The Role of Coloration in Mate Choice and Sexual Interactions in Butterflies

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I. Introduction

Animal color patterns constitute a large and intriguing component of phenotypic diversity. Perhaps most interesting from an evolutionary perspective is the diversity of conspicuous color badges or colored structures thought to act as sexual signals. Studies of these traits, including the famously iridescent peacock’s train (Loyau et al., 2007), and the orange, black, and iridescent spots of guppies (Endler, 1983; Endler and Houde, 1995), have informed our understanding of intraspecific signaling, speciation, and the evolution of exaggerated secondary sexual signals. By using model organisms in target groups such as birds, fishes, and lizards, such work has demonstrated how information regarding phenotypic and/or genetic quality may be encoded in various types of color ornaments (Blount et al., 2003; Grether et al., 2005; Hill and Montgomerie, 1994; Keyser and Hill, 1999). This work offers an explanation for the adaptive value of color signals in mate assessment, and has delivered significant insights into the actions and outcomes of sexual selection.

Curiously, until recent times, attempts to address these issues have rarely used butterflies as subjects. This is surprising on several fronts. First, as noted, butterflies display highly diverse color patterns borne from various combinations of pigments and reflective nanoscale surface structures (e.g., Prum et al., 2006). Few groups rival the sheer diversity of visual signals seen across the day-flying Lepidoptera, and butterfly wings exhibit among the brightest, most chromatic and optically complex colors seen in nature (Prum et al., 2006; Vukusic and Sambles, 2003). Second, butterflies are
well suited to behavioral, physiological, morphological, and genetic investigations. Features of their life history offer novel opportunities for testing ideas about the connections between the mechanisms of color production, the causes of variation in coloration, and the potential information value of color signals. Many species are easily reared in captivity, with relatively rapid generation times (~3–5 weeks), which offers excellent opportunities for manipulative and genetic studies of color signal development. Quantitative genetics, a necessary tool for the in vivo examination of the map between genotype and phenotype, is therefore eminently possible with this system (e.g., Beldade et al., 2002; Kemp and Rutowski, 2007). Their compartmentalized (holometabolous) life cycle also permits controlled examinations of how and when resources are acquired and allocated to coloration, and how individual differences in these processes may be signaled through wing coloration (Kemp, 2007; Kemp and Rutowski, 2007; Talloen et al., 2004). Behaviorally, butterflies are suited to experimentation and observation both in the field and the lab, and because their colors are housed in dead, metabolically divorced cuticular wing material, they are amenable to precise manipulations using straightforward techniques (e.g., Kemp, 2007; Lederhouse and Scriber, 1996; Silberglied and Taylor, 1978; Stride, 1957, 1958).

In this chapter, we review recent efforts to understand visual signaling that have taken greater advantage of the variety of empirical opportunities afforded by the Lepidoptera. The field has been advanced considerably by work in several groups (e.g., Costanzo and Monteiro, 2007; Fordyce et al., 2002; Jiggins et al., 2001; Knuttel and Fiedler 2001; Robertson and Monteiro 2005), but we focus here on the functional, behavioral, developmental, and quantitative genetic studies in two exemplar genera of the subfamily Coliadinae: *Colias* and *Eurema*. This butterfly subfamily has a rich legacy of research into color function and evolution (e.g., Gerould, 1923; Ghiradella, 1974; Ghiradella et al., 1972; Rutowski, 1977; Silberglied and Taylor, 1973; Silberglied and Taylor, 1978; Watt, 1964) and exemplifies the potential that butterflies present for understanding the evolution of color-based sexual signaling in nature.

Further, because the field of color signal evolution is large and fast moving, we wish to limit our focus to female mating biases and the evolution of exaggerated male-limited signals. This is the same focus as used by Darwin (1874) in his presentation of sexual selection theory (see Section III), and we wish to update the debate regarding Darwin’s assertion that female choice drives color exaggeration in butterflies (a debate best summarized over two decades ago by Silberglied, 1984). Separate from this, there is also the increasingly popular possibility that mate choice imposed by males may also potentially select for female phenotypic traits (e.g.,
Bateman and Fleming, 2006). However, at least in insects, the current view is that male mate choice is more likely to select for female traits subject to existing vectors of natural selection, such as body size (Bonduriansky, 2001), rather than display traits such as ornamental wing coloration. Recent modeling efforts indicate further difficulties with the evolution of male mate choice under scenarios when mates are encountered sequentially (Barry and Kokko 2010). Because butterflies are well suited to studying male mate choice, they offer opportunities to test these viewpoints. Kemp and Macedonia (2007), for example, investigated the consequences of a clear, color-based male mating bias to the reproductive biology of differentially attractive conspecific females but found no signature of male choice. Although we treat the issue no further here, excellent additional progress in understanding male choice and its potential influence on female coloration is given by the work of Ellers and Boggs (2002, 2003, 2004a,b), Fordyce et al. (2002), Jiggins et al. (2001), Knuttel and Fiedler (2001), and Sweeney et al. (2003).

II. COLORATION AS A SIGNAL ELEMENT

A. COLORATION, CONDITION, AND HONESTY

The color patterns seen on the external surfaces of animals may have evolved, either wholly or partly, due to their function as visual signals. In some cases, the signals may have defensive function, such as the “warning” colors of noxious or dangerous species (e.g., the coral snake; Brodie, 1993), and the false “eyespots” best seen in larval and adult Lepidoptera (Kodandaramaiah et al., 2009). In other cases, the color patterns may advertise species identity, mate identity, or mate quality. Such signals may have also evolved as secondary sexual characters and are often famously exaggerated, such as the stunning iridescent blue and green train of the peacock. These traits may signal mate attractiveness (in the purely “Fisherian” sense; Fisher, 1930), or they may provide clues to the bearer’s physical or physiological condition, his abilities as a forager or caring parent, the quality of his territory, or the quality or potential compatibility of his genome.

Many sexual signals are described as highly “exaggerated.” Exaggeration in this sense is really equivalent to the extent to which their expression causes a departure from the viability-selected optimum phenotype (Bonduriansky, 2007a; Bonduriansky and Rowe, 2005), that is, the phenotype most conducive to survival and offspring production. By definition, therefore, such exaggerated signals exact viability costs upon their bearers.
Indeed, the theory of honest signaling (Zahavi, 1975) proposes that the very reason such signals are evolutionary stable is because they are costly to express and, therefore, contain “honest” information regarding the ability of their bearer to withstand or pay such costs. Viability costs could arise because signaling is nutritionally or physiologically demanding or because it increases the risks of predation (Lyytinen et al., 2004) or social harassment (Martin and Forsman, 1999). Alternatively, as Maynard Smith and Harper (2003) discuss, the signals may be just plain difficult to achieve or construct, such that only strong and healthy individuals can achieve the greatest levels of expression (see also the arguments of Fitzpatrick, 1998). In either case, not all signalers should be able to display with the same intensity, which means that signal expression can be reliably used as a proxy for mate quality. This insight from the theory of honest signaling has provided a consistent framework for understanding and fruitfully studying the types of signal traits thought to evolve as components of mate signaling systems.

A key prediction from honest signaling theory is that the expression of directionally selected sexual traits, such as sexual ornaments, should covary tightly with phenotypic condition. This is the phenomenon known as “condition-dependence” (Andersson, 1994; Johnstone, 1995; Rowe and Houle, 1996). Strictly defined, this is a form of developmental plasticity whereby investment into discrete aspects of the phenotype varies according to the total pool of available resources (i.e., condition; Rowe and Houle, 1996). Sexually selected traits are expected to be especially condition-dependent compared to naturally selected traits because their close relationship to fitness means that individuals of increasingly higher condition will incur lower marginal costs per unit elaboration of these traits (Grafen, 1990). Perhaps due to the apparent simplicity of this prediction, considerable effort has gone into testing it across a range of animals and traits, including many color-based sexual signals (e.g., Johnsen et al., 2003; Lim and Li, 2007; Masello et al., 2008; Siitari et al., 2007). However, as pointed out by Tomkins et al. (2004) and Cotton et al. (2004a,b), properly defining condition and testing for condition-dependence demand great care. Many studies have fallen short of convincingly demonstrating it for their focal (putatively sexual) traits. In particular, researchers often failed to appreciate that condition is likely to affect most aspects of organismal growth, development, and function (West-Eberhard, 2003), and the key prediction for costly sexually selected traits is for heightened condition-dependent variation, relative to nonsexual metric or somatic traits (Cotton et al., 2004b; indeed, the distinction between sexual and nonsexual traits may not be clear-cut). More recent efforts at examining this prediction have therefore emphasized a comparison among sexual and nonsexual traits in their
relationship to underlying variation in condition (e.g., Bonduriansky, 2007a; Bonduriansky and Rowe, 2005; Cotton et al., 2004a; Kemp, 2008a; Kemp and Rutowski, 2007).

