25

Visual signals: color and light production

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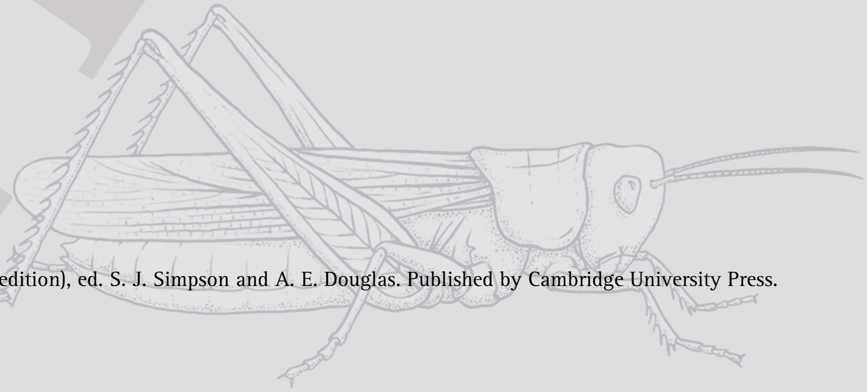
INTRODUCTION

Insects generate a spectacular variety of visual signals, from multicolored wing patterns of butterflies, through metallic-shiny beetles to highly contrasting warning coloration of stinging insects and their defenseless mimics. Section 25.1 explains what colors are and the subsequent sections describe how insect colors result from a variety of physical structures (Section 25.2) and pigments (Section 25.3). Often, several pigments are present together, and the observed color depends on the relative abundance and positions of the pigments, as well as control signs generating color patterns (Section 25.4). The position of color-producing molecules relative to other structures is also important, and this may change, resulting in changes in coloration (Section 25.5). The many biological functions of color in insect signaling are covered in Section 25.6. Table 25.1 lists the sources of color in some insect groups. A small selection of insects also exhibit fluorescence or luminescence (Section 25.7).



The Insects: Structure and Function (5th edition), ed. S. J. Simpson and A. E. Douglas. Published by Cambridge University Press.

Table 25.1 **Some of the principal causes of colors in different insects**

Color	Taxon	Cause of color
Black*	Homoptera, Aphididae	Aphins
	Coleoptera	Melanin
	Diptera	Melanin
	Lepidoptera (larvae)	Melanin
Red	Odonata	Ommochromes
	Hemiptera, Heteroptera	Pterins and carotenoids
	Hemiptera, Coccoidea	Anthraquinones
	Coleoptera, Coccinellidae	Carotenoids
	Diptera, Chironomidae (larvae)	Porphyrin
	Lepidoptera	Ommochromes
Brown*	Lepidoptera	Ommochromes
	Many orders – eye colors	Ommochromes + pterins
Orange	Hemiptera, Heteroptera	Pterins
Yellow	Orthoptera, Acrididae	Carotenoids, flavonoids
	Hymenoptera	Pterins
	Lepidoptera, Papilionidae	Papiliochromes
Brassy yellow	Lepidoptera	Interference color
Bronze	Coleoptera, Scarabaeidae	Interference color
Gold	Coleoptera, Cassidinae	Interference color
	Lepidoptera, Danaidae (pupae)	Interference color
Green	Orthoptera	Insectoverdin (carotenoid + bilin)
	Lepidoptera (caterpillars)	Insectoverdin
	Lepidoptera, Zygaenidae	Interference color
	Diptera, Chironomidae (adults)	Bilin
Blue	Odonata	Interference color and ommochromes
	Lepidoptera	Interference color
	Orthoptera, Acrididae	Carotenoids
Ultraviolet	Lepidoptera, Pieridae	Interference color

Table 25.1 (*cont.*)

Color	Taxon	Cause of color
White	Hemiptera, Heteroptera	Uric acid
	Lepidoptera, Pieridae	Scattering + pterins
	Lepidoptera, Satyridae	Flavonoids
Silver	Lepidoptera, Danaidae (pupae)	Interference color
Iridescence	Coleoptera	Diffraction
	Coleoptera, Scarabaeidae	Interference colors

Note:
* Darkening may result from sclerotization.

25.1 The nature of color

Color is not an inherent property of objects; it is a perceptual attribute that depends on illumination, the spectral reflectance of an object and its surroundings, as well as the spectral receptor types and further neural processing in the animal in question. Thus the same object might appear differently colored to different viewing organisms. A red poppy, for example, is red to human observers, but appears as a UV-reflecting object to a bee pollinator, which does not have a red receptor and, like all insects studied to date, sees UV-A light between 300 nm and 400 nm. For reasons of simplicity, the color terminology in this chapter specifies what a human observer will perceive under daylight conditions. Information about UV is provided separately where available.

Color is generated from white light incident upon an insect when some of the incident wavelengths are eliminated, usually by absorption in its pigmentation, and the remainder are scattered. These scattered wavelengths of the reflected or transmitted components determine the color observed (Fig. 25.1). If all wavelengths are reflected equally then the reflecting surface appears white; if all are absorbed the appearance is black.

Differential reflection of light to produce color occurs in one of two ways: First, the physical nature of the surface may be such that only certain wavelengths are reflected, the remainder being transmitted; second, pigments may be present which, as a result of their molecular structure, absorb certain wavelengths and scatter the remainder. Colors produced by these methods are known, respectively, as physical (or structural) and pigmentary colors. In either case, the color observed is dependent on the wavelengths of light in which the object is viewed, usually sunlight or white light. The colors of insects are usually produced by structures or pigments in the cuticle or epidermis, but, if the cuticle is transparent, the hemolymph, fat body or even gut contents may contribute to the insect's color.

25.2 Structural colors

Various forms of surface and epidermal structures can be responsible for the production of whites and iridescent colors. They also assist in creating the appearance of many black surfaces. These colors may be produced by random scattering or by coherent scattering, such as by interference.

