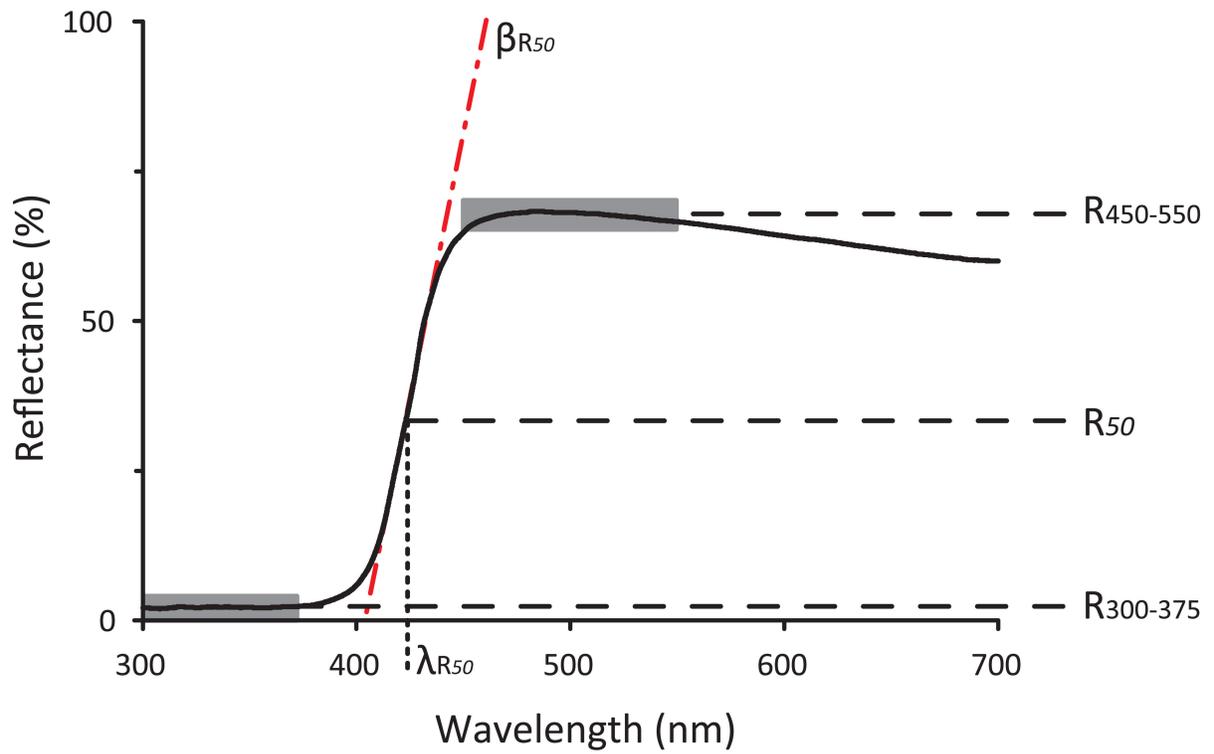
**Note:** See figure A1 for definitions of R<sub>300-375</sub>, R<sub>450-550</sub>,  $\lambda_{R_{50}}$ , and  $\beta_{R_{50}}$ . See “Material and Methods” for description of the ANOVA models used.

<sup>a</sup> Mean difference = mated males – unmated males, estimated when controlling for effects of trial, female, and measurement error. Statistical significance indicates that the mean in question is different from 0 in the reported direction.

<sup>b</sup> *F* values for the main effect of trial. Statistical significance indicates that the phenotypes of males differed between trial cohorts.

\* *P* < .05.

\*\* *P* < .01.



**Figure A1:** Illustration of the parameters extracted from raw spectra of male wing coloration.  $R_{300-375}$  and  $R_{450-550}$  are average reflectance values from 300–375 and 450–550 nm, respectively.  $R_{50}$  is the midpoint reflectance value between  $R_{300-375}$  and  $R_{450-550}$ .  $\lambda_{R_{50}}$  is the wavelength corresponding to  $R_{50}$ .  $\beta_{R_{50}}$  is the slope of a line tangent to  $R_{50}$ . Results from analyses of these parameters are reported in table A1.