From the perspective of signal information content, the presence of condition-dependence means that the level of signal expression, such as the brightness of a color badge, could be used by a receiver to gain insights into (at least) the bearer’s phenotypic condition. If such phenotypic information is relevant to female reproductive fitness—for example, if she gained access to a higher-quality territory or a more nutritious ejaculate—then selection would favor female choice for high-signaling males. Moreover, because “condition” is effectively shorthand for the total pool of resources available for conversion to fitness and encompasses both present and future reproductive potential, achieving high levels of condition will call upon the ability to acquire, appropriate, and conserve nutrients, energy, and other essential resources; the ability to avoid or repel predators and pathogens; and the ability to withstand myriad other environmental stressors and challenges (Tomkins et al., 2004). This suggests that phenotypic condition should depend upon a large proportion of the functional genome and that females could also use condition-dependent signals as a source of information on underlying genetic quality (Hunt et al., 2004; see below). However, these ideas predict that such a signaling system will remain evolutionarily stable only if low-condition/low genetic quality individuals are unable to “cheat,” which predicts in turn that signal traits should be highly developmentally and physiologically integrated (Badyaev, 2004; Rowe and Houle, 1996).

B. The Signaling of Genetic Quality

Empirically testing the theory of honest signaling, particularly as it pertains to genetic quality, continues to pose challenges for the field of behavioral and evolutionary ecology. Phenotypic condition-dependence has proven relatively simple to demonstrate in many signaling systems, but few researchers have investigated whether there is an underlying genetic basis to condition and/or condition-dependent trait expression (Cotton et al., 2004b). Such knowledge is ultimately necessary to appraise what type of information is revealed by the signal and, hence, to fully understand the evolution of the signaling system. However, getting to the bottom of these issues demands a quantitative genetic approach, ideally coupled with realistic environmental manipulations that impinge upon the ability to acquire condition (see below). Such designs and manipulations require known pedigrees and large sample sizes and are obviously very difficult to achieve in many systems.
One approach to investigating the potential genetic basis to a condition-dependent display is to search for genotype-by-environment interactions (GEIs) in the expression of the signal trait (Hunt et al., 2004; Kokko and Heubel, 2008). Simply, a GEI refers to a developmental outcome in which the phenotype that results from a particular genotype is a function of the environment under which the organism developed (or in which the gene(s) of interest were expressed). Alternatively, this phenomenon can be viewed as a specific type of “phenotypic plasticity,” wherein the relationship between phenotype and environment in which it develops is expressed as the “norm of reaction.” A significant GEI can arise for several reasons, including among-environment differences in the rank order of genotypes (equating to a “crossing over” of reaction norms) or differences in the spread of genotypes among environments (equating to differences in genetic variance among environments).

In studies of sexual signaling, the GEI of special interest is that relating to genetic variance for the acquisition of phenotypic “condition,” which is, in turn, signaled by the ornament. Thus, individuals of high genetic quality, that is, in possession of “good genes,” are able to attain high condition regardless of their developmental environment, whereas low genetic quality individuals can only do so in favorable environments. This situation would generate significant GEIs relating to both overall phenotypic condition as well as ornamental trait expression of a nature in which genetic variance for both condition and trait expression increases under stress. As noted for the purely phenotypic study of condition-dependence (Cotton et al., 2004a,b), the crucial evidence is not just significant GEIs but those of a larger magnitude than seen in nonsexual traits and homologous (nonsexually functional) traits in the opposite sex.

Empirically, GEIs for sexual signal expression can be revealed by rearing or housing full- or half-sibling families under a range of differently challenging environments. The best known empirical example of this is given by the work of David et al. (2000) on stalk-eyed flies. Females of this species prefer to mate with males that have their eyes spanned further apart, and male eyes are located on laterally protruding stalks. David et al. (2000) reared full sibling families under three environments of decreasing nutritional quality, and assayed eye span and wing length (a nonsexual trait) in the subsequent adult males and females. Their results are perhaps the clearest demonstration of genetically mediated condition-dependence in the contemporary literature. Whereas males of all families achieved highly exaggerated eye spans in the best environment, and many did so in the mid-quality environment, only several families were able to maintain this high level of trait expression in the most suboptimal environment (and some families suffered markedly). The result is therefore an increasing “fanning”
of family means for ornament expression (i.e., an increase in genetic and phenotypic variance) across environments of decreasing nutritional quality. Importantly, this result held even when male eye span was adjusted for body size, which rules out a simple mechanistic explanation based upon scaling. The same pattern was also not evident for female eye span, or for either male or female forewing length. On average, therefore, male eye span exhibits the greatest degree of phenotypic condition-dependence, but this effect is modulated by genes. Given environmental variation, female choice for exaggerated male eye span would deliver mates in high phenotypic condition and (on average; Kokko and Heubel, 2008) in possession of higher-quality genomes. Whether the exaggerated coloration seen in many male butterflies could function in this way is unknown but well worthy of empirical investigation (see also the commentary of Kemp and Rutowski, 2007, as below). It also has to be considered that the presence of environmental variation, or GEIs, has the potential to in fact erode the genetic information content of the signal, and to constrain the benefits to choosiness in females (see, e.g., Greenfield and Rodriguez 2004). Full treatment of the different views on GEIs in relation to sexual selection is given by Kokko and Heubel (2008).

C. Potential Sexual Signaling Function of Butterfly Color

Much recent effort in testing the signal content of color-based sexual ornaments has focused on vertebrate models, such as birds and fishes. Many of these species have well-characterized mating systems, social systems, and ecological niches, and much is often known about their sensory physiology, and that of major competitors and predators (e.g., Endler, 1991; Endler and Mielke, 2005). This information is, at present, lacking for most of the more than 20,000 described species of butterflies. However, detailed empirical work in several species has provided a solid platform for investigating the full use of wing coloration during social and sexual interactions in this group (e.g., Brakefield, 1996; Kemp, 2007; Kunte, 2009; Oliver et al., 2009; Vane-Wright and Boppre, 1993).

The wing coloration of day-flying Lepidoptera functions primarily for thermoregulation, protection (including crypsis and aposematism), and intraspecific communication (Lederhouse and Scriber, 1996; Vane-Wright and Boppre, 1993). Dorsal and ventral surfaces frequently differ, often markedly so, and sexual dimorphism, genetic polymorphism, and seasonal polyphenism are commonplace (Silberglied, 1984). Above all, the many cases of sexual dimorphism in this group present a strong signature of sexual selection in that the males exhibit the more visually striking color patterns (Rutowski, 1997; Silberglied, 1984). Sometimes, as in the case of
many Nymphalids and Pierids, the dorsal surfaces of male wings are adorned with bright and/or iridescent color pattern elements that are completely absent in the opposite sex. These colors are sometimes obviously presented to females via highly ritualized aerial courtship routines, which support the idea that they evolved as mating signals. There is good evidence across many species that such color patterns are used by females to recognize potential mates. As we outline below, several studies also demonstrate female discrimination based on the expression of the male color signals, which suggests that the function of these traits transcends the mere announcement of species and/or sexual identity (Kemp, 2007, 2008b; Papke et al., 2007).

