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COLOR AS A RELIABLE SIGNAL OF FIGHTING ABILITY IN MALE DAMSELFLIES, CALOPTERYX MACULATA

By

Donna Marie Fitzstephens

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ABSTRACT

COLOR AS A RELIABLE SIGNAL OF FIGHTING ABILITY IN MALE DAMSELFLIES CALOPTERYX MACULATA

By

Donna Marie Fitzstephens

Color may act as an important signal in agonistic encounters by providing reliable information on the resource holding potential of opponents. Males of the damselfly Calopteryx maculata engage in costly agonistic contests which result in the turnover of territory ownership. Male C. maculata exhibit structural interference colors that vary with age and territorial status making color a prime candidate to convey information as a signal. This study examines the mechanisms and significance of color production and color change in a natural population of C. maculata.

Physical and chemical cuticle tests are suggestive of constructive interference colors produced by lamellae in the epicuticle. Transmission electron micrographs provide direct evidence of a multilayer interference reflector. The lamellae of blue males are thinner (6.2nm ± 1.0SD) than the lamellae of green males (6.4nm ± 1.0SD). Wavelength of peak reflectance calculated from lamellar spacing yields values which correspond to the colors observed in the field.

Male C. maculata maintained in the laboratory on a high food diet have significantly higher percent body fat (7.3% ± 2.2SD) and exhibit a significantly smaller color change than similar age/size color males maintained on a low food diet (4.1% ± 2.7SD). High food males also retain their initial color almost twice as long as low food males.

In the field, territorial male C. maculata were younger, reflect shorter wavelength coloration, and were more successful at mating than nonterritorial males. As male C. maculata age, their color changes from short to long wavelength. Fat analysis indicates that short wavelength males have significantly higher fat levels (6.7% ± 2.0SD) than do long wavelength males (4.4% ± 1.6SD). The field and laboratory evidence indicates that fat content is more important than age for determining male color.

Wavelength is reliably related to the measures of body condition (energy reserves) which affect the outcome of male C. maculata interactions. Path analysis indicates that the wavelength of male color is the best predictor of male territorial status. Therefore, color conveys reliable information about male fighting ability as measured by fat content.

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INTRODUCTION

The exchange of information via signals is often an integral part of agonistic encounters (Gardner and Morris, 1989; Hasson, 1994). What guarantees that opponents in aggressive interactions signal 'correct' information (Enquist, 1985)? This problem of signal reliability is a much discussed and controversial topic (Zahavi, 1975, 1977; Enquist, 1985; Grafen, 1990; Harper, 1991).

In general, signals may be reliable if they are biologically correlated with resource holding potential (RHP) or if they are costly. Assessment signals are biologically correlated with RHP and therefore cannot be faked (Harper, 1991). In guppies, color is dependent on food resources; it is therefore an assessment signal biologically correlated with foraging ability (Kodric-Brown, 1985, 1989). Conventional signals are not logically correlated with RHP. Color as a 'badge of status' in birds is not correlated with RHP but is a reliable conventional signal due to the costs of wearing the badge (Rohwer, 1982; Jarvi and Bakken, 1984; Whitfield, 1987; Maynard Smith and Harper, 1988). It has been demonstrated that conventional signals are reliable or 'honest' if they are costly to produce or display (Zahavi, 1975, 1977; Enquist, 1985; Grafen, 1990) with cost being a function of RHP.

Disputes are often settled by asymmetries between contestants (Hammerstein and Parker, 1982; Maynard Smith, 1982; Leimar and Enquist, 1984; Huntingford and Turner, 1987). These asymmetries often reflect physical advantages, residency, or other differences in RHP. In such contests, animals may utilize signals to assess RHP or payoff asymmetries. Despite continued discussion of the presence of cheating in animal signaling systems (Grafen, 1990; Dawkins and Guilford, 1991; Wagner, 1992), theory predicts that signals used for assessment of fighting ability should honestly advertise fighting ability (Maynard Smith and Parker, 1976; Zahavi, 1977; Grafen, 1990). Dishonest signals are evolutionarily unstable because they tend to be ignored or fall into disuse if unreliable (Harper, 1991). Recent models have demonstrated that cheating or dishonesty may persist at low levels in a system (Dawkins and Guilford, 1991). However, as long as a signal is honest 'on average' it will be reliably related to that which it predicts (Johnstone and Grafen, 1993). For discussion of the various definitions and uses of signals see Grafen (1990) and references therein.

Male Calopteryx maculata are brightly colored Calopterygid damselflies with a blue or green thorax and abdomen. Previous studies of damselfly color have concentrated on the family Coenagrionidae. These studies have addressed genetically determined female limited color polymorphisms (Fincke, 1987; Cordero, 1990; Forbes, 1991), temperature related physiological color changes (May, 1976; Conrad and Pritchard, 1989) and developmental color changes associated with maturation (Hinnekint, 1987; Úeda, 1989). No previous studies have addressed the significance of male color differences of in C. maculata or the use of color as a signal in agonistic encounters. This study examined the mechanisms of color production and color change as well as the likelihood that color conveys information as a signal in a natural population of C. maculata. In studying agonistic encounters, it is important to estimate the role that assessment of RHP plays in settling contests. Elucidating the mechanisms of color production will demonstrate how color is related to the biology of C. maculata and as such, will reveal what types of information can be conveyed to opponents through color.

Calopteryx maculata is especially well suited for the study of color as a signal. Adults are relatively large, slow moving damselflies and are therefore easily observed and captured for marking. Territorial males are rather sedentary (see below) defending the same 1-2m of stream each day. Therefore, marked individuals can be observed throughout their lifetime. Finally, there is variation in male color that seems to be related to age and territorial status (Fitzstephens, unpubl), which makes color a prime candidate to convey information about the traits important for turnover in territory ownership.

I will examine the mechanisms of color production in C. maculata by various physical and chemical tests to distinguish among pigment as well as three types of structural colors. A feeding experiment will demonstrate how color change is related to C. maculata feeding behavior and energy reserves. In the field, individual males will be followed throughout their lifetime to determine how color change is related to territorial and reproductive behavior. Finally, path analysis will be used to evaluate the direct and indirect relationships among all variables to determine the strength of the relationships between color and the variables important for territorial defense (age and fat, see below). This analysis will demonstrate that color is reliably related to the measures of body condition important in winning contests and that it is the best predictor of territorial status.

CALOPTERYX MACULATA NATURAL HISTORY

Calopteryx maculata is a stream dwelling Calopterygid damselfly. Average life span including an 11day teneral period is 16-20 days (Waage, 1972). The mating system of C. maculata has been classified as resource-defense polygyny (Alcock, 1987). As with many animals exhibiting resource defense polygyny, C. maculata are sexually dimorphic. Males are bright blue or green with black wings while females are dull brown with translucent brown wings. Males compete to attract females by defending a valuable resource (oviposition sites). Adults are active from mid-June through mid-August; activity peaks during July (Forsyth and Montgomerie, 1987). Adult males are territorial and aggressively defend 1-2m of stream consisting of a perch and at least one oviposition site. Territories are defended from approximately 10:00 to 18:00 on sunny days, and most reproductive activity occurs during this period within 2m of the stream (Waage, 1973). Male aggressive contests may last from 10s to over an hour (Waage, 1988). Females mate almost exclusively with territorial males (Waage, 1979; 1982). Mating lasts 1.0-1.5 minutes and is followed by 12-19 minutes of oviposition during which the female deposits eggs in emergent vegetation while being guarded by the territorial male (Waage, 1979; Forsyth and Montgomerie, 1987). Unguarded females oviposit for 1-2 minutes before being disturbed by males.

The presence of non-territorial, reproductively active males has been documented in several studies of C. maculata (Waage, 1972; 1973; Forsyth and Montgomerie, 1987). Nonterritorial or sneaker males do not defend a specific area. Instead, they pursue and attempt to mate with ovipositing females opportunistically and do not guard females after mating (Forsyth and Montgomerie, 1987). Nonterritorial males cover up to 100m of stream per day and are decidedly surreptitious in their behavior around territorial males. Females often terminate encounters with nonterritorial males by dislodging them with wing flips (Waage, 1973) or evasive flights typically used as mate rejection responses (Waage, 1979; Forsyth and Montgomerie, 1987). The daily copulation rate for territorial males is higher (3.9 matings/male/day) than that for nonterritorial males (0.14-2.8 matings/male/day; Forsyth and Montgomerie, 1987).