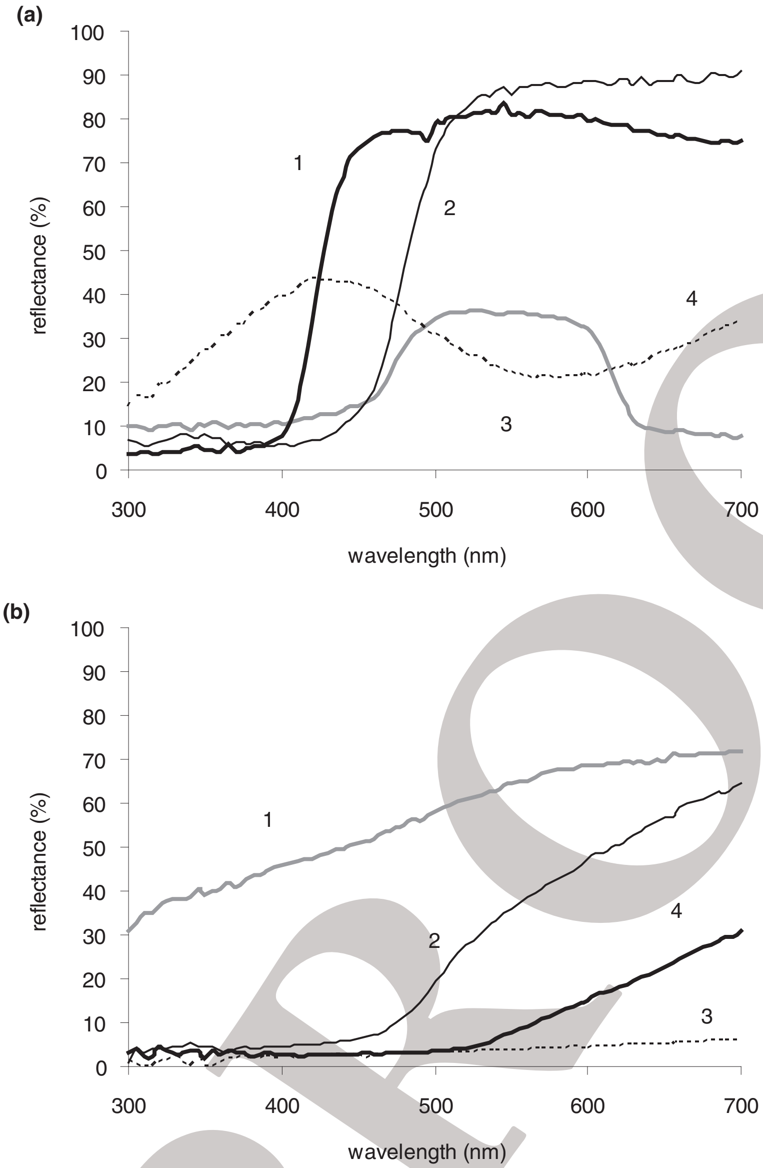


Figure 25.1 Representative reflectance spectra of insect body surfaces. Colors are produced by the absorbance of certain wavelengths, for example by pigments in the insect cuticle; wavelengths that are not absorbed may reach a viewer's eye via reflectance or transmission. (a) Reflectance spectra from butterfly male forewings: (1) the white (and UV-absorbing) wing of the European cabbage white, *Pieris rapae*; (2) the yellow brimstone, *Gonepteryx rhamni* (Europe); (3) the purple (and UV-reflecting) spots on the wings of the Malayan jungleglory, *Thaumantis odana*; and (4) green parts of the wings of the Southeast Asian swallowtail butterfly, *Papilio lorquinianus*.
(b) Reflectance of European bumble bee workers: (1) the white (and UV-reflecting) tip of the abdomen of the large earth bumble bee, *Bombus terrestris dalmatinus*; (2) the yellow band on the thorax of *B. t. dalmatinus*; (3) black parts of the abdomen of *B. t. dalmatinus*; (4) the brownish-red tip of the abdomen of the red-tailed bumble bee, *B. lapidarius*.

25.2.1 Scattering

Light may be scattered (i.e., reflected in all directions) by granules on, or irregularities in, a surface. If these scattering centers are randomly spatially distributed in or on the surface then virtually all incident light is reflected diffusely and the surface appears as a matt white. In some Lepidoptera, such as Pieridae, this results from the presence of densely packed arrays of pterin granules across each white scale's surface, the effect of which is coupled with that of the structure of the scale itself. In some Coleoptera, such as *Cyphochilus* sp., a bright, diffuse white appearance is generated by scattering from interconnected cuticular filaments that are randomly arranged within each white-colored scale. Pearly whites, such as those of *Morpho sulkowski* (Lepidoptera), are produced by the addition of a degree of specular (mirror-like) reflection in addition to the diffuse scattering.

25.2.2 Interference in multilayers

Interference colors result from the reflection of light from a series of neighboring interfaces that are separated by distances comparable with a quarter of the wavelength of light. As a result of wave superposition from these reflections, some

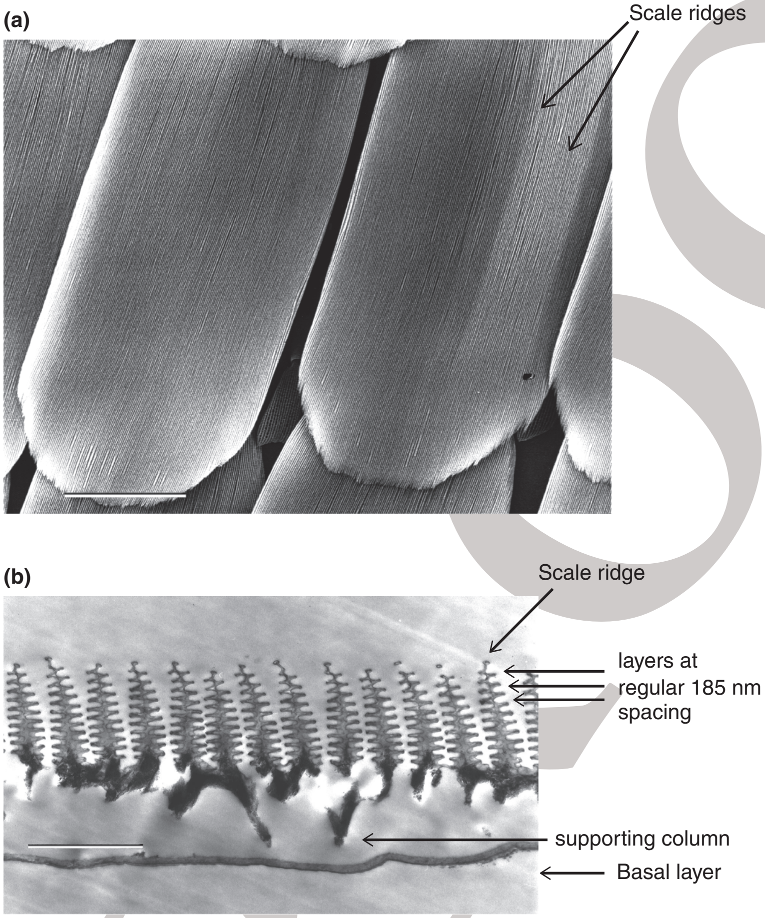


Figure 25.2 Interference color production in different insects. *Morpho* butterfly wing scales. (a) Scanning electron microscope image of two neighboring wing scales showing the ridging structures running the full length of each scale; scale bar = 50 μm. (b) Transmission electron microscope image of a cross-section of part of one of the scales; scale bar = 1 μm. The parallel horizontal layers of cuticle (with a regular spacing of 185 nm) and the air spaces between them are responsible for the interference of the reflected blue color. Notice that the supporting columns are irregularly arranged so only some are in the plane of the section (based on Vukusic *et al*., 1999).

wavelengths are reflected or transmitted in phase and are therefore reinforced, while others are out of phase and are cancelled out. The net result is that only certain wavelengths are reflected or transmitted and the surface appears colored.

Interference colors are common in adult Lepidoptera where the layers producing interference are formed by modifications of the scales. Each of the blue scales of the *Morpho rhetenor* butterfly, for instance, consists of a flat basal plate carrying a large number of near-parallel vertically aligned ridges that run parallel with the length of the scale (Fig. 25.2). Within each ridge are series of horizontal layers, separated by air spaces. Collectively, the horizontal layers in each adjacent ridge form a series of reflecting surfaces, which are spaced such that a blue color is produced by interference. Various optical appearances are produced by a great variety of scale modifications in many different Lepidoptera. For instance, strong ultraviolet reflectance at a peak wavelength of approximately 345 nm is generated in the butterfly *Colias eurytheme* by horizontal layered structures in its scale ridges that are 30–40 % thinner that those typical of iridescent *Morpho* butterflies.