**Appendix B from N. I. Morehouse and R. L. Rutowski, “In the Eyes of the Beholders: Female Choice and Avian Predation Risk Associated with an Exaggerated Male Butterfly Color”**  
(Am. Nat., vol. 176, no. 6, p. 768)

**Detailed Visual Modeling Methods and Extended Results**

**General Modeling Methods**

We modeled the female *Pieris rapae* visual system and two well-recognized avian visual systems (Endler and Mielke 2005) using methods extended from the receptor noise-limited models developed by Vorobyev and Osorio (1998). Photoreceptor outputs ( $q_i$ ) were generated according to a log-linear model of photoreceptor quantum catch with a von Kries transformation using the following formulas:

$$q_i = \ln \left( \frac{Q_i}{Q_i^B} \right),$$

where  $i = 1, 2, \dots, n$ ;  $q_i$  is the quantum catch of photoreceptor  $i$ ,

$$Q_i = \int_{\lambda} R(\lambda) I(\lambda) A_i(\lambda) d\lambda,$$
$$Q_i^B = \int_{\lambda} R^B(\lambda) I(\lambda) A_i(\lambda) d\lambda,$$

where  $\lambda$  is wavelength (nm),  $R(\lambda)$  is the reflectance of a color stimulus at a given wavelength,  $R^B(\lambda)$  is the reflectance of the background,  $I(\lambda)$  is the photon flux ( $\mu\text{mol m}^{-2} \text{s}^{-1} \text{nm}^{-1}$ ), and  $A_i(\lambda)$  is the absorbance of photoreceptor  $i$ . We integrated these equations for the wavelength range 300–700 nm. Descriptions of the measurement protocols for  $R(\lambda)$  and  $R^B(\lambda)$  can be found in “Material and Methods”;  $R^B(\lambda)$  was the average reflectance from samples of background foliage in the mate choice arena;  $I(\lambda)$  was measured in the mate choice arena at 1000 hours midway through the mate choice trials using a spectrophotometer (USB2000, Ocean Optics, Dunedin, FL) outfitted with a UV-Vis fiber optic cable connected to a cosine corrector and calibrated using a standard light source (LS1-CAL, Ocean Optics). Ambient light in the enclosure was characterized by a mix of sun and shade from overhanging nearby trees, similar to the habitat where male and female *P. rapae* were collected. Data for  $A_i(\lambda)$  were from intracellular recordings in *P. rapae* (fig. 3, kindly provided by K. Arikawa) and from the online data set for avian visual sensitivities provided by Endler and Mielke (2005).

Visual systems in both vertebrates and invertebrates interpret the reflectance of surfaces using luminance and color contrast independently (Kelber et al. 2003; Endler and Mielke 2005). We calculated luminance contrast against the background as follows:

$$L = \sum_{i=1}^N \alpha_i q_i,$$

where

$$\alpha_i = \frac{\eta_i}{\eta}$$

The parameter  $\alpha_i$  weights the contribution of photoreceptor  $i$  to luminance contrast on the basis of its abundance in the eye ( $\eta_i$ ) relative to the total number of photoreceptors ( $\eta$ ). Estimates of  $\eta_i$  were derived from histological data for *P. rapae* (Qiu et al. 2002) and from literature convention for avian vision (Endler and Mielke 2005).

Color contrast (contrast with background;  $\Delta S$ ) and color discriminability (contrast between two focal stimuli;  $\Delta S^t$ ) were calculated using the receptor noise-limited color opponency model of Vorobyev and Osorio (1998), extended for hexachromats,

$$\begin{aligned} \Delta S \text{ and } \Delta S^t = & \{[(e_3e_4e_5e_6)^2(\Delta q_1 - \Delta q_2)^2 + (e_2e_4e_5e_6)^2(\Delta q_1 - \Delta q_3)^2 + (e_2e_3e_5e_6)^2(\Delta q_1 - \Delta q_4)^2 \\ & + (e_2e_3e_4e_6)^2(\Delta q_1 - \Delta q_5)^2 + (e_2e_3e_4e_5)^2(\Delta q_1 - \Delta q_6)^2 + (e_1e_4e_5e_6)^2(\Delta q_2 - \Delta q_3)^2 \\ & + (e_1e_3e_5e_6)^2(\Delta q_2 - \Delta q_4)^2 + (e_1e_3e_4e_6)^2(\Delta q_2 - \Delta q_5)^2 + (e_1e_3e_4e_5)^2(\Delta q_2 - \Delta q_6)^2 \\ & + (e_1e_2e_5e_6)^2(\Delta q_3 - \Delta q_4)^2 + (e_1e_2e_4e_6)^2(\Delta q_3 - \Delta q_5)^2 + (e_1e_2e_4e_5)^2(\Delta q_3 - \Delta q_6)^2 \\ & + (e_1e_2e_3e_6)^2(\Delta q_4 - \Delta q_5)^2 + (e_1e_2e_3e_5)^2(\Delta q_4 - \Delta q_6)^2 + (e_1e_2e_3e_4)^2(\Delta q_5 - \Delta q_6)^2] \\ & / [(e_1e_2e_3e_4e_5)^2 + (e_1e_2e_3e_4e_6)^2 + (e_1e_2e_3e_5e_6)^2 + (e_1e_2e_4e_5e_6)^2 + (e_1e_3e_4e_5e_6)^2 + (e_2e_3e_4e_5e_6)^2]^{(1/2)}, \end{aligned}$$