CHAPTER 1

A MULTILAYER INTERFERENCE REFLECTOR IN THE EPICUTICLE OF MALE CALOPTERYX MACULATA

INTRODUCTION

Colors may have a physical (structural) rather than a chemical (pigment) basis (Mason, 1926). Pigment colors result from absorption of selective constituents of white light. Physical colors depend on the structure of the cuticle and may be due to scattering, reflection, refraction, or diffraction of incident light (Neville, 1975; Hinton, 1976). See examples below.

Cromartie (1959) provides an extensive review documenting the occurrence of carotenoid, anthraquinone, aphin, anthroxanthin, anthrocyanin and pterin pigments in the following insect families: Orthoptera, Lepidoptera, Coleoptera, Phylloterida and Aphididae.

Mason (1926) established general criteria by which physical or structural colors can be differentiated from pigment colors. Physical colors can be altered or destroyed by pressure, distortion, swelling, shrinking, and immersion in a medium of the same refractive index as the structure. Unlike pigment colors, structural colors are unaffected by bleaching or chemical reagents unless the tissue is altered. Chemical digestion of the integument of insects exhibiting structural colors releases only black or brown pigments.

The most commonly discussed types of structural colors include scattering by particles (Tyndall colors), diffraction grating, and interference by thin films. Tyndall colors arise by scattering of shorter wavelength light and transmission of longer wavelengths when light penetrates a system of randomly dispersed particles (Mason, 1926; Neville, 1975). The resulting color depends on particle size. If white light penetrates a heterogeneous system composed of bodies embedded in a medium, the refractive index of which is different from that of the bodies, and the particle diameter is small compared to the wavelength of light, the scattered light will be blue (Onslow, 1921). The smallest particles reflect violet, larger ones reflect deep to pale blue, and the largest ones reflect white. The most extensive evidence of Tyndall colors in insects was found in Coenagrionid damselflies (Mason, 1926; Charles and Robinson, 1981) and the following dragonflies: Anax, Erythemis, and Libellula (Mason, 1926).

Structural colors can occur in insects having a cuticle with suitably spaced surface ridges (Anderson and Richards, 1942). These systems behave as diffraction gratings. Diffraction colors are produced by a series of evenly spaced parallel groves or ridges on the epicuticle. White light is scattered from the lines and the resulting waves either reinforce or cancel each other. In diffuse light, the resulting spectra overlap so much that the original color is significantly dimmer or reduced to dull black. Diffraction colors differ from other structural colors in that they can only be seen from certain directions relative to the striations (Hinton, 1976; Hinton and Gibbs, 1971). Diffraction colors as the major source of color have been reported in beetles of the following families: Scarabaeidae, Phalacridae, Cerambycidae, Gyrinidae, Carabidae, Staphylinidae, Torridincolidae, and Silphidae (for review see Neville, 1975).

The most widely cited system of structural color production in insects is that of constructive interference colors produced by thin films or lamellae in the epicuticle (Richards, 1951; Fox and Ververs, 1960; Simon, 1971; Schultz and Rankin, 1985). Differences in color correspond to differences in uniformity or thickness of the layers. Constructive interference of waves occurs between light reflected at successive interfaces. Diffuse illumination does not cause interference colors to disappear (Mason, 1927). As the angle of viewing is increased from grazing incidence to a direction normal to the cuticle surface, color produced by multilayer interference changes from shorter to longer wavelengths (Onslow, 1921).

Swelling and pressure also alter the appearance of interference colors in predictable ways (Mason, 1927). Swelling (with water for example) increases lamellar spacing and shifts color to longer wavelengths. Increasing pressure decreases lamellar spacing, shifting in color to shorter wavelengths. These color shifts are reversed upon removal of the swelling agent or release of the pressure.

Males of the damselfly Calopteryx maculata are conspicuous having black wings and a brightly colored blue or green thorax and abdomen. Females are more cryptic, having translucent brown/gray wings and a brown thorax and abdomen. Preliminary observations suggested that color in male C. maculata may be structural in nature and change over an individual's lifetime (Fitzstephens, pers obs). The objective of this study was to determine the nature of male C. maculata coloration and to reveal the microstructure of the color producing components of the cuticle. Determining the nature of color production and how it is related to the biology of C. maculata is the first step in the determination of what information is provided by color in this species.

MATERIALS AND METHODS

I assessed the relationship between age and color of male C. maculata, from 15 June to 15 August 1991 at Augusta Creek, approximately three miles from the Kellogg Biological Station in Southwestern Michigan. I performed field comparisons of the color of young versus old males by netting and marking them using a Testor's © paint marker with a unique number on the hindwing (Hinnekint, 1974). Male age refers to the number of days elapsed since an individual was first marked at the stream (Forsyth and Montgomerie, 1987). This categorization does not include the 11 day teneral period which males spend away from streamside (Waage, 1972). I performed pairwise comparisons for color between young and old males by viewing the males side by side at grazing incidence to the cuticle surface and assessing their color as either ‘blue’ or ‘green’. Grazing incidence refers to a view parallel to or at 0^{o} to the cuticle surface. A more quantitative assessment of color (see next section and results) determined that males subjectively labeled as ‘blue’ or ‘green’ in fact reflected different wavelengths. I selected males for comparison by walking the stream and capturing the first male encountered that fit one of the two age categories. I then captured the next male encountered of the opposite age category. For these tests, young males were those marked fewer than five days prior to the test, while old males were those marked more than 10 days prior to the test (Marden and Waage, 1990).

I also performed pairwise comparisons of the age of ‘blue’ versus ‘green’ males by netting one blue and one green male and recording the number of days elapsed since that individual was marked. I obtained pairs by capturing the first male encountered of one color category (‘blue’ or ‘green’) and then capturing the first male encountered of the other color category. I used each male in only one field comparison. The color of male C. maculata did not change with time of day or with sunny versus shady locations.

From June through August 1992 and 1993, I quantified the color of ‘blue’ and ‘green’ male C. maculata. During this time, I assessed the color of each male at normal incidence by comparison to Munsell Color Chips (Munsell, 1976a). Normal incidence refers to a view perpendicular or at 90^{o} to the cuticle surface. Comparison at normal incidence provided a more reliable color assessment than comparison of color at grazing incidence (pers obs). Munsell color notation was later translated into a wavelength (Munsell, 1976b).

To determine whether the colors were produced by pigments or some type of structural colors, I collected 20 blue and 20 green male C. maculata and subjected them to a variety of physical and chemical tests. I first viewed each cuticle in diffuse light to determine whether the color changed with lighting conditions, as would be expected for structural colors. To examine positional color change, I viewed each cuticle from a direction at grazing incidence to the surface and then rotated the cuticle for viewing normal to the surface, noting the progression of color change. To distinguish between epicuticle and exocuticle as the source of color, I immersed the cuticles of five blue and five green males in 8% KOH at 60 °C and monitored color change every 30 minutes (Schultz and Rankin, 1985). Immersion in dilute KOH dissolves the epicuticle, separating it from the rest of the cuticle. Separation of the epicuticle will reveal whether the color is produced in the epicuticle or the remainder of the cuticle. If the epicuticle is the source of the color, the blue/green color will disappear and the resulting cuticle will be black. I bleached the cuticle of five blue and five green males by immersion in 10% hydrogen peroxide at 26 °C and monitored color change every 30 minutes (Mason, 1927). Hydrogen peroxide will bleach pigment colors and alter interference colors if melanin is a component of the interference reflector (Schultz and Rankin, 1985). I immersed five blue and five green cuticles in water to swell the exoskeleton. After noting any color change, I allowed the cuticles to dry to determine if the initial color could be restored. I subjected the remaining five blue and five green males to shrinking by compressing the cuticle with a dissecting needle and noting any color change (Mason, 1927).

I collected an additional five blue and five green males and obtained transmission electron micrographs (TEM's) of their cuticle for microstructural analysis of the presumed color producing layer. TEM's were obtained from the same abdominal segment of each male. Transmission electron microscopy was performed by the Center for Electron Optics at Michigan State University.