Interference colors in other insects are produced by reflection at the interfaces of layers in the cuticle which differ in refractive index. The refractive indices of the alternating layers in the pupa of the danaid butterfly, *Euploea*, are 1.58 and 1.37. In jewel beetles (Buprestidae) and tiger beetles (Cicindellidae), these layers are in the exocuticle, but in tortoise beetles (Cassidinae) and some butterfly pupae they are in the endocuticle.

In some scarab beetles the reflecting surfaces are layers of chitin/protein microfibrils with a common orientation in the transparent exocuticle. Because their arrangement changes progressively in successive layers, creating helical orientation (Section 16.2), any given orientation recurs at intervals and, if the intervals are within the range of wavelengths of light, interference colors are produced. Due to this helical fibril orientation, the reflected light is circularly polarized (CP). The clockwise or anticlockwise orientation of fibrils through the exocuticle determines the right-handed or left-handed nature of the circular polarization. In the majority of cases, left-handed CP light is reflected from scarabs that exhibit this helical structure. However, in very few cases, *Plusiotis resplendens* for instance, both left- and right-handed CP light are reflected concurrently due to the presence of a multi-component helical structure.

Whatever the nature or position of the reflecting surfaces which form the layer interfaces, the wavelength of the reflected light depends on their spacing. Viewing the surface from a more oblique angle is equivalent to increasing the optical path length between successive surfaces so that the color changes in a definite sequence as the angle of viewing is altered. This change in color with the angle of viewing is called iridescence and is a characteristic of interference colors.

A constant spacing between the reflecting surfaces produces relatively pure colors (Fig. 25.3a), as in the scales of blue *Morpho* butterflies (Fig. 25.2), or the moths *Urania leilus* and *Chrysiridia rhipheus*. In the majority of insect multilayer examples, spectral purity is enhanced when the reflecting layers are backed by a layer of melanin-based pigment which offers broad-band absorption to the light that is transmitted through the multilayer. Color conspicuousness is often enhanced by the presence of a strongly black border surrounding the brightly colored area, particularly so in some butterfly and weevil color patterning.

Broad-band colors such as gold, silver and bronze, by contrast, are produced if the spacing changes systematically with increasing distance from the surface of the cuticle. For example, in the endocuticle of the pupa of *Euploea*, and the exocuticle of *P. resplendens*, the thickness of the paired layers changes systematically (Fig. 25.3b). This is referred to as a *chirped* layer structure. It produces interference over a broad range of wavelengths (Fig. 25.3b) and, in the cases of these insects, creates a gold mirror

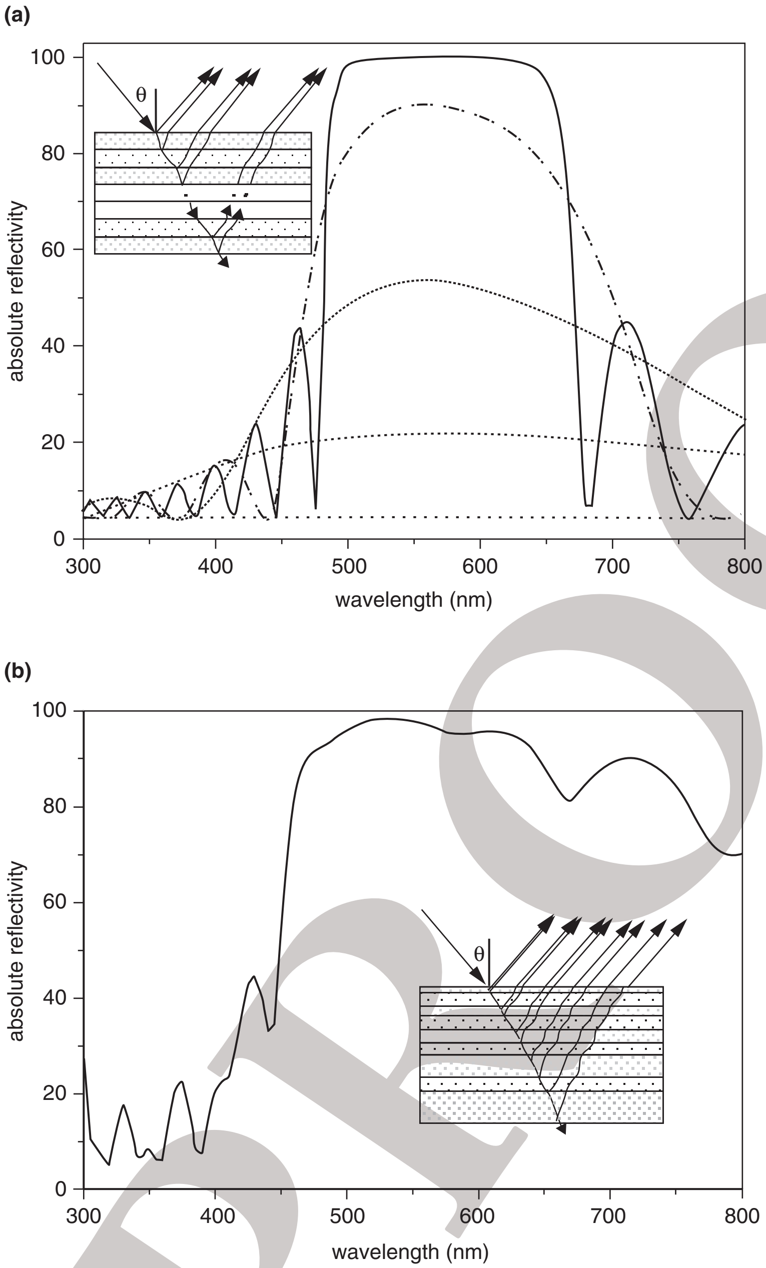


Figure 25.3 Theoretical modeling showing the spectral distribution of reflected intensities at multiple cuticular layers. (a) Reflected intensities from 1, 2, 3, 5 and 10 layers of cuticle, respectively, resulting in the increasing reflectances shown. In each case, air is the spacer layer and the normal incidence case is presented (dimensions used for modeling: cuticle layers 90 nm, and air layers 140 nm). Inset: a schematic diagram representing multiple reflections within a multilayer system leading to constructive interference. This multilayer comprises a single set of layer dimensions and this leads to a relatively narrow band of reflected wavelengths. (b) Reflection from a chirped multilayer (comprising ten cuticle layers of consecutively increasing thickness, spaced by air) producing the appearance of metallic gold. Inset: schematic diagram of multiple reflections in a chirped multilayer, showing the increasing thickness of cuticle layers through the system.

appearance to human eyes. Silver interference colors can be produced in the same way.

The peak reflected wavelength produced by interference, and also to some extent the brightness of the reflected colors, also depends on the refractive indices of the materials forming the layers. The horizontal layers in the ridges on blue *Morpho* wing scales are uniformly separated by approximately 120 nm. Together with the thickness of the layers themselves, approximately 80 nm, and the refractive index of the cuticle material, approximately 1.56, the system produces a bright blue interference color. The paired layers in the exocuticle of the scarab beetle *Heterorrhina* are of similar thickness, but the interference color produced is green due to the different refractive indices of the paired cuticular layers compared with the air–cuticle interfaces in *Morpho*.