In trying to understand the evolution of color-based signaling, it is important to appraise what kind of information may be encoded within the standing levels of signal variation. In butterflies, males donate not only sperm to females but also a package of nutrients comprising a structure known as a spermatophore and accessory gland secretions. Radiolabeling experiments have revealed that these male-derived nutrients are incorporated into egg production and that females use them for somatic maintenance (Boggs and Gilbert, 1979; Boggs and Watt, 1981). Females of some species are also thought to forage for matings in order to supplement their lifetime resource budget (Kaitala and Wiklund, 1994; Karlsson, 1998). Moreover, at least in Colias eurytheme, we know that females can receive differential fitness benefits from mating with different members of the population (Rutowski et al., 1987). This raises the possibility that evolution may have favored females that discriminate among potential mates to receive direct benefits. However, it is not known whether such choice occurs in butterflies, let alone whether it is based upon wing coloration. The only known attempt to investigate this empirically (that we are aware of) produced negative results (Kemp et al., 2008). Aside from direct benefits, there is also the possibility that female butterflies could obtain indirect benefits from mating with more strikingly colored males. This encompasses the possibility that such females could generate more attractive and/or viable offspring due to receiving “good” or “complementary” genes from their partner. These possibilities are treated at length later in this chapter.

III. BUTTERFLY COLOR: IS IT A SEXUAL SIGNAL?

As one of nature’s most colorful animal groups, butterflies have been called upon in the study of visual-based mating preferences, and such preferences have been successfully demonstrated across a range of species (e.g., Ellers and Boggs, 2003; Fordyce et al., 2002; Robertson and Monteiro,
Data have steadily accumulated in support of the idea that wing coloration is used (at least) to recognize potential mates, but until recently, few experimenters have set out to isolate behavioral responses to intraspecific signal variation. Most manipulations have therefore amounted to the entire removal or substitution of specific color pattern elements, rather than graded manipulations of color element size, brightness, hue, or chromaticity. As a consequence, it has remained largely unclear whether the expression of butterfly wing colors may serve to indicate phenotypic and/or genetic mate quality, as appears to be the case in other well-studied animal groups. A second interesting (but slightly unfortunate) point is that manipulations in butterflies have tended to target relatively inconspicuous (e.g., Fordyce et al., 2002; Wiernasz, 1995) or sexually monomorphic (e.g., Robertson and Monteiro, 2005) color pattern elements. Theory predicts that mate-quality indicators should evolve to be highly exaggerated, that is, to depart significantly from the optimum phenotype for viability and that production and/or maintenance costs should lead to their sex limitation (thereby leading to the evolution of sexual dimorphism; Bonduriansky, 2007a,b; Bonduriansky and Chenoweth, 2009). Third, many more studies have addressed male mate choice rather than female mate choice in butterflies, mainly because the former is far easier to measure and manipulate (Silberglied, 1984). But, as Darwin (1874) observed, where sex dimorphism occurs in butterflies, it is nearly always the males which exhibit the more obviously striking color pattern. Theory also indicates that male choice is unlikely to strongly select for arbitrary or maladaptive advertisement traits such as wing coloration (Bonduriansky, 2001). This suggests that, at least in terms of understanding the evolution of exaggerated wing patterns in butterflies, inadequate attention has been given to the study of female mate choice. In turn, these issues may be collectively responsible for butterflies, as a group, rarely featuring in the theoretically grounded literature on how and why exaggerated sexual traits evolve in nature.

More recent efforts have, however, sought to examine the signal relevance of visually exaggerated, male-limited color patches. In this section, we outline this work by focusing on empirical progress in two exemplar species: *C. eurytheme* and *Eurema hecabe*.

**A. CASE #1: COLIAS EURYTHEME**

The orange sulfur butterfly, *Colias eurytheme*, is a common and widespread North American species that has been used extensively in studies of physiology, life history, genetics, and behavior. As suggested by its common name, the wings of both sexes of *C. eurytheme* are predominately orange
The dorsal wing surface of both sexes has a band of black around the distal margin, and in males, the orange color is overlain by a bright and iridescent ultraviolet (UV) reflectance (Fig. 1), which is thought to be the primary sexual signal. The history of the study of this species illustrates nicely how interest in the role of color in mate choice in butterflies has developed, how the approaches to these questions have evolved, and the strengths and weaknesses of butterflies as a system for addressing these questions.

1. Hybridization and Mate Choice in Colias

Interest in mate choice in *C. eurytheme* quickly followed the observation that this species naturally hybridizes with its close relative *C. philodice* where the two species occur sympatrically. Hybridization was first described in the 1930s, and was then followed by work on the occurrence and consequence of interspecific matings (e.g., Hovanitz, 1949). These investigations were especially motivated by the fact that hybrids produced viable offspring but yet introgression between the two species was never complete. In areas of sympathy, hybrids occurred but always at a lower
frequency than the parental phenotypes, which was proposed to be a result of female preference for conspecific mates. However, Hovanitz (1949) reported on the occurrence of interspecific matings in the field, and reported little evidence of female preference for conspecific mates.

Field investigation of these phenomena was facilitated by the occurrence of these species in fields of commercially grown alfalfa (*Medicago sativa*), a perfectly acceptable larval host for both. This connection with alfalfa cultivation not only expanded the range of sympatry but also produced dense populations of both species due to abundant larval foodplant. In the 1960s and early 1970s, Taylor (1972) took advantage of the opportunity presented by sympatric populations of exceptional density in cultivated alfalfa fields in south central and southeastern Arizona. His work suggested that females do in fact prefer intraspecific mates and that interspecific matings only resulted from males mating with freshly emerged females before their wings had hardened and they could effectively reject heterospecific suitors.

2. **UV Iridescence and Species Recognition in Colias**

   Additional interest in this system was stimulated by Silberglied and Taylor’s (1973) observation that males of *C. eurytheme* but not *C. philodice* have brilliant iridescent UV coloration on their dorsal wing surfaces (Fig. 1). This interspecific difference in male coloration was proposed to be an important cue in species recognition by females. This idea was tested by Silberglied and Taylor (1978) in a series of impressive experiments that explored the role of color in female mate selection in these species. Their experiments also took advantage of the hugely dense populations that were available in Arizona. At the right time in the summer, they were able to collect thousands of pupae to obtain males, but equally as important, virgin females. Females in these species mate more than once but after the initial mating are unreceptive for days before mating again (Rutowski and Gilchrist, 1986). Efficient studies of mate choice depend upon a good supply of virgin females who are certain to be receptive.

   Silberglied and Taylor (1978) conducted two major experiments in which manipulated males were presented to virgin females in a large flight cage. In the first, they used color markers to manipulate male color across a wide range of the spectrum visible to humans. The markers also obliterated UV reflectance. The results indicated that coloration in the human-visible range of the spectrum was not important in species identification. The second experiment presented females with males whose dorsal wing surfaces were covered with transplanted dorsal wing surfaces of other conspecific, heterospecific, and hybrid males. In these manipulations, they only transplanted the nonblack regions of wing, which presented various combinations of diffuse yellow, orange, and iridescent UV. The results indicated that
male-specific color character might have evolved in the context of female mate preference. However, there was a potential confound in their wing transplant study. Silberglied and Taylor suspected that a male chemical signal used by females in mate choice (Grula et al., 1978) might be arising from special scales found in the black margins of the dorsal wing surface. This is why their transplants involved only the nonblack areas of the dorsal wing surface. As it turned out, there is a chemical signal that stimulates virgin females to assume a receptive posture, but it arises from an area on the ventral forewing and spreads out over all wing surfaces, especially the dorsal hindwing and forewing (Rutowski, 1980). These areas were included in Silberglied and Taylor’s (1978) wing transplants, an observation which weakens their conclusions regarding the role of color in mate selection by females.