RESULTS

In 48 of 52 pairwise color comparisons of young versus old male C. maculata, the color of the younger male was judged blue while that of the older male was green ($X^2 = 81.7$, df = 2, $P < 0.0001$). Only one older male was judged blue while its younger counterpart was judged green. In three cases the color of both the young and the old male was judged blue.

In 49 pairwise comparisons for age (days marked) between blue versus green males, the mean age (± SD) of the blue males was 3.0 days ± 3.2 SD while that of green males was 12.1 days ± 7.3 SD (Wilcoxin signed ranks test, $T_S = 7$, $N = 49$, $P < 0.001$)

Viewing in diffuse light did not reduce the intensity or alter the color of any of the 20 blue or 20 green cuticles. This means that the colors are not produced by diffraction. When viewed at grazing incidence to the surface, all 20 blue cuticles were deep to medium blue while all 20 green cuticles were blue-green to green in color. When the angle was increased to normal incidence, the 20 blue cuticles gradually progressed to bright green while that of the 20 green cuticles progressed to yellow-green. These color changes are suggestive of constructive interference colors.

Figure 1 reveals the Munsell Chip-quantified colors of 617 'blue' and 314 'green' male C. maculata. The resolution of the Munsell Chips used for color assessment is 0.5-5.0nm. The ability of C. maculata to distinguish among these colors is being investigated. The subjective color assessment of whether a male was 'blue' or 'green' was made upon viewing flying or perching animals which approximates a view at grazing incidence to the cuticle surface. The Munsell color assessments were made from a view normal to the cuticle surface. At normal incidence, 'blue' males look bright green and 'green' males look yellow-green. Translation of Munsell color notation into wavelengths indicated that blue males exhibited short normal incidence wavelengths of 509nm or 525.5nm while green males exhibited long normal incidence wavelengths of 550nm or 558nm (Figure 1). Two males reflected a normal incidence wavelength of 559nm. No color matches were found for the color chips corresponding to the intermediate wavelengths. The mean wavelength of 'blue' males (520.1nm ± 7.7SD) was significantly shorter than that of 'green' males (550.7nm ± 2.3SD; Mann-Whitney U = 0.00, $P < 0.0001$).

Chemical treatments of cuticle produced the same results for both blue and green cuticle. Portions of C. maculata cuticle treated with 8% KOH gradually lost their color. During the first 30 minutes, the color of both green and blue cuticles lengthened to red/orange. After one hour, all cuticle fragments were devoid of color and became a flat black/brown. The solution of dissolved epicuticle was brown. This suggests that the epicuticle is the source of the bright blue/green male colors. Fragments of cuticle bleached in 10% hydrogen peroxide changed to longer wavelength green during the first 30 minutes. After three hours the intense green color began to dull and continued to dull slowly over the next eight hours. This suggests that the color is structural rather than chemical in nature.

Assessments of the physical tests were made while viewing the cuticles at normal incidence in the field. Therefore 'blue' cuticles initially looked bright green and 'green' cuticles initially looked yellow-green. Swelling the cuticles with water resulted in the five 'blue' cuticles turning a lighter green while the five 'green' cuticles turned pale yellow-green. The color change was reversed upon

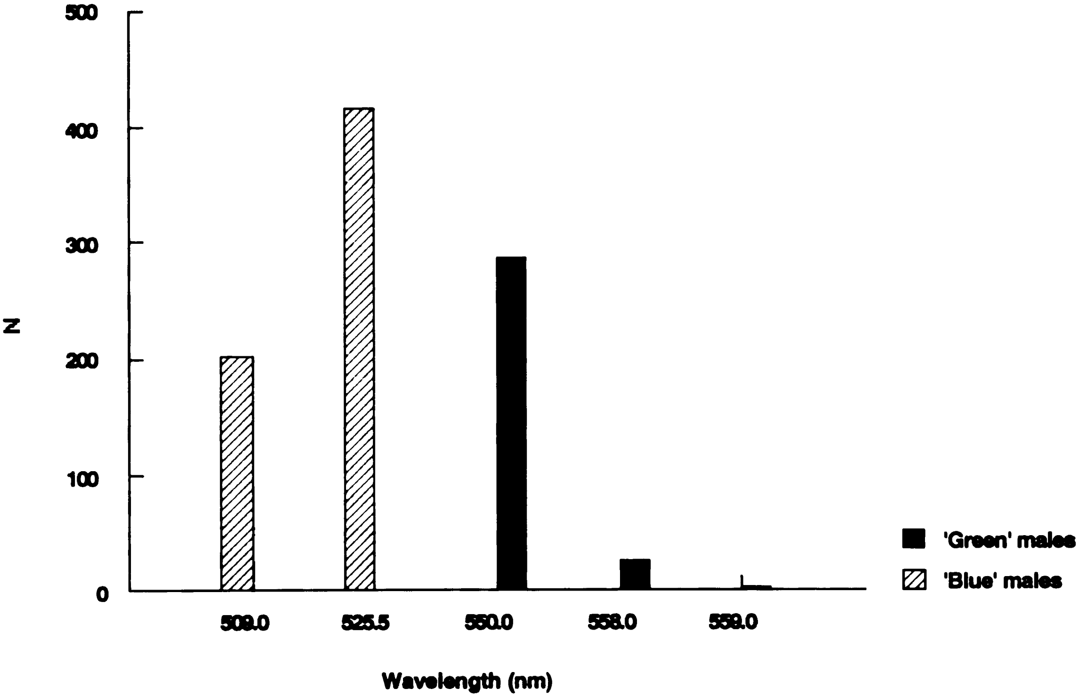

Figure 1. The wavelengths of color reflected by male Calopteryx maculata categorized as 'blue' or 'green'. (N = sample size)

drying. Compressing the cuticles resulted in changes to slightly shorter wavelength colors. The five initially bright green 'blue' cuticles turned blue-green while the five initially yellow-green 'green' cuticles turned bright green. The color change was reversed upon release of the pressure. These changes associated with position, pressure, and swelling are suggestive of constructive interference colors.

TEM's of both blue and green cuticles revealed a series of electron dense bands separated by electron lucent bands in the epicuticle (Figure 2), providing direct evidence of a multilayer interference reflector. The cuticles of blue and green male C. maculata did not differ with respect to the number of electron lucent or electron dense bands. Cuticles of blue males had thinner electron lucent bands as well as thinner electron dense bands than cuticles of green males (Table1; Figure 2).

Table 1. Mean number and spacing of electron lucent and electron dense lamellae in the epicuticle of blue versus green male Calopteryx maculata.

		Number of lamellae (±SD)		Width of lamellae (mm ±SD)	
Male Color	N	lucent	dense	lucent	dense
Blue	5	6.3 ± 1.0	6.3 ± 1.0	96.1 ± 4.6	56.0 ± 2.6
Green	5	6.4 ± 1.0	6.4 ± 0.9	103.6 ± 2.3	60.0 ± 1.9
Statistic	df=8	t=0.24ns	t=0.24ns	t=3.28*	t=3.28*

* $P < 0.05$

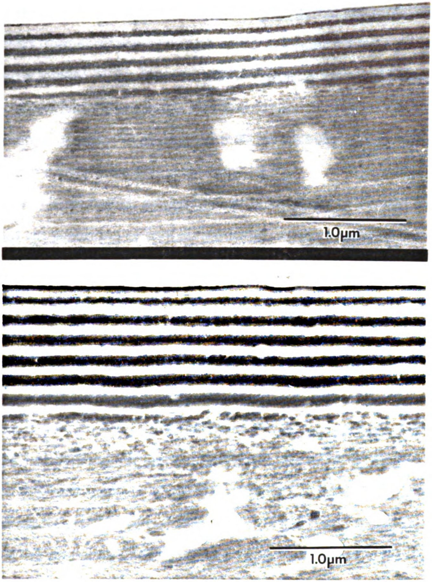

Figure 2. Transmission electron micrographs of the outer exoskeleton of blue (top) and green (bottom) male Calopteryx maculata showing alternating layers of electron lucent and electron dense material in the epicuticle.