pentachromats,

$$\begin{aligned} \Delta S \text{ and } \Delta S^t = & \{[(e_3e_4e_5)^2(\Delta q_1 - \Delta q_2)^2 + (e_2e_4e_5)^2(\Delta q_1 - \Delta q_3)^2 + (e_2e_3e_5)^2(\Delta q_1 - \Delta q_4)^2 \\ & + (e_2e_3e_4)^2(\Delta q_1 - \Delta q_5)^2 + (e_1e_4e_5)^2(\Delta q_2 - \Delta q_3)^2 + (e_1e_3e_5)^2(\Delta q_2 - \Delta q_4)^2 \\ & + (e_1e_3e_4)^2(\Delta q_2 - \Delta q_5)^2 + (e_1e_2e_5)^2(\Delta q_3 - \Delta q_4)^2 + (e_1e_2e_4)^2(\Delta q_3 - \Delta q_5)^2 + (e_1e_2e_3)^2(\Delta q_4 - \Delta q_5)^2] \\ & / [(e_1e_2e_3e_4)^2 + (e_1e_2e_3e_5)^2 + (e_1e_2e_4e_5)^2 + (e_1e_3e_4e_5)^2 + (e_2e_3e_4e_5)^2]^{(1/2)}, \end{aligned}$$

and tetrachromats,

$$\begin{aligned} \Delta S \text{ and } \Delta S^t = & \{[(e_3e_4)^2(\Delta q_1 - \Delta q_2)^2 + (e_2e_4)^2(\Delta q_1 - \Delta q_3)^2 + (e_2e_3)^2(\Delta q_1 - \Delta q_4)^2 \\ & + (e_1e_4)^2(\Delta q_2 - \Delta q_3)^2 + (e_1e_3)^2(\Delta q_2 - \Delta q_4)^2 + (e_1e_2)^2(\Delta q_3 - \Delta q_4)^2] \\ & / [(e_1e_2e_3)^2 + (e_1e_2e_4)^2 + (e_1e_3e_4)^2 + (e_2e_3e_4)^2]^{(1/2)}, \end{aligned}$$

where

$$e_i = \omega \sqrt{\frac{\eta_j}{\eta_i}}$$

The parameter  $e_i$  is an estimate of noise originating from photoreceptor  $i$ , scaled to the G photoreceptor for *P. rapae* ( $j = G$ ) and to the UV or V photoreceptor in the avian visual models ( $j = UV$  or  $V$ ). The former scaling attributes the lowest noise level to the most common photoreceptor type in the *P. rapae* eye (G), whereas the latter is based on literature precedent (Endler and Mielke 2005). The parameter  $\omega$  is the standard deviation of the noise in a given photoreceptor, roughly equivalent to the Weber fraction (Vorobyev and Osorio 1998). We used a  $\omega$  value of 0.01 for *P. rapae* on the basis of the high color acuity reported from a similar butterfly visual system (Koshitaka et al. 2008) and 0.05 for our avian visual system modeling (Endler and Mielke 2005).

For color contrast ( $\Delta S$ ),  $\Delta q_i = q_i(\text{focal stimuli}) - q_i(\text{background})$ . However, because of the von Kries transformation and log-linear model of photoreceptor responses,  $q_i(\text{background}) = 0$ , and the equation therefore simplifies to  $\Delta q_i = q_i(\text{focal stimuli})$ . For color discriminability ( $\Delta S^t$ ),  $\Delta q_i = q_i(\text{focal stimuli 1}) - q_i(\text{focal stimuli 2})$ . In our study, focal stimuli for this latter calculation correspond to the average  $q_i$  value for a given animal type (e.g., female, mated male, unmated male).

**Table B1**  
ANOVA results from comparison of mated and unmated male coloration in studies 1 and 2 using alternative *Pieris rapae* visual models

	$\Delta S$		$L$	
	Estimated mean difference $\pm$ SE <sup>a</sup>	Trial <sup>b</sup>	Estimated mean difference $\pm$ SE <sup>a</sup>	Trial <sup>b</sup>
Study 1:				
5:				
No UV	-.23 $\pm$ .08**	8.56**	.08 $\pm$ .01**	9.43**
No V	1.88 $\pm$ .23**	14.60**	.07 $\pm$ .01**	7.64**
No B	1.90 $\pm$ .24**	14.65**	.07 $\pm$ .01**	7.42**
No G	1.84 $\pm$ .21**	14.97**	.07 $\pm$ .01**	6.43**
No PR	1.76 $\pm$ .20**	14.34**	.07 $\pm$ .01**	6.67**
No DR	1.80 $\pm$ .21**	14.33**	.07 $\pm$ .01**	7.18**
4:				
No type II	2.18 $\pm$ .26**	14.44**	.07 $\pm$ .01**	7.01**
Study 2:				
5:				
No UV	-.94 $\pm$ .37*	1.86	.02 $\pm$ .01	3.00*
No V	7.51 $\pm$ 2.15**	1.98	-.01 $\pm$ .01	7.65**
No B	7.41 $\pm$ 2.17**	2.03	-.01 $\pm$ .01	7.88**
No G	8.37 $\pm$ 2.39**	1.83	-.02 $\pm$ .01**	6.46**
No PR	6.95 $\pm$ 1.96**	2.00	-.02 $\pm$ .01*	7.24**
No DR	7.10 $\pm$ 2.01**	2.03	-.01 $\pm$ .01	7.70**
4:				
No type II	8.78 $\pm$ 2.52**	1.96	-.02 $\pm$ .01*	7.61**