The brightness of the reflected color significantly increases with the number of reflecting layers. Generally, the insects that exhibit higher layer numbers are brighter in appearance, although this additional brightness may be offset by the presence of absorbing pigment within the layers in the system. In *Morpho rhetenor*, for instance, there are 8–12 horizontal layers per ridge and in *Morpho didius* there are 6–8 layers per ridge; quantitative measurements indicate that *M. rhetenor* is significantly brighter, reflecting 80% of incident blue light, while *M. didius* reflects approximately 40%. Similar numbers of layers are present in the cuticles of *Chrysochroa raja* and other iridescent buprestid beetles. By contrast, the metallic gold or silver cuticles of pupae of danaid butterflies can have over 200 reflecting layers. They too reflect approximately 80% of the incident light over a broad band of wavelengths (Fig. 25.3); the reflectivity is not higher due to optical absorption by the layers' constituent materials.

Apart from colors of the human visible spectrum, ultraviolet is also produced as an interference color. This occurs in some butterflies of the Pieridae. In *Eurema*, for example, certain scales possess analogous scale structures to those of *Morpho*; however, their reflecting layers are approximately 55 nm thick and are separated by air spaces of approximately 80 nm. This system reflects light with a peak wavelength of approximately 365 nm. The brightness of a similar ultraviolet-reflecting system in male *Colias eurytheme* butterflies has been shown to be an indication of their fitness to conspecific females. The number of their intra-ridge reflecting layers, a variable which controls this brightness, appears to be affected by larval food quality.

Interference is responsible for the iridescence of the membranous wings of many different insects, particularly Odonata. Notable for their intensity and spectral purity are the hindwings of the dragonfly *Neurobasis chinensis*. In these iridescent wings, an approximately 1 μm thick multilayer on the upper side of the membrane is backed by an approximately 2 μm layer of melanin-based optical absorber for enhanced color contrast.

25.2.3 Coherent scattering in other structures

While interference in mainly one-dimensional multilayering is the origin for the majority of structural colors in insects, there are examples of other insect optical structures which have two- or three-dimensional order. The brightly colored wing scales of *Parides sesostris* and *Teinopalpus imperialis* butterflies comprise good examples of such three-dimensional structures, which is commonly referred to as photonic crystals (Fig. 25.4). In their scales, filaments of cuticle are interconnected periodically in all three spatial dimensions, forming a distinct crystalline geometry with a lattice constant that is on a length-scale of 200–30 nm. The coherent scattering conferred by this structure offers strong light manipulation and, much like the multilayer system in one dimension, prevents the propagation of light through the crystal system. It is this which

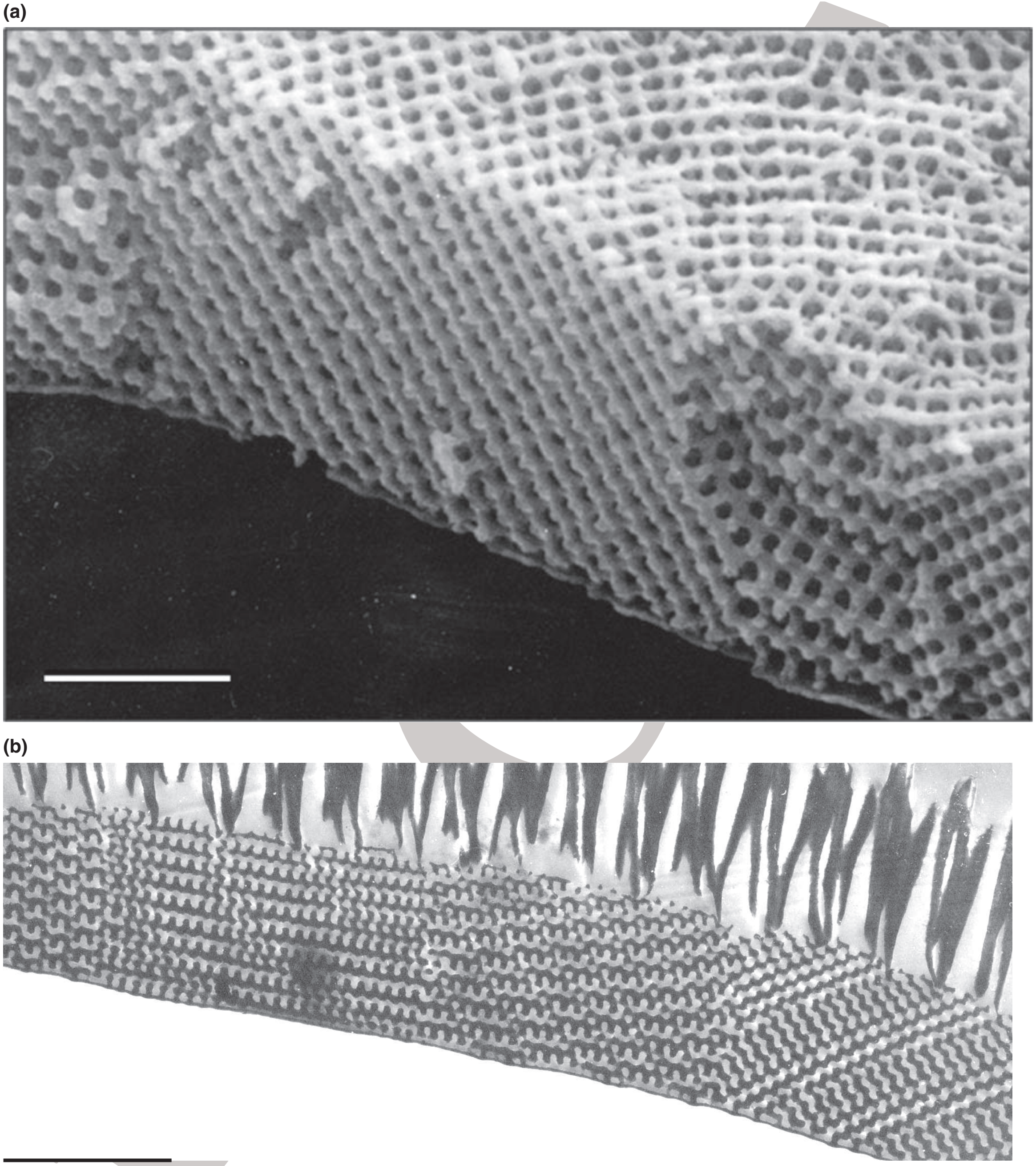


Figure 25.4 The three-dimensional photonic crystal present in the wing scales of the butterfly *Parides sesostris*. (a) Scanning electron microscope image showing the filaments of periodically interconnected cuticle with a fixed lattice constant enabling the scattering of a narrow band of color; scale bar = 1 μm. (b) Transmission electron microscope image of the scale region shown in (a); scale bar = 1.5 μm. This image reveals the highly geometric pattering that is associated with the two-dimensional projections of the actual three-dimensional crystal structures captured in various orientations from micron-sized domain to neighboring intra-scale domain within the scale. This variation in the angular orientation of each neighboring domain's photonic crystal leads to an overall color averaging effect.

results in certain wavelengths being reflected, producing a color appearance for the viewer. Many colored weevils, such as *Eupholus magnificus* and *E. schoeneri*, also exhibit this form of structure in their elytral and body scales. Commonly, in the lepidopteran and coleopteran scales that exhibit this three-dimensional photonic crystal structure, small grains of individual photonic crystal regions within each scale are oriented in different directions so that they reflect specific different colors. As an ensemble, their overall effect is to create additive color mixing and render the color as angle-independent rather than truly iridescent.