DISCUSSION

The most likely cause of color in male C. maculata is constructive interference by thin films or lamellae in the epicuticle. When the angle of viewing the cuticle is changed from grazing to normal incidence the color progressed from shorter wavelength blues to longer wavelength greens as predicted (Onslow, 1921; Mason, 1927). Swelling the cuticle lengthened wavelengths while the converse was true for compressed cuticle. The direction of these color changes in addition to their reversibility is also indicative of constructive interference colors (Mason, 1927).

It is unlikely that C. maculata color is the result of Tyndall effects (color produced via scattering of light by particles), as the positional color changes are uncharacteristic of Tyndall blues. Also, the green color would require the presence of a yellow pigment (Neville, 1975) which was not detected in the chemical tests. Diffraction gratings are also unlikely to be the major source of color in male C. maculata, since the color does not dim in diffuse light nor disappear at certain angles of viewing (Hinton, 1976).

The source of the structural colors in male C. maculata is the lamellate epicuticle. Abdominal fragments treated with 8% KOH to remove the epicuticle exhibited a loss of color. Bleaching in 10% H_2O_2 did not alter the hue but reduced the intensity of color. This dulling of color with bleaching likely indicates the presence of melanin as an absorbent background to the interference reflector (Mason, 1927; Schultz and Rankin, 1985).

The alternate layering of electron lucent and electron dense materials in the epicuticle as indicated by the TEM's is characteristic of a multilayer interference reflector. Other studies of multilayer interference reflectors in insects suggest that the system is composed of electron dense melanin layers separated by chitin (Durrer and Villiger, 1972; Schultz and Rankin, 1985). This may also be the case for C. maculata; however, it is difficult to detect small amounts of melanin. The brown-stained solution from dissolving the epicuticle in dilute KOH may indicate the presence of melanin in the epicuticular layers, however, melanin may also have been leached from the exocuticle (Richards, 1967; Schultz and Rankin, 1985).

If we assume that the multilayer interference reflector consists of layers of chitin and melanin, with refractive indices of 1.5 and 2.0 respectively, the wavelength of peak reflectance such a system can be calculated with the formula:

$$\lambda_{max} = 2\ (n_L d_L + n_D d_D)$$

where λ_{max} is the wavelength of peak reflectance, n_L and n_D refer to the refractive indices of the electron lucent and electron dense layers respectively while d_L and dD refer to the thicknesses of the electron lucent and electron dense layers respectively (Neville, 1975; Schultz and Rankin, 1985). Calculating the wavelength using refractive indices of 1.5 and 2.0 and mean thickness for the electron lucent and electron dense layers of blue and green cuticles respectively yielded wavelengths of 512nm for blue males and 552nm for green males. These numbers closely correspond to the wavelengths reflected by these males as indicated by the field comparisons with Munsell Chips. In the field, 'blue' males reflected normal incidence wavelengths of 520.1nm ± 7.7 SD (range: 509nm to 525.5nm) while 'green' males reflected normal incidence wavelengths of 550.7nm ± 2.3SD (range: 550nm or 558nm).

According to color quantification with Munsell Chips, blue males had shorter wavelength coloration that green males. Due to positional color changes characteristic of interference colors described earlier, future work will refer to ‘blue’ *C. maculata* as short wavelength males and ‘green’ *C. maculata* as long wavelength males.

The present study is the first to address color production and color change in a Calopterygid damselfly. This study also presents the first indication of a multilayer constructive interference reflector in a damselfly epicuticle. Tyndall effects are responsible for the blue colors of Coenagrionid damselflies (Mason, 1926; Charles and Robinson, 1981) as well as the colors of the following dragonflies: *Anax*, *Erythemis*, and *Libellula* (Mason, 1926). The current field evidence indicates that male *C. maculata* change color from blue to green as they age, suggestive of swelling of the epicuticular lamellae responsible for color production. The exact mechanism and significance of color change with age in this species will be discussed in subsequent chapters.

CHAPTER 2

DECREASING FAT LEVELS WITH AGE RESULT IN COLOR CHANGE IN MALE *CALOPTERYX MACULATA*

INTRODUCTION

Males of the damselfly Calopteryx maculata appear bright blue or green as a result of constructive interference by thin epicuticular lamellae (see Chapter 1). Transmission electron microscopy indicates that short wavelength colors (512nm) are produced by epicuticle with thin lamellae while longer wavelength colors (552nm) are produced by thicker lamellae in the epicuticle. In the field, males with shorter wavelength coloration (509-525nm) are younger than males with longer wavelength coloration (550-559nm).

Young male C. maculata have higher fat levels and are more likely to win territorial contests than are old male C. maculata (Forsyth and Montgomerie, 1987; Marden and Waage, 1990). Fixed body size at emergence, combined with the energetic demands of territoriality and reduced foraging time of territorial males compared to nonterritorial males results in energy depletion and declining resource holding potential with age (Forsyth and Montgomerie, 1987).

The relationship between age and color, and age and fat or energy reserves suggests that declining fat levels with age may result in the observed change in the wavelength of male coloration. The objective of this study was to examine the relationship between fat levels and male color and to discern the relative importance of fat versus age for color change. I met this objective by manipulating the fat reserves of same age/color males and monitored the rate and degree of color change.

MATERIALS AND METHODS

From 15 July to 15 August 1993, I collected male *C. maculata* from a stretch of Augusta Creek in the Kellogg Forest approximately six miles from the Kellogg Biological Station in Southwestern Michigan. I collected only those males with minimal wing wear and that were similar in size and color. After netting, I measured each male's forewing length and assessed its color by comparison with Munsell Color Chips at normal incidence to the cuticle surface (Munsell 1976a). Damselflies were maintained in the laboratory facilities at the Kellogg Biological Station. I housed males individually in quart-sized glass jars, placed on their sides, and with fine mesh screen covering the mouth of the jar. The bottom of each jar was covered with thick wettened filter paper. I randomly assigned males to one of two treatment groups to determine the effects of high or low food consumption on color change. I fed each male individually two times per day by presenting live *Drosophila melanogaster* to them with a forceps (Hinnekint, 1987). I fed males in the low food treatment two *D. melanogaster* per feeding (4/day) and males in the high food treatment , five *D. melanogaster* per feeding (10/day). Four *D. melanogaster* is the lowest number of prey that will maintain *C. maculata* in the laboratory for at least 10 days (Fitzstephens, unpubl.). Five *D. melanogaster* is the largest number of prey that *C. maculata* can comfortably consume at one feeding without force feeding (Fitzstephens, unpubl.).

During the first feeding bout of each day, I assessed each male's color by comparison with Munsell Chips at normal incidence to the cuticle surface. Munsell color notation was later translated to a wavelength (Munsell 1976b). After 10 days of feeding, I dissected the males for assessment of fat and water content. I determined fat content by subtracting lean dry body mass from dry body mass, dividing by dry body mass and multiplying by 100%. Lean dry body mass was determined following fat extraction for four hours with refluxing chloroform (Marden 1989; Marden and Waage 1990). A measure of percent water that was independent of fat content was determined by subtracting dry mass from fresh mass, dividing by lean fresh mass and multiplying by 100% (Marden and Chai, 1991).

RESULTS

A total of 55 male C. maculata were captured and maintained in the laboratory as described above. At the start of the experiment, the 25 males assigned to the high-food treatment and the 30 males assigned to the low-food treatment did not differ with respect to size or the wavelength of color (Table 2). Over the 10 days on their respective diets, low-food males experienced a significantly greater positive change in the wavelength of color reflected than high-food males (Table 3). This resulted in the low-food males exhibiting significantly longer wavelength coloration than high-food males at the end of the study (Table 3). Low-food males also changed color faster than high-food males. High-food males retained their initial color almost twice as long as low-food males (Table 3).

High-food males had a significantly higher fat but not water content than did low-food males (Table 4). The values of percent fat content reported here for high-food and low-food males are similar to those reported elsewhere for short wavelength males (6.7% ± 2.0SD) and long wavelength males (4.4% ± 1.6SD) respectively (see Chapter 3).

Table 2. Mean wing length and initial wavelength of color for high food and low food male Calopteryx maculata.

Male	N	Wing Length (mm ± SD)	Initial Wavelength (nm ± SD)
High food	25	29.8 ± 1.1	517 ± 8.4
Low food	30	29.6 ± 0.7	517 ± 8.4
Statistic	df=53	t=0.91ns	t=0.14ns

Table 3. Mean latency to change color, final and change in wavelength for high food and low food male Calopteryx maculata.