3. Intraspecific Mate Choice in Colias

Silberglied and Taylor’s work focused on interspecific and not intrasexual variation in coloration, and utilized dramatic manipulations of male color. In contrast, sexual selection as a hypothesis for male coloration focuses on female preferences acting on existing phenotypic variation among conspecific males. This variation, and its consequences for male mating success, has been the key issue in several studies since Silberglied and Taylor’s pioneering work. Rutowski (1985) tested for female mate preferences in C. eurytheme by presenting virgin females to free-flying conspecific males in the field and noting the form and outcome of the ensuing courtships. Like many butterflies, females of this species can reject males via two characteristic methods: first, by assuming postures that prevent males from coupling, and second, by ascending high into the air which is apparently used as a signal of unreceptivity (Rutowski, 1978). Although color was not assessed quantitatively in this study, the presented virgin females were found to mate preferentially with young males in the middle of the size range. UV reflectance declines with age due to wing wear and scale loss (Kemp, 2006a), which was suggested as the proximate cue used in mate assessment by females (Rutowski, 1985).

A similar experimental design was used by Papke et al. (2007) to address the relative roles of chemical and visual signals in the mating success of C. eurytheme. The results showed that out of a number of color and pheromonal parameters measured, bright UV coloration was the best predictor of whether or not a male would be accepted by a female in mating.

In sum, there is substantial evidence that females in C. eurytheme attend to male coloration in the UV and prefer males with bright signals. However, there are two caveats to this conclusion in this species. First, there have still
been no studies in which manipulations have produced male color that varies in the way that it does in nature, and examined the effect of these manipulations on male attractiveness. Second, it is not clear whether variation in male coloration leads to variation in male mating success in the field. In one study, a comparison of male coloration between naturally occurring copulating males versus their unattached counterparts found no differences between the two groups (Kemp, 2006b). However, this study was necessarily conducted at the height of the breeding season, and in alfalfa fields when the butterflies were at high population density. This high density of breeding animals means that many of the copulating females may have been freshly emerged adults, and such females are constrained in their ability to exercise precopulatory mate choice (Taylor, 1972). Ideally, work on this species would seek to investigate mating patterns under conditions of lower population density, or among females that have the confirmed ability to reject courting males (such as nonvirgin females).

B. CASE #2: *Eurema hecabe*

Known as the large or common grass yellow, *E. hecabe* is a small, slow-moving coliadine butterfly that is widely distributed throughout the Indo-Pacific. Like *Colias eurytheme*, it is a well-studied species, having been called upon for investigations of seasonal development, reproduction and polyphenism (Jones, 1987a,b, 1992), reproductive strategies (Hiroki and Kato, 1996; Hiroki and Obara, 1997; Hiroki et al., 1998), and host-seeking behavior (Hirota and Kato, 2001, 2004), among other things. Recently *E. hecabe* has also emerged as a model system for studying host-endosymbiont dynamics (Hiroki, 2002; Narita et al., 2007a,b,c), based principally upon the discovery that it harbors (and is reproductively manipulated by) the bacterial endosymbiont *Wolbachia* (Werren, 2008). As we discuss below, part of the reason for *E. hecabe*’s popularity as a study species across many different empirical contexts is because of its small size, ease of use in behavioral trials, and ease of large-scale culturing in the laboratory.

The wing coloration of this species is similar to the general pattern of *C. eurytheme* (as described above), in that the wings of both sexes are predominantly yellow/orange (yellow in *Eurema*, orange in *Colias*) and are framed by a margin of black on their dorsal surface. Males also have bright UV iridescence across most of the yellow areas of their dorsal wings. *E. hecabe* differs from *C. eurytheme* (and almost all other butterflies) in that females possess these markings as well, albeit only on the proximal half of their dorsal forewings, and which are only about half as bright as the homologous male UV (Kemp, 2008a). Females also have slightly less chromatic yellow, slightly brighter black margins, and greater suffusion
of black into the yellow regions, which collectively results in them possessing a less striking color pattern than that of their male counterparts. Again, this is also true for *C. eurytheme*, and for many other Coliadine species, which is consistent with the notion that wing coloration (i.e., intrawing signal contrast) is generally under stronger selection in males.

The similarity of the *E. hecabe* color scheme to that of *C. eurytheme* has allowed the former species to be used (at least) to validate earlier findings in the latter (i.e., Papke et al., 2007; Rutowski, 1985; Silberglied and Taylor, 1978). However, due to its smaller size, *E. hecabe* has proven considerably more versatile as a behavioral subject. This is best evidenced by the work of Kemp (2008b), in which he performed a series of experiments designed to evaluate whether *E. hecabe* females prefer the UV-brightest males. As we describe below, Kemp’s (2008b) program was conceived to exploit the full spectrum of empirical opportunities offered by this system, and coupled manipulations of ambient light and butterfly wing color, in small and large enclosures, with nonmanipulative observational studies of mating patterns in the wild. Such a coupling is desirable because it combines the benefits of “in vitro” observation of natural behavioral patterns with the power of experimental manipulation to isolate causality among candidate variables. Regrettably, however, this combination of empirical approaches is not realistically achievable in many systems.

Kemp’s (2008b) first experiment involved a manipulation of ambient light. Because the trait of primary interest consists of light reflected in the UV range, its visual expression will be directly determined by the intensity of UV in the illumination spectrum. As anyone who has been afflicted with sunburn can attest, unfiltered sunlight contains adequate UV light (although it is interesting to note that atmospheric scattering causes a reduction in the relative intensity of shorter wavelengths reaching the earth’s surface—which is why sunlight appears yellowish). Kemp’s (2008b) experiment was established by releasing virgin male and female *E. hecabe* into two replicate 1-m$^3$ cages, one of which was covered by UV-absorbing plastic film (the “UV-minus” arena) and the other which was covered by a non-colored “neutral density” film (the “control” arena). He then monitored the rate at which matings occurred in each cage, substituting each observed copulating pair with a fresh virgin male and female. As predicted, if UV patterning plays a crucial role during mating interactions, copulations accumulated six times faster on average in the control arena (Fig. 2A). Ultimately, however, this experiment only tells us that having UV illumination is important, which could apply for many reasons, not the least of which are changes in male and/or female behavior under a highly visually altered environment.
In order to isolate the relevance of male UV wing markings, Kemp (2008b) then established two further experiments, one in the 1-m³ cages and one in a large (6 × 5 × 4 m) outdoor insectary. In both cases, the enclosures were covered with open-weave mesh, such that the ambient light spectrum was virtually unchanged from that of natural sunlight. The manipulative element consisted of a UV-absorbing solution painted onto male wings (which consisted of the plant flavonoid rutin dissolved in ethanol, as used earlier by Robertson and Monteiro, 2005). In the UV-minus treatment group, this solution was applied to the male dorsal surfaces, thus effectively halving the brightness of reflected UV, while members of the control group were painted on their otherwise UV-absorbing ventral surfaces. Both groups were then released in equal numbers into the enclosures,

![Figure 2](image_url)

**Fig. 2.** Results of experiments conducted into the behavioral significance of UV coloration in *Eurema hecabe*. (A) The frequency of matings between virgin individuals housed under full spectrum (dark column) versus UV-minus (light column) illumination. (B, C) The frequency of matings achieved by visually unaltered ("control"; dark columns) versus UV-dulled ("UV−"; light columns) males in mating experiments conducted in (B) 1-m³ cages and (C) an outdoor insectary.
together with an equivalent number of virgin females, and copulations were monitored. The results, indicated by Fig. 2B–C, were clear and consistent: copulations were significantly more likely to involve males from the control group, in both experiments, and in almost precisely the same ratio. Coupled with this, there was also evidence in both experiments that control males achieved copulations with larger females (Fig. 3A–B). While not specifically predicted \textit{a priori}, this result is nevertheless also consistent with a situation where UV-brighter males are more attractive, in that they were able to mate with more reproductively valuable females. Because the male UV-signal was dulled (rather than removed completely), these results indicate that females prefer to mate with males that have more brightly colored wings. Importantly, as Kemp (2008b) was careful to point out, the range of color manipulation used in this experiment also falls within the naturally occurring range of brightness variation in this species; hence, the observed effect is unlikely to represent a mere artifact of an unnatural or extreme experimental manipulation.