25.3 Pigmentary colors

Pigments appear colored because they absorb certain wavelengths of light, the unabsorbed remaining light being scattered by various nanostructures. The energy of absorbed light is dissipated as heat. Which particular wavelengths are absorbed depends on the molecular structure of the compound. Particularly important in the production of color are the number and arrangement of double bonds, C=C, C=O, C=N and N=N. Particular functional groups are also important. The $-NH_2$ and $-Cl$ radicals, for example, shift the absorptive region of a compound so that it tends to absorb longer wavelengths. The color-producing molecule, known as a chromophore, is often conjugated with a protein molecule, forming a chromoprotein. Insects are able to synthesize most of their pigments, but not flavonoids or carotenoids which are, consequently, acquired through diet. The sources of some other pigments, found only in a few insects, are unknown. The black or brown of hardened cuticle often results from sclerotization (Chapter 16). However, cuticular hardening and darkening are not necessarily tightly linked. This is illustrated by albino strains of the locust *Schistocerca*, which have a hard but colorless cuticle.

25.3.1 Pigments that are synthesized

Melanin Dark cuticle is often the result of the presence of the pigment melanin, a nitrogen-containing compound which has been demonstrated in the cuticles of Blattodea, Diptera, adult and some larval Lepidoptera, and in Coleoptera. It is typically present as granules in the exocuticle. Insect melanin is a polymer of indole derivatives of tyrosine. Like sclerotization, melanization involves the production of DOPA and dopaquinone (Fig. 25.5). In *Drosophila*, various genes involved in pigment synthesis (*ebony*, *tan*) and patterning (e.g., *bric-a-brac*) have been identified. The synthesis of black melanin synthesis from DOPA requires the *yellow* gene in *Drosophila*, which is in turn regulated by the Engrailed and Abdominal-B proteins. Both bind to *cis*-regulatory sequences of the *yellow* gene; the former represses expression, while the latter activates it.

There are numerous pleiotropic interactions between color appearance and other traits, because the biochemical pathways for synthesis of cuticular pigmentation and other traits are interlinked, as demonstrated by studies of pigmentation mutants in various species of *Drosophila*. The genes *yellow*, *ebony* and *tan* all affect behavior in addition to pigmentation; they are not only expressed in epidermal cells but also in neurons, where their products are involved in synthesis of neurotransmitters; *yellow* has been implicated in sex-specific behavior. Melanin is also involved in wound-healing, immune responses and the prevention of desiccation. These pleiotropies might place important constraints on evolutionary change in expression of these genes because a response to selective pressures on any one of the traits involved, e.g., cuticular pigmentation, might affect multiple other traits that all contribute to the fitness of the insect.

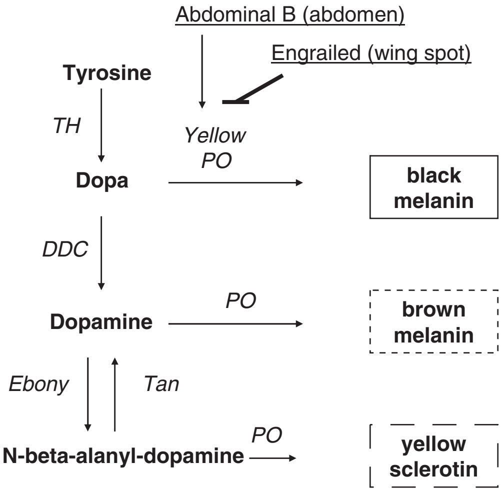


Figure 25.5 Melanin synthesis pathways in *Drosophila*. Pigments and their precursors are shown in bold, enzymes are shown in italics (DDC, dopadecarboxylase; PO, phenyloxidases; TH, tyrosine hydroxylase). In different cells different components of the pathway might be expressed, resulting in different pigmentation of the cuticle. Two proteins controlling expression of the *yellow* gene by binding to its *cis*-regulatory sequences are shown on top (underlined): in *D. melanogaster* males, the Abdominal-B protein activates the yellow gene, resulting in sex-specific pigmentation of the abdomen. In *D. biarmipes*, males but not females have a conspicuous black spot on the wing, and Engrailed protein represses the *yellow* gene and thus the generation of wing spots. Note that dopa is also a key intermediate in cuticular sclerotization and, because of this, melanization and sclerotization may sometimes be linked together (redrawn from Wittkopp and Beldade, 2009).

Pterins Pterins are nitrogen-containing compounds, all having the same basic chemical structure, a pyrazine ring and a pyrimidine ring, but differing in the radicals attached to this nucleus. They are synthesized from the purine guanosine triphosphate, and this synthesis involves the *rosy* and *purple* genes, which control eye color in *Drosophila*. Not all pterins appear colored. Some pterins are important metabolically as cofactors of enzymes concerned with growth and differentiation and may act as controlling agents in these processes. They often occur together with pigments of another group, the ommochromes, because they are cofactors of the enzymes involved in ommochrome synthesis. The vitamin folic acid also contains a pterin.

Pterin pigments can be white (leucopterin and isoxanthopterin, which absorb only in the ultraviolet), yellow (e.g., xanthopterin or dihydroxanthopterin, which absorb in the blue, but not necessarily as strongly in the UV-A) and orange to red (e.g., erythropterin, which absorbs blue, but not as much in the UV-A). They are important pigments in lepidopteran scales, where they are concentrated in pigment granules located on the crossribs. Leucopterin and xanthopterin are common in the wings of Pieridae, supplementing the structural white (Section 25.2.1). Males and females of the Japanese butterfly, *Pieris rapae crucivora*, both appear white to human observers, but only males' wings are adorned with beads, resulting in a sexual dichroism when viewed by conspecifics, such that only the males strongly absorb ultraviolet. In the orange sulfur butterfly, *Colias eurytheme*, pterin pigments interact with iridescent structural coloration, effectively amplifying the ultraviolet signal in males but not females. The yellow of the brimstone butterfly, *Gonepteryx*, is due to chrysopterin, the brighter color of the male resulting from a higher pigment concentration than is present in the female, while the red of the orange-tip butterfly, *Anthocharis*, is due to erythropterin. The yellows of Hymenoptera are produced by granules of pterins in the epidermis.

The pterins are also abundant in compound eyes, occurring with ommochromes in the screening pigment cells of the ommatidia. In this situation they are sometimes components of granules, but are often in solution. Several different pterins are present, not all of them colored. Their functions in the eyes are not fully understood, although some pterins are engaged in circadian rhythm regulation. They accumulate with age in the eyes of higher Diptera, and, because they are products of purine degradation, they may provide a means of storage excretion. The progressive accumulation provides a means of aging these insects. However, no comparable accumulation occurs in the eyes of the moth *Pectinophora*, although changes do occur in the pterins.

Ommochromes Ommochromes are yellow (xanthommatin), red (ommatin) and brown pigments usually occurring in granules coupled with proteins. Ommochromes (and their precursors such as kynurenin) typically absorb ultraviolet and might thus have a photoprotective function. The granules also contain accumulations of calcium. Xanthommatin is the most widely distributed ommochrome and is usually present wherever ommochromes are found.