		Latency (days ± SD)	Wavelength (nm ± SD)	
Male	N	to change	Final	Change
High food	25	6.9 ± 2.6	526 ± 15.5	9 ± 11.0
Low food	30	3.5 ± 2.1	539 ± 12.4	22 ± 6.5
Statistic	df=53	t=5.31***	t=3.54**	t=5.73***

** $P < 0.001$, *** $P < 0.0001$

Table 4. Mean fat and water content for high food and low food male Calopteryx maculata.

Male	N	%Fat (± SD)	%Water (± SD)
High food	25	7.3 ± 2.2	7.5 ± 2.0
Low food	30	4.1 ± 2.7	7.4 ± 2.0
Statistic	df=53	t=4.68***	t=0.18ns

*** P < 0.0001

DISCUSSION

The relationship between diet and color has been studied in other odonates. McVey (1985) demonstrated that in males of the dragonfly Erythemis simplicicollis, decreasing food consumption decreased the rate of male color maturation. McVey's study examined color change from juvenile to mature color. The present study, which examines color change from young mature male C. maculata to old males, indicates an increasing rate of color change with decreasing food consumption.

Fat levels are more important than age for determining the wavelength of color reflected by male C. maculata. Males that were maintained on a high food diet had a higher percent body fat than males maintained on a low food diet. These males with higher percent body fat experienced a significantly smaller color change than similar age males with lower percent body fat. The high fat males retained their initial color almost twice as long as did low body fat males.

The decrease in percent body fat must increase the lamellar spacing in the epicuticle to result in the observed color change from blue to green. The initial high percent body fat may compress the lamellae thereby resulting in the reflection of shorter wavelength blues. A decrease in body fat releases that pressure causing the lamellar spacing to widen and reflect the longer wavelength green color. Calopteryx maculata exoskeleton is malleable compared to other insects exhibiting constructive interference colors and is likely to be susceptible to these slight changes in internal pressure (Schultz, pers. com.)

Marden and Waage (1990) also concluded that fat or energy is more important than age for determining the winners of aggressive contests in male C. maculata. They demonstrated that differences in fat or energy reserves are an important asymmetry between male C. maculata engaging in wars or attrition or escalated territorial contests. Young territorial males had more body fat than old territorial males and winners of fights had significantly more body fat remaining at the end of contests than did losers of fights (Marden and Waage, 1990). Marden and Waage concluded that the ability of relative age to predict relative energy was less than the observed ability of relative energy to predict winners of contests. The authors did not; however, find evidence of assessment of fat reserves in male C. maculata. The relationship presented here between fat reserves and the wavelength of color reflected by male C. maculata suggests that color might provide an index which males may use to assess each others fat levels.

CHAPTER 3

COLOR RELIABLY SIGNALS FIGHTING ABILITY IN MALE <u>CALOPTERYX</u> <u>MACULATA</u>

INTRODUCTION

This chapter investigates the role of color as a signal of male resource holding potential (RHP) in a natural population of Calopteryx maculata.

The presence of territorial and nonterritorial males has been well documented in C. maculata populations (Waage, 1972; Forsyth and Montgomerie, 1987). Nonterritorial, sneaker, males are previously successful territorial males that have adopted an alternative strategy later in their lifetime as their resource holding potential decreases (Forsyth and Montgomerie, 1987). Male C. maculata often engage each other in aerial contests, a primary mechanism for turnover in territory ownership (Marden and Waage, 1990). Marden and Waage (1990) established that in C. maculata populations, winners of contests have significantly more fat (energy reserves) than do losers and that young territorial males have higher fat levels than old territorial males. They conclude that fat content or energy reserves were more often correlated with winning contests than size or physical attributes related to fighting ability. Forsyth and Montgomerie (1987) hypothesized that fixed body size at emergence, combined with the physical and energetic demands of territoriality and the reduced foraging time of territorial males compared to nonterritorial males results in energy depletion and declining resource holding potential with age.

Male C. maculata exhibit constructive interference colors on their thorax and abdomen that change from blue to green with age (see Chapter 1). Munsell color assessment indicates that ‘blue’ males exhibit short normal incidence wavelengths that range from 509nm to 525nm while ‘green’ males exhibit longer normal incidence wavelengths that range from 550nm to 558nm. To avoid confusion, all color assessments will refer to normal incidence and 'blue' males will be referred to as 'short-wavelength' males while 'green' males will be referred to as 'long-wavelength' males. Transmission electron micrographs of the cuticle of male C. maculata indicate that the increase in wavelength results from an increase in epicuticular lamellar spacing which may be related to fat content or energy reserves. Male C. maculata maintained in the laboratory on a high food diet had significantly higher percent fat and exhibited a significantly smaller color change than males on a low food diet (see Chapter 2). High-food males also retained their initial color almost twice as long as low-food males.

In this study, I investigated the relationships among male C. maculata age, size, energy reserves and color and how these variables affected male territorial behavior and mating success in the field. I then use this information to evaluate the potential for color to act as a useful signal for assessment of male fighting ability in this species.

MATERIALS AND METHODS

The study site consisted of a 100m stretch of Augusta Creek approximately three miles from the Kellogg Biological Station in Southwestern Michigan, USA. From 16 June through 20 August of 1992 and 1993, I collected data on sunny or partly cloudy days by netting male C. maculata, measuring their forewing length (Southwood 1968), and individually marking them. I censused the population daily at 10:00, 13:00, and 16:00 by walking the length of the study site and capturing male C. maculata. During each census, I marked all animals not previously seen on the study site and mapped the location of all territorial and nonterritorial males. As described in Chapter 1, male age refers to the number of days elapsed since an individual was first marked at the stream (Forsyth and Montgomerie, 1987). Territorial males were those that perched on or near floating oviposition material and defended these sites by chasing away intruding males. Nonterritorial males were those that were highly mobile, visiting locations on the stream up to 100m apart on a single day. Nonterritorial males did not defend specific locations along the stream and were often chased away from oviposition sites by territorial males (Forsyth and Montgomerie, 1987).

Every day, I captured males once for assessment of color at normal incidence by comparison with Munsell Color Chips (Munsell, 1976a). Munsell color notation was later translated into a wavelength (Munsell, 1976b). Other data collected ad libitum between census times included the location, outcome and identity of males observed in copulatory attempts. During copulation, the male mounts the female from above forming the tandem position. Copulation typically lasts one to two minutes after which the female initiates oviposition (Waage, 1973). I only recorded as successful copulations those interactions in which the complete sequence of events occurred. I recorded an unsuccessful copulation when females were successful in actively refusing males using wing flaps or evasive flights.

I collected a sample of 30 short-wavelength and 30 long-wavelength males for analysis of fat and water content. After assessing each male's color with Munsell Chips, I determined percent body fat and percent water as described in Chapter 2.

Finally, I utilized path analysis (Li, 1981) to investigate hypothetical causal relationships among the variables. Path analysis is essentially a series of multiple regressions and correlations structured by prior knowledge of the biological system and can be used to predict important interactions (Wootton, 1994). The relationships among variables in a system are represented by a path diagram based on knowledge of the biology of the system (Li, 1981; Kingsolver and Schemske, 1991). In a path diagram, single-headed arrows reflect a causal relationship between two variables while double-headed arrows represent correlations without causation. The path coefficient indicates the strength of association of two variables by providing a relative measure of the amount of variation explained by each causal variable and the sign of the interaction. Path coefficients are estimated as the partial regression coefficient standardized by the ratio of the standard deviations of the independent and dependent variables (Li, 1991; Wootton, 1994). Path coefficients represent the direct contribution of the predictor variables to the predicted variables, holding other variables constant. The degree to which the variation in a variable is accounted for can be calculated by summing the products of each path coefficient with the respective variables' correlation coefficient (Li, 1981).

A single path diagram representing causal relationships among variables can be analyzed to estimate the relative strengths of interactions among variables. Path analysis also distinguishes direct from indirect effects. Conclusions based on this type of analysis are to be treated as predictions that indicate experiments to be conducted rather than as conclusions in themselves (Wootton, 1994).