Manipulative demonstration of mating preferences is crucial for determining causality, but questions inevitably remain regarding whether and how such results transfer to the animal’s natural ecological context. Following the earlier approaches with \textit{C. eurytheme} (as outlined above; Kemp, 2006b; Papke et al., 2007; Rutowski, 1985), Kemp (2008b) attempted to bridge this gap for \textit{E. hecabe} by examining the phenotypic characteristics of individuals found in copula at a high-density field mating site in Cairns, Australia. The results presented an intriguing mix of contradiction and corroboration in relation to the experimental findings. First, and most intriguingly, comparison of 161 copulating males with 188 free-flying males indicated that in-copula individuals were older and actually possessed significantly less bright markings than their free-flying counterparts. The most parsimonious explanatory model of male “status” (in-copula vs. free-flying) was one containing UV brightness as the sole predictor variable. Second, and this time consistent with the experimental findings, among the males that were copulating, male UV brightness was correlated (along with their body size) with the size of their mate (Fig. 3C). Thus, males that were larger and in possession of brighter UV wing markings tended to copulate with larger females (male size and UV brightness were orthogonal factors).

How can this intriguing set of results be reconciled? As described for \textit{C. eurytheme} (above), one possibility is that a contrast between in-copula versus free-flying males may not truly represent a random sample of “attractive” versus “unattractive” males. The roles of individual males in such a “snapshot” of mating activity may bear little relationship to their lifetime mating success (hence, attractiveness; Kemp, 2006b). Second, it is also
Fig. 3. Experimentally and naturally observed relationships between the putative attractiveness of male *Eurema hecabe* (the brightness of their dorsal wing UV markings) and the body size of their female mating partner. (A, B) The average size difference between females that mated with visually unaltered (“control”) versus UV-dulled (UV−) males in mating experiments conducted in 1-m³ cages (panel A) and an outdoor insectary (panel B). (C) Covariance between the UV brightness of field sampled in-copula males (x-axis) and the size of their female partner (y-axis). The open circles and dashed line represents the entire sample of copulating pairs, captured from a high-density mating site in tropical Australia, while the closed circles and solid line represents the subset of females that were confirmed to be nonvirgins at the time of this mating. The stronger relationship in the latter sample is consistent with the presence of nonchoosy females in the former sample. Reproduced from Kemp (2008b) with the permission of Oxford University Press.
salient to note that due to the practicality of accessing workable numbers of copulating butterflies over a reasonable timeframe, studies of this nature can only realistically be conducted at high-density mating sites. These sites may or may not accurately represent the ecological context under which courtship and mating usually takes place. Moreover, because the densities are high, and activity is centered upon localized breeding sites, males at these sites are able to profitably locate virgin adult females as they have just emerged from their pupae. As noted earlier for *C. eurytheme*, newly emerged females are unable to reject male advances (Taylor, 1972), which means that precopulatory female mate selection is circumvented. The situation therefore effectively becomes one of scramble competition for matings. Mindful of this possibility, Kemp (2008b) dissected the female members of sampled copulating pairs in order to determine their mating history, which he then used as a basis for definitively identifying nonpupal mating females. Tantalizingly, an analysis including only these females indicated considerably stronger covariance between male UV brightness and female body size. This more closely corroborates the experimental findings and suggests that male UV coloration does play a role in the aerial courtships of free-flying butterflies. The lack of close corroboration between field and lab-manipulative results is nevertheless a significant issue and may stand testimony to the true complexity of mating signal evolution in this group.

IV. BUTTERFLY COLOR: MECHANISMS AND PRODUCTION COSTS

If we accept that the conspicuous wing markings of *Colias* and *Eurema*, and similar color traits in other butterflies (e.g., *Hypolimnas bolina*; Kemp, 2007), do indeed function for mate attractiveness, which appears most likely, the crucial question then becomes: Why? Do females benefit from receiving information signaled by the trait? Does such information transcend the mere signaling of species and/or sexual identity? Specifically, what information is signaled by the color trait, and how is such information encoded during development? As outlined earlier, these questions are best tackled using the explicit context of honest signaling theory (Zahavi, 1975), which predicts that signal exaggeration is costly and/or difficult for the average individual to achieve. One approach to testing this prediction is to attempt to measure the production costs directly, or via examination of the environmental, phenotypic and genetic basis of color signal variation (which we treat in Section V). Another popular approach is to examine the underlying mechanisms of color signal production, and then use such information to fuel *a priori* arguments for why certain color signals should be costly to express. However, this approach has been seriously criticized
(Griffith et al., 2006), primarily on the basis of its overly simplified application, and an almost exclusive focus on a single color mechanism (namely, carotenoid pigment-based coloration; as reviewed by Griffith et al., 2006).

Here we provide an overview of butterfly color-production mechanisms, along with what is known about their potential nutritional, physiological, and developmental costs. While mindful of the limitations of a priori cost-based argumentation (as outlined by Griffith et al., 2006), we feel that an understanding of these mechanisms is important for a holistic appraisal of signal function and evolution. Given that color is a product of light interacting with morphological structures and chemical pigments, selection acting upon color characteristics will lead to changes in those underlying structures and chemicals. Efforts to understand the evolution of color-based signaling, the paths it can and might be likely to take, and the sources and causes of variation in color signals must therefore be informed by an understanding of the structures and chemicals involved. From a more practical perspective, an understanding of color mechanisms may also guide more effective attempts to manipulate butterfly wing coloration in behavioral experiments (as in Section III).

In butterflies, all color signals are produced by the modification and reflection of incident light. There are no known cases of bioluminescence in this taxon, and while some pigments are known to fluoresce in the visible wavelengths when stimulated by light (Rawson, 1968), there is limited evidence that fluorescence contributes to visible or functional aspects of butterfly coloration (although see Vukusic and Hooper, 2005). The largest color signaling surfaces in butterflies are the wings, and the colors that contribute to the patterns on the wings are a result of properties of the scales and their distribution on the wings. Each scale acts like a pixel in a digitized image contributing one point of color to the image. In what follows, we will discuss the mechanisms known to contribute to the color of an individual scale and then take up how scales work together to create pattern. Finally, as noted above, we will assess the potential of the various mechanisms to provide a proximate basis for encoding in the resulting coloration information about the sender, that is, to produce a potentially honest signal.

A. SCALES AND COLOR PRODUCTION

Butterfly scales are flattened hairs that are attached by their proximal end to the surface of the wing integument. Scales can vary dramatically in form, but a typical scale is about 200 μm long and 100 μm wide. They are arranged in a shingle-like array with each scale resting at an angle of 10–20° relative to the surface of the wing integument. Two distinct classes of scales, known as “cover” and “ground” scales are recognized, with the former situated
externally to the former. Sometimes the cover scales are transparent, and may manipulate the colored light reflected off underlying ground scales (e.g., Vukusic et al., 1999; Yoshioka and Kinoshita, 2004). In other cases, the cover scales are most heavily endowed with color-producing structures and pigments (Fig. 4A), and contribute most to the wing’s appearance. Across butterflies, the various scale surfaces and underlying wing membrane are enormously varied in their structural and optical characteristics (e.g., Ghiradella 2010; Prum et al., 2006; Stavenga et al., 2010) and in their contribution to the appearance of the wing (Stavenga et al., 2006).