Ommochromes, principally xanthommatin, are widely distributed in insects as screening pigments in the accessory cells of the eyes, usually associated with pterins. They are also present in the photoreceptor cells. It is thought that this is their original function in insects, and that its function in integument coloration in several insect taxa is derived. Yellow, red and brown body colors are produced by ommochromes in the epidermis. Xanthommatin turns from yellow to red upon reduction. The pink of immature adult *Schistocerca* is due to a mixture of ommochromes. Red Odonata, and probably also the reds and browns of nymphalid butterflies, are due to ommochromes, while in blue Odonata a dark brown ommochrome provides the background for the production of structural blue believed to stem from coherent scattering from quasi-ordered arrays of particles in the endoplasmatic reticulum of pigment cells underlying the cuticle. Epidermal ommochromes sometimes directly underlie cuticular melanin, and in these cases they do not contribute to the insect's color.

The ommochromes are a group of pigments derived from the amino acid tryptophan via kynurenine and 3-hydroxykynurenine (both of which can also function as yellow pigments themselves). The biochemical pathway leading to their production involves the two enzyme genes *vermilion* and *cinnabar* (coding for tryptophan 2,3 dioxygenase and kynurenine 3-monooxygenase, respectively) and the ommochrome precursor transporter gene *white*. Oxidative condensation of 3-hydroxykynurenine gives rise to the ommochromes (Fig. 25.6). In larval *Drosophila*, kynurenine production takes place primarily in the fat body, but its conversion to 3-hydroxykynurenine occurs in the Malpighian tubules, where it is stored. At metamorphosis, the 3-hydroxykynurenine is transported to the eyes, where the ommochromes are formed. In the adult fly, the whole process can take place in the cells of the eye, although this is normally supplemented by kynurenine and 3-hydroxykynurenine synthesized elsewhere. The scale-forming cells on butterfly wings also have the capacity to synthesize ommochromes from tryptophan or from 3-hydroxykynurenine. Transport of ommochromes in the hemolymph is achieved by specialized binding proteins.

Ommochrome production is the only way in which insects can remove tryptophan, which is toxic at high concentrations such as may occur at times of high protein turnover. A transitory increase in tryptophan occurs at metamorphosis in holometabolous insects, often followed by the production of ommochromes. In Lepidoptera ommochromes are accumulated in the meconium, the accumulated waste products of the pupal period which are voided immediately following eclosion. They are responsible for its characteristic red/brown coloration. Accumulation of ommochromes in the integument causes the larva of the puss moth, *Cerura*, to turn red just before pupation. Some of the ommochrome produced at the time of pupation contributes to the screening pigment in the eyes of

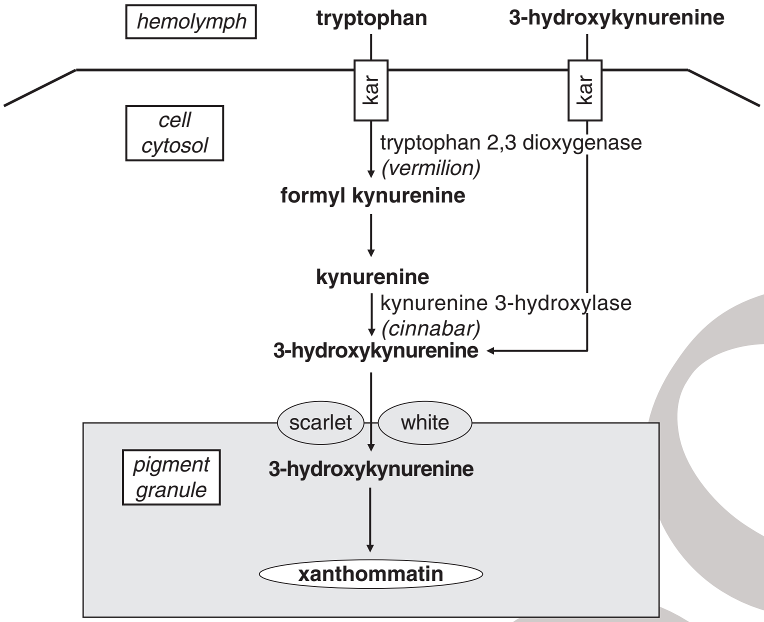


Figure 25.6 Ommochromes synthesis in the eye of *Drosophila melanogaster* (and similarly in butterfly wing patterns). Tryptophan is apparently moved into the cell by the amino acid transporter karmoisin (kar). The *vermilion* gene encodes for tryptophan 2,3 dioxygenase, an enzyme that catalyzes tryptophan's conversion to formyl kynurenine. Hydrolysis of formyl kynurenine results in kynurenine, whose transformation into 3-hydroxykynurenine in turns requires the *cinnabar* gene (and its product, kynurenine 3-hydroxylase). 3-hydroxykynurenine is also generated elsewhere in the body and taken up directly from the hemolymph. White and scarlet are transporter molecules that form a dimer localized in the surface of the pigment granules; they have been implicated in transporting ommatin precursors into the granule. Oxidative condensation of 3-hydroxykynurenine generates the ommochromes. Synthesis of other ommochromes involves the same pathway, differing in the final steps (after figure 1 in Reed and Nagy, 2005).

the adult. Red fecal pellets containing ommochromes are produced by locusts during molting or starvation. At these times, excess tryptophan is likely to be liberated from proteins that are broken down during structural rearrangement or that are used for energy production.

Tetrapyrroles There are two major classes of tetrapyrroles: the porphyrins, in which the pyrroles form a ring, and the bilins, which have a linear arrangement of the pyrroles. The bilins are usually associated with proteins to form blue chromoproteins. Biliverdin occurs in many hemimetabolous insects, but is also found in Neuroptera and some Lepidoptera, although the latter usually contain other types of bilins. Associated with a yellow carotenoid, these pigments are responsible for the greens of many insects. Sometimes the pigments themselves are green. In *Chironomus* (Diptera), bilins derived from the hemoglobin of the larva accumulate in the fat of the adult and impart a green color to the newly emerged fly. In *Rhodnius*, the pericardial cells become green due to the accumulation of bilins derived from ingested hemoglobin.

A porphyrin having an atom of iron in its center is called a heme molecule and this forms the basis of two important classes of compounds, the cytochromes and the hemoglobins. In each case the heme molecule is linked to a protein. All insects are able to synthesize cytochromes, which are essential in respiration, the different cytochromes differing in the forms of their heme groupings. Normally they are only present in small amounts and so produce no color, but where they are present in high concentrations, as in flight muscle, they produce a reddish-brown color.

Some insects living in conditions subject to low oxygen tensions contain hemoglobin in the hemolymph, and these are colored red by the pigment showing through the integument. In *Chironomus* (Diptera) larvae the hemoglobin is in solution in the hemolymph, while in the larva of *Gasterophilus* (Diptera) it is in hemoglobin cells. Many other insects contain hemoglobin in tracheal tissue and the fat body. Hemoglobin serves a respiratory function, but perhaps also serves as a protein store and enables the aquatic hemipteran *Anisops* to regulate its buoyancy (Section 17.9).

Papiliochromes Papiliochromes are yellow and reddish-brown pigments known only from the swallowtail butterflies, Papilionidae. Papiliochrome II is pale yellow and is formed from one molecule of kynurenine, derived from tryptophan, and one molecule of β-alanine.