Prior to this section of the study, relationships among variables are discussed in terms of pairwise comparisons. These relationships can be organized into a coherent account of C. maculata territorial and mating behavior using path analysis. This will allow for the partitioning of direct versus indirect interactions while eliminating from the analysis variables with no significant direct effects on other variables. In this chapter, path analysis will be used to determine the relative strengths of the biological interactions depicted in Figure 3. Figure 3 represents the positive and negative relationships among variables important for *C. maculata* territorial and mating behavior as discussed prior to my study. I will incorporate my data into a path analysis of these interactions to factor out insignificant variables and to determine the relative strengths of the remaining interactions for predicting male *C. maculata* territorial and mating success. I used information of *C. maculata* natural history to construct the interaction diagram as a starting point for path analysis. I included paths in the path diagram only if the partial regression in the underlying multiple regression was statistically different from zero. The path analysis will demonstrate the relationship between color and the measures of body condition important in *C. maculata* contests as well as the degree to which color contributes to our understanding of territorial behavior.

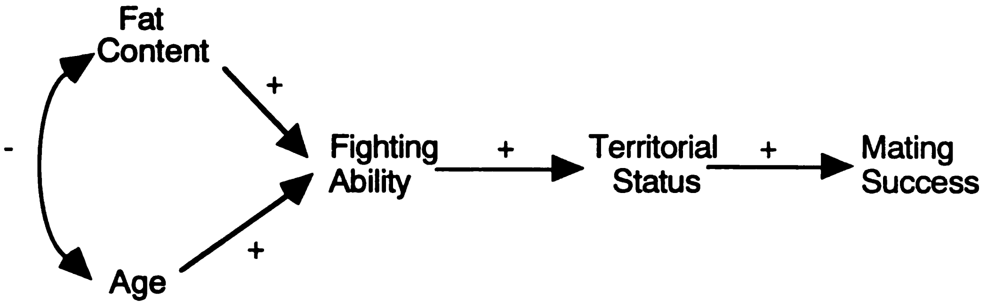

Figure 3. Interaction diagram constructed using information from Calopteryx maculata natural history. This diagram is to be used as a starting point for path analysis.

RESULTS

I marked a total of 932 individual male C. maculata during the two year study for a total of 2008 animal observations (Figure 4). The size of individuals at first capture, as indicated by forewing length, declined throughout the season (Kendall's tau = -0.22, $P < 0.0001$). This negative relationship between size at marking and day of season is common in odonate studies (Harvey and Walsh, 1993; Tsubaki and Ono, 1987; Banks and Thompson, 1985; Harvey and Corbet, 1985). This seasonal effect accounted for a small percentage of the variation in size ($r^2 = 11.6\%$). Since the size of C. maculata is fixed at emergence (Forsyth and Montgomerie, 1987), the seasonal change in emergence size sets up a pattern of older males (those that emerged early in the season) being larger than younger males (those that emerged later in the season). Therefore, size (the physical dimensions of the damselfly) should not be confused with fat content which is variable and changes throughout an individuals lifetime. In addition to size and fat content, fresh body mass will be reported in this study. However, the results involving body mass should be interpreted with caution as body mass incorporates both body size (which is static) and body fat (which is dynamic). Since the statistical analyses yielded similar results for each year analyzed separately as well as combined, we report the results for 1992 and 1993 combined. Territorial males were significantly smaller in body size and younger than nonterritorial males (Table 5). Despite the statistical significance, the differences in size as measured by wing length are probably not biologically meaningful. The sample sizes were very large thus exaggerating the significance of the very small differences in wing length. Also, the size differences are most likely the result of the seasonal decline in emergence size. Territorial males also reflected shorter wavelength colors than did nonterritorial males (Table 5). Of the 143 males exhibiting both territorial and nonterritorial behavior during their lifetime, all began as territorial males and later switched to nonterritorial.

I observed a total of 275 mating attempts involving marked males during the study. Significantly more mating attempts by territorial males were successful (203 of 210) than were mating attempts by nonterritorial males (1 of 65, $\underline{X}^{\underline{2}}$ = 229.6, df = 1, $P < 0.001$). Successful males were smaller and younger than unsuccessful males (Table 6). Again, the relationship with size is most likely the result of the size/emergence day pattern. Successful males also reflected shorter wavelength coloration than unsuccessful males (Table 6).

Wavelength was positively correlated with damselfly size (Kendall's tau = 0.10, $P < 0.01$) as well as with age (Kendall's tau = 0.29, $P < 0.0001$). As damselflies aged, their color changed from short wavelength to longer wavelength. Since size is fixed at emergence, the positive relationship between size and color is due to the fact that older (and therefore larger) males reflect longer wavelengths. I analyzed a sample of 30 short wavelength males and 30 long wavelength males for fat and water content. The mean body mass of short wavelength males (65.24mg ± 5.25SD) was slightly but significantly smaller than that of long wavelength males (69.03mg ± 5.94SD; Mann-Whitney $\underline{U}$ = 291, $P < 0.05$). The mean fat content of short wavelength males (6.7% ± 2.0 S.D.) was significantly higher than that of long wavelength males (4.4% ± 1.6 S.D., Mann-Whitney $\underline{U}$ = 764, $P < 0.0001$). Short wavelength males also had a significantly higher water content (7.5% ± 2.1 S.D.) than did long wavelength males (7.3 % ± 1.4 S.D., Mann-Whitney $\underline{U}$ = 710, $P < 0.001$). Short wavelength males have a smaller body size and lower body mass than long wavelength males. The relationship between wavelength and body size reflects the age/size relationship established by the trend of decreasing emergence size

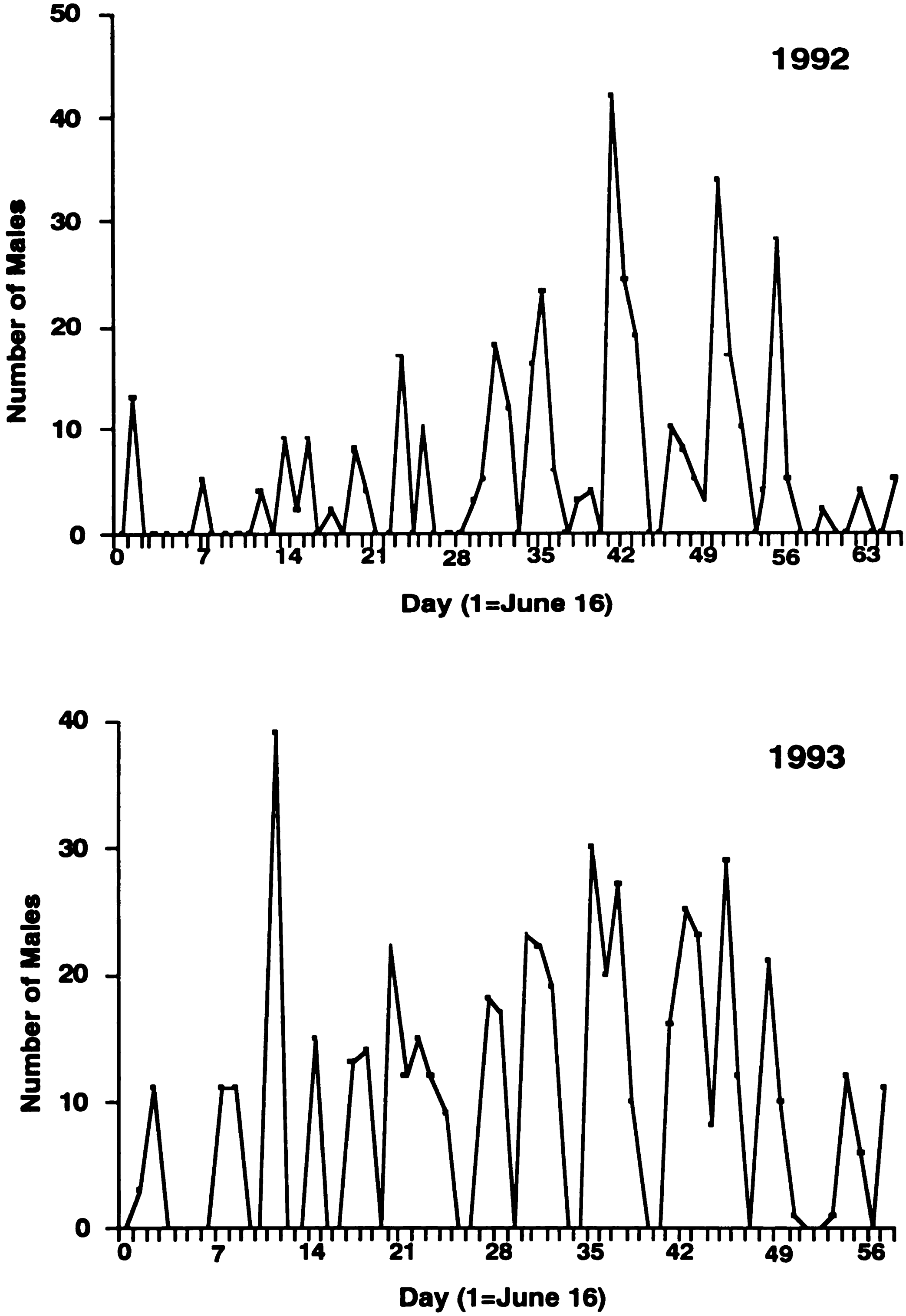

Figure 4. The number of individual male <u>Calopteryx</u> <u>maculata</u> marked on each day of the study.