**Fig. 4.** Male wing coloration and wing scale morphology in the orange sulfur butterfly, *Colias eurytheme*. (A) The male dorsal wing photographed in “human-visible” light. The lighter wing areas appear yellowish orange to the human observer, but also reflect bright and iridescent ultraviolet (UV) over a restricted range of viewing angles. The inset in (panel A) shows a 1000× magnification Scanning Electron Micrograph (SEM) of a single scale from this region of wing. Far from being smooth, the upper surface of a lepidopteran wing scale is seen to feature a series of longitudinal ridges running its full length. (B) A higher magnification (18,000×) SEM of the male *Colias* wing scale showing the ridges and the underlying scale ultrastructure in more detail. The globular structures below the ridges are granules of pteridine pigment, responsible for both broadband scattering of light and absorption of short wavelengths. (C) An 18,000× magnification Transmission Electron Micrograph (TEM) of a cross-section through a male *Colias* wing scale, clearly indicating the vertical ridges and the horizontally oriented lamellae borne down each of their sides. These are the structural elements responsible for producing the brilliant, limited-view UV reflectance in this species. A similar structural architecture generates the iridescent UV of male *Euarena hecabe*, and the iridescent or metallic coloration of many other butterflies. The embedded scale bars represent 30 μm (panel A), 1.6 μm (panel B), and 1.5 μm (panel C). Reproduced from Kemp et al. (2006) with the permission of John Wiley and Sons.
Each scale is formed during the later stages of the pupal instar from a single cell that secretes an outer shell of cuticle (Ghiradella, 1974, 1984, 1994). This balloon-like shell collapses and forms a flat scale with a lower sheet of cuticle which is intact and flat, and an upper surface which can have a complex architecture of ridges, holes, flat areas, supportive cross members, and other structures. It is these upper surface structures that usually make the major contributions to the light reflected from the scale, although the lower scale membrane may have optical function as well (e.g., Stavenga et al., 2006; Vukusic and Hooper, 2005).

B. PIGMENTS AND BUTTERFLY COLORATION

Scales may contain pigments that selectively absorb certain wavelengths of light that would otherwise be scattered or reflected from the scale, thus generating color. Several major classes of pigments have been extracted from the wings of butterflies (Nijhout, 1991). These pigments differ in their properties in ways described below that might affect the information content of the color signals they produce.

Pterins absorb in the short wavelength region of the spectrum, and contribute to the reflection of what humans perceive as reds, oranges, and whites. Interestingly, pterins are reported to be the most nitrogen-rich pigments known, and so in species whose growth and development is routinely limited, pterin deposition in the scale may be limited, and so coloration might be indicative of the quality of the individual. This has been proposed specifically for the whites in the Family Pieridae who feed on crucifers, which are nitrogen poor (Morehouse and Rutowski, 2010a,b). An alternative view is that pteridines are by-products of routine metabolic processes and therefore unlikely to be particularly costly in their acquisition and expression.

Melanins are the pigments often involved in the production of blacks and browns. What are the potential costs of melanin deposition? As a derivative of the common amino acid tyrosine, melanins have been proposed by some to be cheap to produce (e.g., Badyaev and Hill, 2000). However, melanin plays the role of encapsulating foreign materials in the insect immune response, which means that investments of melanin into wing coloring may compete with requirements for immunocompetence (Stoehr, 2006). A recent study with a moth revealed a negative correlation between melanin deposition in the wings and the strength of the immune response (Mikkola and Rantala, 2010); however, the coloration of this animal is a naturally selected cryptic color. In the case of sexual signals, high expression of black may be indicative of the individual’s ability to invest in coloration while maintaining a responsive immune system (but see also Stoehr, 2010).
Flavonoids also absorb short wavelengths and so contribute to yellows and other longer wavelength colors, especially in the lycaenid butterflies (blues and hairstreaks). Like carotenoids in birds, these pigments cannot be synthesized by butterflies and must be obtained in the larval diet (Knuttel and Fiedler, 2001). As such, they could serve as a signal of success at acquiring larval resources. Flavonoids are also reported to have antioxidant properties (Rice-Evans et al., 1995) that might bolster their potential as a cue to signaler health and quality.

The last major class of butterfly pigments is the ommochrome, which is a compound derived from the common amino acid tryptophan (Linzen, 1974). This substrate is believed to be relatively common, albeit highly variable among different host plants (Linzen, 1974), which suggests no major or universal production costs to generating this class of signal. However, ommochrome-based wing colors in butterflies have not been thoroughly investigated for their potential to encode salient mate-quality information.

The exact placement of pigments in the scale structure is not well known except for the pterins. Pterins are deposited in granules or beads that are suspended from the structures that make up the upper surface of the scale (Giraldo and Stavenga, 2007; Morehouse et al., 2007). Recent work has demonstrated that these beads also contribute to the broadband scattering from the scale surface (Morehouse et al., 2007; Stavenga et al., 2004). High levels of pterin deposited in the scale then have the effect of making the wings more chromatic as a result of more UV absorption and more scattered light in the longer wavelengths. No other pigment is known to be deposited in packages whose physical structure contributes to color production over and above the absorption of light by the contained pigments.

C. SURFACE STRUCTURES AND BUTTERFLY COLORATION

Incident light interacts with physical structures on a surface, such as that of a scale, and these interactions can result in the reflection of some wavelengths and not others, thereby producing color. In general, these photonic interactions depend on the size and arrangement of the structures and on a difference in refractive index between the cuticle that makes up the structures and the surrounding medium (typically air). There are several broad categories of structures that function as photonic mechanisms to produce colorful reflections with different sorts of properties (Ingram and Parker, 2008).

General nano- and microstructures on scales: Particles and structures on the scale whose dimensions are of similar magnitude to light wavelengths may diffusely scatter incident light, thereby producing noniridescent
reflection. Because the intensity of the scattered light is proportional to the inverse of the cube of the wavelength, light scattered by an array of similarly sized particles tends to be most intense in the short wavelengths. However, because the dimensions of small structures on a butterfly scale are usually highly variable, the spectrum of scattered light is often very achromatic. In fact, butterfly scales without pigment typically appear white to human eyes (Rutowski et al., 2005, 2007).

**Diffraction gratings:** When there are closely spaced and periodic micro-ribs or other ridges on the scale surface, each ridge can act as a scatterer and splits the incident light into its component wavelengths (e.g., Vukusic et al., 1999). This produces a reflection with a dominant hue that varies according to the angle of viewing (such as seen on the surface of a CD or DVD disk).

**Thin film layers:** Incident light will be reflected from both the upper and lower surface of a thin film. The beam reflected from the lower surface passes back through the film and subsequently combines with the upper surface reflection in ways that can be constructive or destructive, depending on wavelength. This produces reflection that varies in hue and intensity with angle of viewing, that is, an iridescent color. The structures that produce thin film iridescence in butterfly scales typically consist of multilayers of cuticle and air (Ghiradella et al., 1972; Ingram and Parker, 2008; Vukusic and Sambles, 2003). Constructive interference is the mechanism responsible for production of the iridescent UV in *C. eurytheme* and *E. hecabe* and is illustrated in detail for the former species in Fig. 4.

**Photonic crystals:** Some scales are made of materials that have highly ordered, periodic, and three-dimensional structures that permit some wavelengths to pass and not others (e.g., Michielsen and Stavenga, 2008; Poladian et al., 2009). These structures, by virtue of their three-dimensional optical functionality (i.e., their ability to manipulate the flow of light in three dimensions), are of significant interest for their ability to inspire future technologies (Vukusic and Sambles, 2003).

From an honest signal perspective, there are conflicting views concerning the potential costs and/or developmental challenges involved with producing the physical structures responsible for generating structural coloration. One view holds that the production of these structures may rely heavily on self-assembly organization principles, and that once the conditions for self-assembly are established they proceed with the organism incurring little cost other than the building materials (Ghiradella, 1989; Michielsen and Stavenga, 2008; Prum et al., 2009). This viewpoint has been used to predict little variation (across and within individuals) in the surface layer nanostructures, and therefore little phenotypic variation in the structurally
produced color signal (e.g., Endler 1983, pp. 184). However, this “invariant self-assembly” viewpoint is overly simplistic in not considering how a concerted process of self-assembly may magnify important physiological processes, for example, the developmental/nutritional challenges involved with constructing an appropriate starting condition for self-assembly to occur. Self-assembly per se is not a sufficient condition to conclude that these signals will always be phenotypically and genetically invariant. An alternative view is that for these structures to produce a maximally bright and/or chromatic signal, they need to be built with great precision and consistency, which is potentially costly and/or difficult for an individual to achieve (Kemp and Rutowski, 2007). This view predicts high levels of phenotypic variation and condition-dependence in these color signals (Rowe and Houle, 1996). As we discuss in Section IV.D, evidence obtained in butterflies is accumulating in support of these predictions (e.g., Kemp, 2006a, 2008a; Kemp and Rutowski, 2007), although further work is needed to properly address this question.