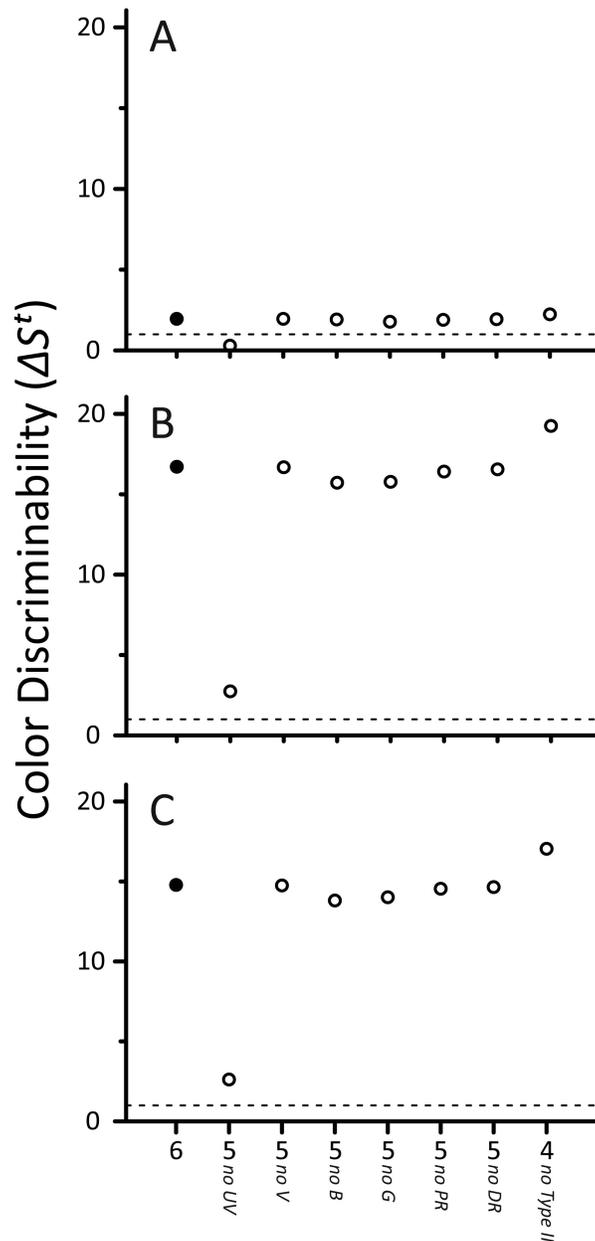
**Note:** See appendix B for definitions of  $\Delta S$  and  $L$  and “Material and Methods” for description of the ANOVA models used. In the left column, numbers represent the number of photoreceptor types in the modeled visual system, followed by the omitted photoreceptor type. For the tetrachromat (4: no type II), type II ommatidia were omitted from the color vision system, thus removing both V and DR photoreceptor types.

<sup>a</sup> Mean difference = mated males – unmated males, estimated when controlling for effects of trial, female, and measurement error. Statistical significance indicates that the mean in question is different from 0 in the reported direction.

<sup>b</sup>  $F$  values for the main effect of trial. Statistical significance indicates that the phenotypes of males differed between trial cohorts.

\*  $P < .05$ .

\*\*  $P < .01$ .



**Figure B1:** Color discriminability ( $\Delta S^t$ ) of mated males versus unmated males (A), mated males versus females (B), and unmated males versus females (C) from study 1, when viewed through alternative visual system models of female *Pieris rapae*. Alternative visual models are listed along the abscissa; numbers correspond to number of photoreceptor types in a given visual system, followed by the photoreceptor type omitted. Hexachromatic vision results (solid circles) are presented again for reference. For the tetrachromatic visual system (4; no type II), type II ommatidia were omitted from the color vision system, removing both the V and the DR photoreceptor types. The threshold below which two stimuli are indistinguishable is represented by the dotted horizontal line ( $y = 1$ ).