Table 5. Mean (± SD) size (forewing length), age, and wavelength of territorial versus nonterritorial male *Calopteryx* *maculata*.

Male	Size (mm)	Age (days)	Wavelength (nm)
Territorial (N=618)	29.1 ± 1.1	1.5 ± 2.8	520.2 ± 8.1
Nonterritorial (N=171)	29.4 ± 1.1	5.0 ± 6.5	550.5 ± 3.0
Mann-Whitney *U*	$1.1x10^5$ **	$1.3x10^5$ ***	$1.9x10^5$***

** $P < 0.001$, *** $P < 0.0001$

Table 6. Mean (± SD) size (forewing length), age, and wavelength for successfully mated versus unsuccessful male *Calopteryx maculata*.

Male	Size (mm)	Age (days)	Wavelength (nm)
Successful (N=210)	29.4 ± 0.9	1.9 ± 3.6	523.6 ± 10.8
Unsuccessful (N=65)	29.6 ± 1.0	6.0 ± 7.1	548.2 ± 11.2
Mann-Whitney *U*	8.4×10^3 *	10.0×10^3 **	1.3×10^4 **

* $P < 0.05$, ** $P < 0.001$

with day of season. Body mass is also confounded by this relationship since it incorporates body size as well as fat content.

Path analysis was used to create a model depicting the hypothesized causal relationships among all variables relative to mating success. Figure 5 represents the diagram that best explains the variation in territorial and mating behavior. This diagram represents a 20% increase in explanatory power over the many alternative path diagrams created from the data. This path diagram will also be used to determine the relative strengths of the interactions depicted in Figure 3. The hypothesis predicts that mating success is determined primarily by male territorial status. No other variables measured accounted for a significant portion of the variation in mating success according to this path diagram. Male territorial status was determined by wavelength. Age, fat and water content directly contributed to male color; wavelength increased as age increases and as fat and water decrease. Table 7 shows the correlations and path coefficients used for determining the direct contributions of variables. The total contribution of fat and water content and age toward wavelength can be calculated by multiplying each path coefficient with the respective variable's correlation with wavelength and summing over all three variables (Li, 1981). This calculation indicated that age and fat and water content accounted for 54.5% of the variation in the wavelength of male color. By the same argument, wavelength accounted for 81.0% of the variation in male territorial behavior which in turn accounted for 84.6% of the variation in mating success (Figure 5). If we omit wavelength as a variable, the resulting path coefficients from age, fat and water content to territorial status are much lower than the paths between age, fat and water content to wavelength in the previous path diagram (Figure 6). These three variables directly contribute to only 39.1% of the variation in male territorial status compared to their 54.5% determination of wavelength and to the 81.0% determination of territorial status by wavelength alone (Figure 6). Therefore, path analysis indicates that age, fat and water content are better predictors of color or wavelength than they are of territorial status directly. Color or wavelength is a better indicator of male territorial status than are age, fat and water content combined. The variables ‘day of season’, ‘year’, ‘body mass’ and ‘forewing length’ are not represented on the diagram because they had no significant effects on the other variables (i.e. the partial regression in the underlying multiple regression was not significantly different from zero).

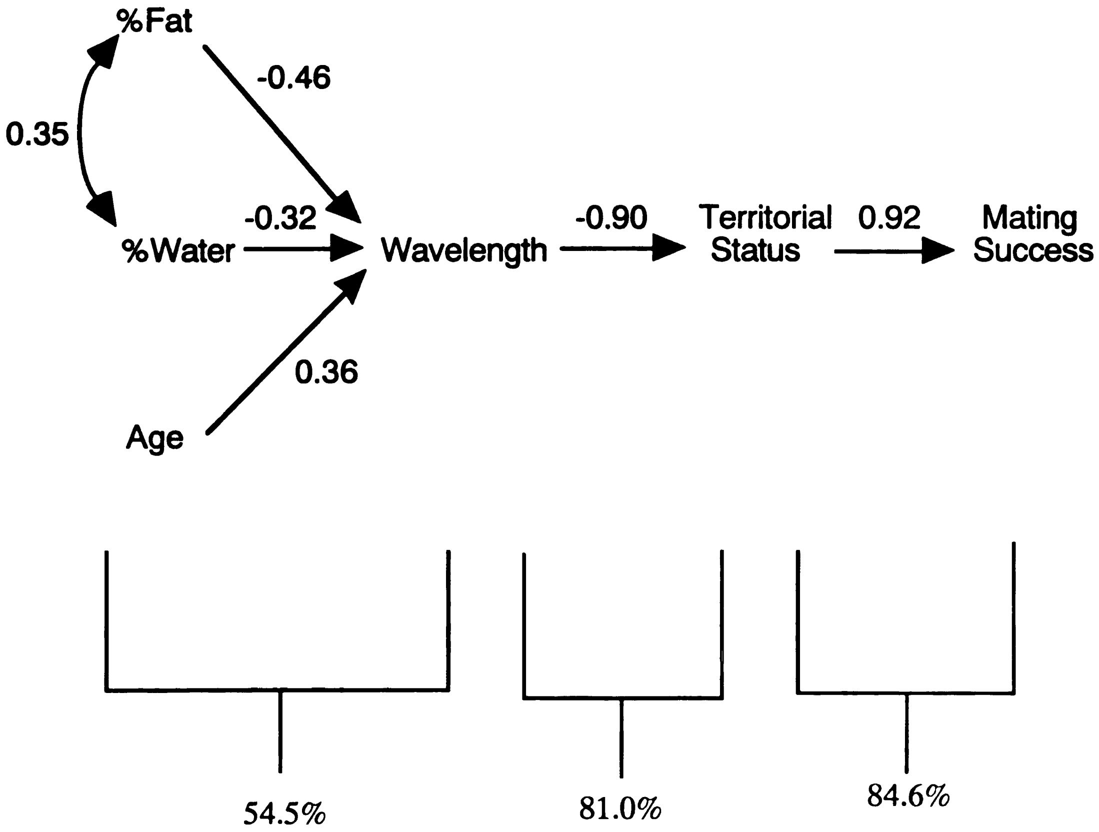

Figure 5. Path analysis of the relationship of the predictor variables (age, %fat, % water, and wavelength) with male territorial status and mating success. This path diagram represents an hypothesis of causation for evaluation. Single-headed arrows represent a causal relationship between two variables while double-headed arrows denote correlations without causation. The degree to which the variation in a variable is accounted for (indicated below diagram) can be calculated by summing the products of each path coefficient with the respective variables' correlation coefficient (Li, 1981).