D. Multiple Contributors to Color on a Single Scale

The color produced by butterfly wing scales is typically a product of one or, more typically, several of these individual mechanisms. For example, the reflectance spectrum of the scale of a male sulfur butterfly begins with the broadband scattering of light off the underlying scale ultrastructure and granules of pteridine pigments (Rutowski et al., 2005). The short wavelengths of scattered light are, in turn, absorbed by these pigments, so that only the yellow, orange, and red wavelengths leave the scale surface. However, projecting above the scattering structures and pigment granules is a complex array of ridges (of 1–2 μm height) from which lamellae extend horizontally (Fig. 4B–C). These lamellae act as a thin film multilayer that produces an iridescent and highly limited-view UV reflection. The result is a bimodal reflection curve that has a diffuse peak in the red–yellow and a strong but highly directional peak in the UV. The involvement of multiple mechanisms can have important consequence for the signal and the information it might contain. In this case, the pteridine pigments absorb short-wave light that would otherwise be scattered diffusely off the scale ultrastructure, such that the only UV contained in the signal is that from the array of lamellar layers on the scale. Because the lamellar-generated UV is highly directional (i.e., only visible over a limited range of above-wing viewing angles), the presence of such UV-absorbing pigment ensures that the wing is entirely UV-dark when viewed from orientations unsuitable for seeing the lamellar UV. In this sense, the underlying pigment amplifies the limited-view nature of the structurally produced UV. The UV component
of the wing color signal therefore only displays information about the quality of the construction of the complex lamellae (Rutowski et al., 2005), such as the density and/or arrangement of structural reflectors (e.g., Kemp et al., 2006).

E. MULTIPLE SCALES ACTING TOGETHER

Although the mechanisms of color production by individual scales have been studied in detail, far less is known about how scales work together to produce color signals. These effects have the potential to significantly influence the quality and potential information content of the signals. For example, in *Morpho* butterflies, the male’s iridescent blue scales produce an intense signal visible over a relatively broad range of above-wing viewing angles. These scales are overlain by clear scales that, via diffraction, broaden the angle over which the iridescence is visible. This effect is presumed to enhance the perception of the signal by intended receivers (Yoshioka and Kinoshita 2004). As another example, in *Pieris rapae* (the cabbage white), overlapping layers of scales lead to brighter reflectance than would occur if the scales did not overlap (Stavenga et al., 2006). These kinds of optical interactions between scales and their potential consequences for color signal production, perception, and evolution remain understudied.

V. BUTTERFLY COLOR: WHAT DOES IT ACTUALLY SIGNAL?

As noted earlier, appraising the physiological and developmental basis of color production may provide clues into its costs, and henceforth the potential honesty and information content of the resulting visual signal. However, that a particular form of coloration is theoretically costly to express does not necessarily predispose it as an evolutionarily stable sexual signal, nor does it exclude alternative mechanisms from attaining costly levels of exaggeration (Griffith et al., 2006). Benefits-based models of sexual selection predict that natural selection will favor signaling systems in which salient, fitness-enhancing information is conveyed, which in butterflies may be the quality or compatibility of a male’s genome, and/or his ability to provide direct benefits such as a nutritious ejaculate. While all of these things may be difficult to appraise (let alone to anticipate for species with vastly different ecologies), valuable insights can be gained by examining the environmental, phenotypic, and genetic correlates of color signal variation. Such data are only now accumulating for butterfly coloration (e.g., Talloen et al., 2004); here we outline what is known for one of our two focal species, *C. eurytheme*.
As outlined in Section III, female *C. eurytheme* prefer males bearing bright dorsal UV markings. Brightness and other reflectance characteristics of this trait vary with adult age (Kemp, 2006a), larval nutrient acquisition (Kemp and Rutowski, 2007), and thermal stress experienced during the pupal stage (Kemp and Rutowski, 2007). Younger males have brighter UV markings, as do individuals that experienced a more nutritious larval diet and a more thermally stable period of pupal development. Hence, UV brightness in this species contains information on a male’s phenotypic condition, as well as providing a window into his prior developmental “experiences.” Notably, the coincident pigment-based color of male wings—the yellowish orange—also varies according to age and larval nutrition, but the magnitude of variation (and therefore the potential signal information content) is much less than that exhibited by the structurally generated UV (Kemp, 2006a; Kemp and Rutowski, 2007). This is an important point because putative sexual traits are not only simply expected to be condition-dependent, but relatively more so in comparison with traits less strongly subject to sexual selection (Cotton et al., 2004b).

The phenotypic quality information encoded within the wing colors of male *C. eurytheme* is potentially salient because (as noted earlier), male-derived nutrients are known to contribute to female fitness traits (Boggs and Gilbert, 1979). Selection should favor—if at all possible—female choosiness for males capable of donating viable and nutritious ejaculates. This could be achieved through choosing UV-bright mates (to the extent that such males experienced high-quality juvenile environments). However, whether this is the ultimate reason why females have evolved such preferences stands to be demonstrated. In follow-up work, Kemp et al. (2008) addressed this possibility by mating virgin females with free-flying males in two separate experiments and then assaying male coloration, ejaculate size, and the fitness traits of their female mates. Intriguingly, there was no relationship between male coloration and the mass of their ejaculate, and only weak covariance between male coloration and female fecundity, fertility, and longevity. Given that any benefits of choice need to be balanced against any costs to being choosy (e.g., Kaitala and Wiklund, 1995), these results suggest that females are not likely to gain meaningful direct benefits from choosing mates based on their dorsal UV coloration.

There is also the possibility that bright coloration may signal indirect benefits (i.e., genetic viability). Because the dorsal UV of a *C. eurytheme* male depends upon an array of nanoscale surface structures, this component of male coloration could signal viability through its ability to magnify physiological and/or developmental processes (Fitzpatrick, 1998). This hypothesis proposes that males of higher genetic quality (i.e., possessing a more adapted or more internally harmonious genome) may be able to build
the necessary fine-scale structures with greater precision, especially in the face of environmental stress. Kemp and Rutowski (2007) tested two predictions of this hypothesis in *C. eurytheme*, both of which are based on the fact that the adult phenotype (including wing structures) is constructed over several days during metamorphosis. First, they predicted that more viable individuals should be able to maintain bright UV expression even when subjected to limitations in the building blocks of the adult phenotype. The second prediction was that more viable individuals should be able to achieve greater architectural precision in their nanostructures even when faced with physiological stress (i.e., a highly variable thermal environment). Based on the theory formalized in Section II.B, these predictions were evaluated according to the presence of GEIs; specifically, a GEI of the nature of which genetic variation is magnified under environmental stress.

By rearing family groups in different nutritional environments, and subjecting them to heat and cold shocks during metamorphosis, Kemp and Rutowski (2007) were able to appraise the GEIs as a formal test of whether some families (i.e., some genetic combinations) withstood stress better than others, and whether this was signaled via their wing coloration. As we note above, in both manipulations, heightened stress reduced the expression of male UV, and color traits were also strongly heritable. However, GEIs were uniformly absent, weak, and/or not of the predicted nature. Genetic variance in UV brightness, for example, which is the key sexual parameter (Papke et al., 2007), actually decreased significantly in the stressful environment. This result argues against the viability indicator hypothesis in this species, at least in terms of the signaling of good genes for mitigating poor juvenile environments. However, there are other ways in which the expression of UV coloration, which is highly heritable, could be genetically correlated with viability. The fact that UV expression is strongly condition-dependent means that any genetic basis to the acquisition of condition, under natural conditions, would be signaled by the male’s adult wing coloration (as well as his overall body size, the main vector of condition; Kemp 2008a). In this way, male UV could function as a revealing indicator of good genes for things like the ability to choose quality microhabitats and nutritious plant foliage, the ability to avoid predation or resist infection, and/or the ability to avoid or withstand stressful environments. This insight, and its suggested lines of enquiry, has been developed only through work in the butterfly system, as far as we are aware. However, effective tests of this idea will require the quantitative genetics of condition-dependence to be assayed under the most natural conditions possible. This is likely to prove challenging, although it may be achievable using large seminatural outdoor enclosures.
VI. DISCUSSION AND FUTURE DIRECTIONS

In this chapter, we have provided a selective review of the production, signaling function and information content of butterfly wing coloration. This presents, in some sense, an update of the past reviews of Silberglied (1984), and later Vane-Wright and Boppre (1993), Rutowski (1997), and Wiklund (2003), although we also acknowledge the progress being made on areas of butterfly coloration outside our present review (e.g., Ellers and Boggs 2002, 2003, 2004a,b; Fordyce et al., 2002; Jiggins et al., 2001; Knuttel and Fiedler (2001); Sweeney et al., 2003). Our review is also an attempt to float butterflies to the mainstream as an alternative, but presently under-utilized, candidate system for advancing our understanding of sexual selection and the evolution of color-based mate-quality indicators.