Quinone pigments The quinone pigments of insects fall into two categories: anthraquinones (violet, blue and green) and aphins (red or purple). Both occur as pigments only among Homoptera (Hemiptera), the former in coccids (Coccoidea) and the latter in aphids only.

Anthraquinones are formed from the condensation of three benzene rings. In the coccids they give the tissues a red, or sometimes yellow, coloration. The best known is cochineal from *Dactylopius cacti*. The purified pigment is called carminic acid. It is present in globules in the eggs and fat body of the female, constituting up to 50% of the body weight. The larva contains relatively little pigment.

Aphins are quinone pigments formed from the condensation of three, in the monomeric forms, or seven benzene rings, in dimeric forms. They are found in the hemolymph of aphids, sometimes in high concentration, and impart a purple or black color to the whole insect. Neriaphin is a monomeric form. Quinone tanning is known to produce dark cuticle.

25.3.2 Pigments obtained from the food

Carotenoids Carotenoids are a major group of pigments that are lipid soluble and contain no nitrogen. They are built up from two diterpenoid units joined tail to tail. In nearly all insect carotenoids, the central chain contains 22 carbon atoms with nine double bonds, and each of the end groups contains nine carbon atoms. There are two major groups of carotenoids: the carotenes, and their oxidized derivatives, the xanthophylls.

Yellow, orange and red are commonly produced by carotenoids, the color depending largely on whether or not the terminal groups are closed rings and on their degree of unsaturation. If the carotenoid is bound to a protein, the color may be altered, sometimes even resulting in a blue pigment. Insects cannot synthesize carotenoids and consequently must obtain them from the diet. Uptake from the food is, at least to some extent, selective. Orthopteroids preferentially absorb carotenes, while lepidopterans favor xanthophylls. Some post-ingestive modification of the carotenoids may occur.

Carotenoids can occur in many different tissues and in all stages of development. A number of structurally different carotenoids may be present in one insect. The possible metabolic functions of carotenoids in insects are not well understood. In other organisms they protect cells from damage due to photo-oxidation by light, but their importance to insects in this regard is not known. They are also the source of retinal in the insect's visual pigments. Apart from producing the reds and yellows of many insects, in combination with a blue pigment, often a bilin, they produce green. Green produced by a carotenoid with a bilin is sometimes known as insectoverdin. In some insects, the ability to sequester carotenoids might have functions beyond pigmentation; carotenoids also confer advantages with respect to photo-oxidative stress induced by ultraviolet radiation.

Flavonoids Flavonoids are heterocyclic compounds commonly found in plants. In insects they are mainly found among the butterflies and are common in Papilionidae, Satyridae and Lycaenidae as cream or yellow pigments. At least in some species, flavonoids are present in all developmental stages, including the egg, indicating that the flavonoids are stored in internal tissues of the adult as well as in the scales. Although some flavonoids function as deterrents to herbivores, it is clear this is not efficient for all insects. Where they occur in insects, flavonoids are acquired exclusively from herbivory, so the flavonoids present in their bodies reflect what is present in the host plants. Some post-ingestive modification of structure does occur so that the flavonoids in the insect are not exactly the same as those in the host. Perhaps these changes are produced by the insects themselves, but it is possible that their gut flora is responsible.

25.4 Color patterns

In general, insects do not have a uniform coloring, but have specific and often finely detailed patterns. The genetics and evolutionary developmental biology have been especially well studied in *Drosophila* and in the Lepidoptera. The development of black spots on fruit fly wings as well as the widespread lepidopteran eyespot patterns (with concentric rings of different colors) is controlled by a series of organizing centers from which morphogens diffuse outwards, creating a concentration gradient. Many of the wing-patterning genes first identified in *Drosophila*, such as *apterous*, *wingless*, *Distal-less*, *engrailed* and *cubitus interruptus*, are also involved in lepidopteran eyespot formation, where they are deployed in specific regions of the wings, often at specific times during development. In butterflies the wing pattern is produced by thousands of small overlapping scales. Morphogens govern the development of pigment in each scale-forming cell and different concentrations induce different pigments. Studies with tissue transplants have shown that the size of the eyespot is determined primarily by properties of tissue at the center, while the specific colors in each ring depend on response thresholds with which the host tissue responds to the chemical signal diffusing from the center. As diffusion extends uniformly outwards in all directions around an organization center, a symmetrical pattern is produced. It is also possible that a band of color rather than a series of separate rings can be produced by organization centers that are close together so that the patterns they produce grade into each other. On the forewings of many butterflies (Papilionoidea) there are rows of organizing centers that produce series of spots across the wing (Fig. 25.7). In general, it appears that each scale contains only a single pigment, although examples of scales with two pigments are known. The pigment produced by each scale-forming cell depends on synthesis within the cell. Although some intermediates may be manufactured elsewhere, scale color is not determined by selective uptake of presynthesized pigment. It seems, therefore, that the morphogens moving between epidermal cells regulate the synthetic machinery within each cell.

25.5 Color change

Color changes are of two kinds: short-term reversible changes which do not involve the production of new pigment, and long-term changes which result from

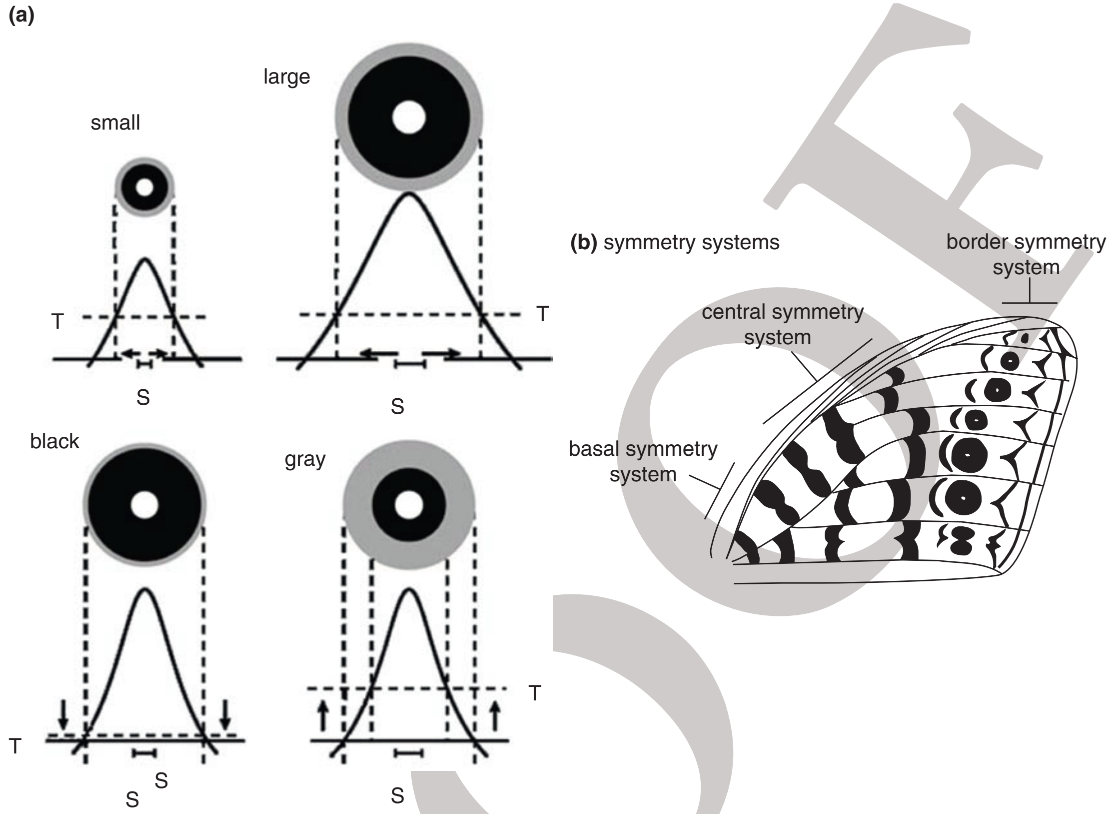


Figure 25.7 Color patterning in the forewing of a Nymphalid butterfly. A string of organizing centers on the wing's border generates a series of circular eyespots. Whether similar organizing centers generate the more proximal bands is not known (after Nijhout, 1994).