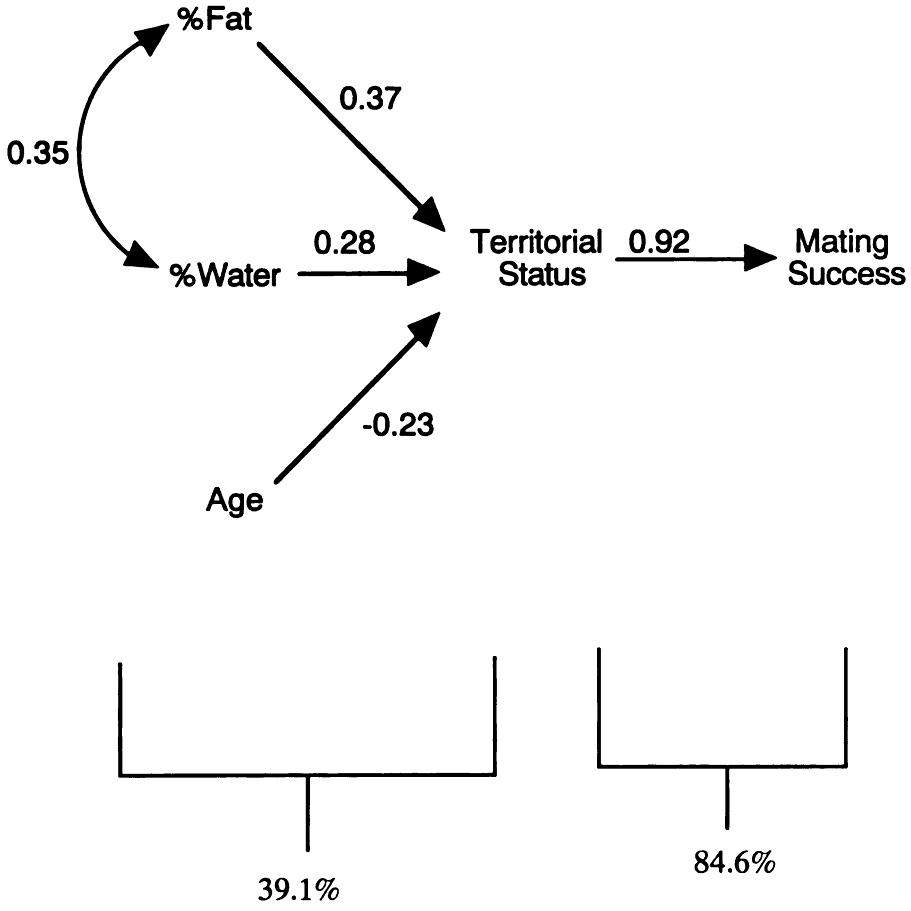

Figure 6. Alternative path diagram resulting from the exclusion of wavelength as a predictor variable.

Table 7. Correlation and path coefficients for calculation of the contributions of predictor variables to predicted variables in Figures 5 and 6.

Path	Correlation	Path Coefficient
Territorial Status to Mating Success	0.92	0.92
Wavelength to Territorial Status	-0.90	-0.90
%Fat to Wavelength	-0.57	-0.46
%Water to Wavelength	-0.48	-0.32
Age to Wavelength	0.36	0.36
%Fat to Territorial Status	0.51	0.37
%Water to Territorial Status	0.44	0.27
Age to Territorial Status	-0.36	-0.23

DISCUSSION

Territorial male C. maculata were smaller in terms of both size and body mass, younger and more successful during mating attempts than nonterritorial males. Marden and Waage (1990) concluded that size and mass are not important in damselfly aggressive interactions. Path analysis of the data supported this conclusion. That territorial males were smaller than nonterritorial males is simply be an artifact of the negative relationship between day of emergence and hence, age, with size. That territorial males are younger than nonterritorial males, and that the 143 males exhibiting both territorial and nonterritorial behavior began as territorial and switched to nonterritorial behavior, is similar to other examples of age related behavior patterns in odonates (Tsubaki and Ono, 1987; Campenella and Wolf, 1974). Forsyth and Montgomerie (1987) also demonstrate that in C. maculata the probability of adopting an alternative reproductive behavior such as sneaking increases with male age.

Successfully mated male C. maculata are younger and smaller than unsuccessful males. This is most likely because females mate almost exclusively with territorial males, which tend to be younger and smaller than nonterritorial males. Territorial male C. maculata are able to control female access to oviposition sites, exchange matings for guarding (Waage, 1979; 1982) and exclude nonterritorial males.

This study demonstrates an age-related color change that is also related to territorial behavior in male C. maculata. Territorial males exhibited significantly shorter wavelength coloration than nonterritorial males. Wavelength was also shown to increase with both size, body mass and age. Again, the relationship with size is most likely an artifact of the relationship between size and day of marking resulting in older males being larger than younger males. Since size is a component of mass, body mass is also confounded by the size/emergence day relationship. Therefore, as C. maculata males age, their color changes from short to long wavelength and many switch from territorial to nonterritorial behavior.

The importance of fat or energy reserves in male C. maculata aggressive interactions has been suggested in a number of studies (Forsyth and Montgomerie, 1987; Marden and Waage, 1990). Marden and Waage (1990) show that winners of escalated contests among male C. maculata have significantly more fat or energy reserves than losers. They also show that young territorial males have significantly higher fat levels than old territorial males. The present study indicates that the wavelength of male color is also related to fat, as well as water content in this species. Short wavelength males had a significantly higher fat and water content per unit body mass than long wavelength males.

Path analysis of the hypothesized causal relationships among the variables predicts that male C. maculata mating success is affected most directly by male territorial status (Waage, 1973, 1979). Male territorial status is affected most directly by male wavelength. The variables age, fat and water, which have previously been shown to vary with male territorial and aggressive behavior (Forsyth and Montgomerie, 1987; Marden and Waage, 1990), make their greatest direct contribution to male wavelength variation rather than to male territorial behavior directly. The variables day of season, male size (forewing length), body mass, and year do not make a significant direct contribution to any other variables. These variables may affect the predicted variables but only through their relationships with the other more important predictor variables of age, fat and water.

Path analysis predicted that age, fat and water content are better indicators of color or wavelength than they are of territorial status directly. Color or wavelength is a better indicator of male territorial status than are age, fat and water content combined. Path analysis has organized the many pairwise comparisons of this and previous studies into a coherent picture of male C. maculata territorial and mating behavior. Path analysis has indicated the relative importance of variables having significant direct effects on territorial and mating behavior (age, fat and water content) while eliminating from the analysis the variables whose affects occur only through their interaction with other more important variables (wing length, body mass, day of season, year). Color is reliably related to the measures of body condition (energy reserves) which have been shown to affect the outcome of male C. maculata interactions. Age and energy reserves account for 54.5% of the variation in wavelength which in turn accounts for 81.0% of the variation in territorial status. Without wavelength, our accounting of the contributions to territorial status is only 39.1%. Thus, wavelength greatly increases the ability to predict male territorial status. The high predictability of territorial status from wavelength alone (81.0%) suggests that color is a reliable signal or index of fighting ability. Color may be used to assess energy reserves while the significant positive relationship between wavelength and fat content maintains a level of reliability or honesty in the signal. Future research must focus on testing whether or not C. maculata make use of the information provided by color.

SUMMARY AND CONCLUSIONS

The exchange of information via signals is often a means by which opponents in agonistic encounters can assess each other's resource holding potential or fighting ability. I investigated the role of color as a signal of fighting ability in males of the territorial damselfly Calopteryx maculata. I accomplished this by elucidating the mechanism of color production and color change to reveal the kinds of information provided by color. I also conducted field observations to establish the relationship of color with territorial and mating behavior.

In my study, I was able to demonstrate that the bright blue and green colors of male C. maculata are caused by constructive interference by thin lamellae in the epicuticle. The lamellae consist of alternating layers of electron lucent chitin and electron dense melanin. Male C. maculata change from blue to green as they age. Fat levels; however, are more important than age for determining the wavelength of color reflected. This is the first study to demonstrate a multilayer interference reflector in an odonate cuticle. It is also the first study to address the role of color and color change in a natural population of C. maculata.

Field data and path analysis indicate that territorial male C. maculata are younger, have higher percent body fat, and are more successful at mating than nonterritorial males. These results are similar to those reported in other studies of C. maculata. What is new and exciting about the present study is that color, a variable previously unmeasured in C. maculata populations is revealed to be the best predictor of territorial behavior, with territorial males reflecting shorter wavelength color than nonterritorial males. Furthermore, color is shown to be reliably related to the measures of body condition (fat or energy reserves) known to affect the outcome of C. maculata aggressive interactions.

These results indicate that color provides reliable information by which male C. maculata can assess each other's fat levels or energy reserves as they indicate fighting ability. What remains to be shown is the extent to which males utilize the information provided by color.

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