As noted earlier, color-based visual signaling in butterflies has provided valuable insights into evolutionary phenomena such as speciation (Chamberlain et al., 2009; Mavarez et al., 2006), reinforcement (Kronforst et al., 2007), and aposematism and mimicry (Kapan, 2001; Kunte, 2009), to name a few. In sexual signal terms, we have long understood the wing patterns of males and sometimes females to function during courtship for the recognition of potential mates (Fordyce et al., 2002; Nielsen and Watt, 2000; Robertson and Monteiro, 2005; Rutowski, 1977; Silberglied and Taylor, 1978; Stride, 1957, 1958; Sweeney et al., 2003; Wiernasz and Kingsolver, 1992). Studies such as those in Colias (Papke et al., 2007, Rutowski 1985), Eurema (Kemp 2007), and Hypolimnas (Kemp 2007) illustrate that female choice can also target the qualitative aspects of color signal expression, a finding which then opens the door for the examination of male-limited butterfly colors as potentially informative sexual signals. As Sections II–V outline, male coloration in several of these species is condition-dependent. The pattern of relative condition-dependence across different wing pattern elements agrees with theoretical predictions (Cotton et al., 2004b; Rowe and Houle, 1996; Tomkins et al., 2004) based on their relative importance as components of male attractiveness. The particularly high level of condition-dependence in iridescent coloration (Kemp 2006a, 2008a; Kemp and Rutowski, 2007) supports the theory that structurally colored signals are not simply invariant products of some self-assembly process. In addition, male wing color traits show high levels of additive genetic variation, which is expected for sexually selected traits (Rowe and Houle, 1996) and which could allow for the signaling of indirect benefits. The one existing test of this hypothesis, based on the key prediction for genetically mediated condition-dependence, found no support for the signaling of indirect benefits (Kemp and Rutowski, 2007). However, condition was assayed under very restrictive and unrealistic laboratory conditions,
which may have prevented or devalued the expression of genes that would normally greatly influence condition in the wild. Butterflies, by virtue of their tractability for seminatural environments, offer excellent opportunities for more realistic assays of condition-dependent trait expression, and for gathering much-needed data on the quantitative genetics of this parameter (Cotton et al., 2004a).

Despite the potential utility of this system, the few examples outlined here (Section IV; also see Kemp, 2007) represent most of what is known about the signal relevance of qualitative aspects of wing color expression, such as the brightness, hue, and/or relative size of certain color elements (although also see Breuker and Brakefield, 2002; Robertson and Monteiro, 2005). Without such information it is difficult to chart the limits to butterflies as a system for understanding the broader evolution of sexual signaling. Given their suitability for lab-based breeding experiments, developmental manipulations, and quantitative genetics, butterflies seem well placed to evaluate sexual selection models, such as sensory drive (Endler and Basolo, 1998), chase away (Holland and Rice, 1998), viability indicator (Møller and Alatalo, 1999), and genic capture (Rowe and Houle, 1996). The novel spread of color-production mechanisms in this group (Prum et al., 2006; Vukusic and Sambles, 2003) could also provide insights into how selection can generate mechanistic (as well as visual) diversity in animal coloration.

We suggest that students of sexual signaling in butterflies should seek to demonstrate convincingly, first and foremost, that individuals are sensitive to qualitative variation in the color signals of conspecifics. Behavioral experimentation to examine this question should ideally use wing color manipulations of a nature that targets the primary vector of signal exaggeration. For some species this will mean graded manipulations of signal brightness or chromaticity (e.g., Kemp, 2007, 2008b), for others it may mean manipulating the size or occurrence of particular elements of the wing pattern. In all cases, the extent of these qualitative manipulations should fall within the bounds of naturally occurring signal variation. Appropriate controls should be used in order to avoid the pitfalls of prior efforts (e.g., Silberglied and Taylor, 1978). Finally, conclusions reached using lab- or insectary-based experimentation should be validated against data obtained in more natural field-based settings. This has been achieved in studies of some species (e.g., Hypolimnas bolina; Kemp, 2007), but has proven challenging in others (as we have outlined for Colías and Eurema in Section IV). Part of this challenge stems from the highly dispersed, cryptic, and ephemeral nature of butterfly copulations, a well-maligned fact among lepidopteran biologists (see, e.g., Silberglied, 1984; Rutowski, 1997).
Given that data support the existence of female preferences for male coloration, well-designed breeding experiments could be used to reveal what information (if any) is encoded within the expression of the male color signal. These efforts should focus, in the first instance, on evaluating the honest color-based prediction of heightened condition-dependence (*sensu* Zahavi, 1975; Rowe and Houle, 1996). This will demand well-designed experiments that avoid the empirical and logical limitations of past work (summarized by Cotton et al., 2004a,b). As outlined earlier, in the case of *C. eurytheme*, the UV component of male wing coloration is more generally and strongly affected by phenotypic stress—and henceforth more strongly condition-dependent—than its accompanying pigment-generated yellowish orange (Kemp and Rutowski, 2007). This agrees well with theory. This pattern is also true of *E. hecabe* (Kemp, 2008a), and further work on this species has indicated that most of the naturally occurring variation in phenotypic condition (and condition-dependent wing coloration) can be approximated by limiting individual’s access to high-quality larval nutrition (Kemp, 2008a). If the same is true across many butterfly species, then quantifying and manipulating condition may be easier to achieve than in many other systems.

Along with questions relating to visually exaggerated male coloration, the butterfly study system also seems well placed to deepen our understanding of sexual dimorphism. Various authors have noted that differences between the sexes are pervasive in the group, and attributed such differences to the operation of sexual selection (e.g., Rutowski, 1997). While prior empirical efforts have set their sights on the evolution of sexual dimorphism in butterflies *per se*, again we wish to emphasize the potential to illuminate more general theories of the concept. Recent theory has cast the evolution of sex dimorphism in the context of intralocus sexual conflict (Bonduriansky, 2007a,b; Bonduriansky and Chenoweth, 2009) and implicated epigenetics and condition-dependence as key brokers of phenotypic divergence between the sexes. Research using butterfly models may not only illuminate these processes, but has broader potential for testing and refining this developing body of theory. An excellent lead in this regard is given by the exploitation of butterfly wing patterns to understand the workings of phenotypic evolution and evolutionary development (e.g., Beldade et al., 2002).

Finally, although we have focused throughout on intraspecific approaches to examining behavior, morphology, development, and genetics, we also appreciate that phylogenetically controlled comparative studies will provide a highly informative and complementary empirical approach. Indeed, the comparative method has been fruitfully applied to the study of the evolution of sexual color signals in sulphur butterflies (Brunton 1998;
Brunton and Majerus, 1995; Kemp et al., 2005). Whereas examining intra-specific color variation can furnish direct insights into signal function, expression costs, and information content, examination of interspecific patterns of wing color variation has the potential to guide our understanding of how and when particular signals and signaling systems evolve. Ideally, and ultimately, researchers will draw upon both levels of analysis in appraising the color signals of butterflies, and in using this system as a model for the broader study of sexual signal evolution.

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