the formation of new pigments and are not usually reversible. Short-term reversible changes are called physiological changes. Color changes involving the metabolism of pigments are called morphological color changes.

25.5.1 Physiological color change

Physiological color change is unusual in insects. It may occur where colors are produced physically as a result of changes in the spacing between reflecting layers, or where color is produced by pigments as a result of pigment movements.

Tortoise beetles change color when they are disturbed. Normally, the beetles are brassy yellow or green, but can change to brown-orange or red within minutes, presumably a form of aposematic coloration. In the Panamanian species *Charidotella egregia*, the gold coloration of the undisturbed state is generated by a chirped multilayer reflector, where porous patches in each layer contain humidity. A reduction in the state of hydration makes the cuticle translucent, revealing the red pigmentation underneath. Rehydration restores the original color.

The elytra of the Hercules beetle, *Dynastes*, are normally yellow due to a layer of spongy, yellow-colored cuticle beneath a transparent layer of cuticle. If the spongy cuticle becomes filled with liquid, as it does at high humidities, light is no longer reflected by it but passes through and is absorbed by black cuticle underneath, making the insect black. In the field, these changes probably occur daily, so that the insect tends to be yellow in the daytime, when it is feeding among leaves, and dark at night, making it less conspicuous.

Physiological color changes involving pigment movements are known to occur in the stick insect *Carausius*, in the grasshopper *Kosciuscola* and in a number of blue damselflies (Zygoptera, Odonata). All these insects become black at night due to the movement of dark pigment granules to a more superficial position in the epidermal cells (Fig. 25.8). *Kosciuscola* is blue during the day as a result of coherent scattering from small granules, less than 0.2 μm in diameter, believed to be composed mainly of white leucopterin and uric acid. At night, the blue is masked by the dispersal of larger pigment granules among the small reflecting granules which also disperse through the cell. Similar masking of structurally scattered blues occurs in a number of damselflies; in brown individuals of the stick insect *Carausius*, ommochrome granules occupy a superficial position at night, causing the insect to become darker, while during the day they occupy a proximal position in the epidermal cells, making the insects paler. Green specimens of *Carausius* do not change color because they lack ommochromes. The granules move along the paths of microtubules which may be responsible for the movements. Similar microtubules are present in the epidermal cells of *Kosciuscola*.

The color change in *Kosciuscola* and damselflies is temperature dependent. The insects are always black below a temperature which is characteristic of the species, but usually about 15°C. At higher temperatures they tend to become blue, and, in some species, the change to blue is enhanced by light. Here, the epidermal cells are to some extent independent

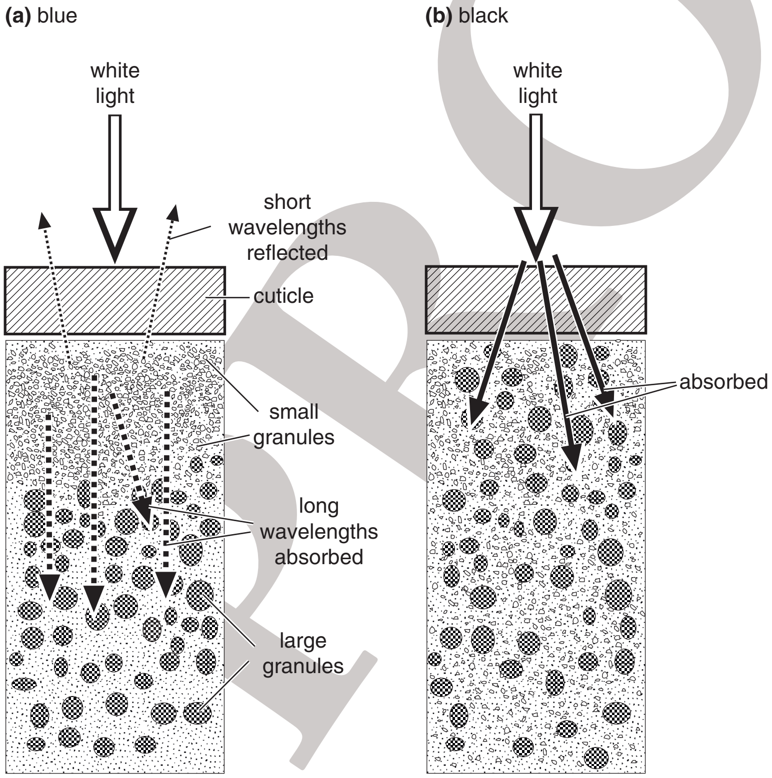


Figure 25.8 Physiological color change in *Kosciuscola*. The two diagrams have the same numbers of large and small granules (based on Filshie *et al.*, 1975). (a) At moderately high temperatures, the larger granules are restricted to the proximal parts of the epidermal cells. Short wavelengths of light are scattered by the small granules in the more distal parts of the epidermal cell, longer wavelengths travel further into the cell and are absorbed. The insects appear blue. (b) At lower temperatures, the pigments are generally dispersed through the cell. All the light is absorbed by the larger granules and the insects appear black.

effectors, responding directly to stimulation. This is true of the change from black to blue in the damselfly *Austrolestes*, but the reverse change is controlled by a secretion released from the terminal abdominal ganglion. The significance of these changes is unknown, but they may be thermoregulatory. Dark insects absorb more radiation than pale ones so they may warm up more rapidly in the mornings and become active earlier than would be the case if they remained pale.

25.5.2 Morphological color change

Changes in the amounts of pigments can occur in response to a variety of external and internal factors.

Ontogenetic changes Many insects change color in the course of development. For example, the eggs of the plant-sucking bug *Dysdercus nigrofasciatus* (Hemiptera) are white when laid, becoming yellow as the embryo develops. The first-stage nymph is a uniform yellow color when it hatches, but becomes orange and then red. In the second instar, white bands appear ventrally on some of the abdominal segments (Fig. 25.9). These become more extensive and white bands are also present dorsally in the later stages. In the final larval instar, the red becomes less intense, especially in the female, and the adults are yellow with white stripes. The yellow and red colors of this insect are produced by three pterins, the proportions of which change through development to produce the different colors. The white bands are formed from uric acid.

It is also common for caterpillars to exhibit a regular change in color during development. The larva of the puss moth, *Cerura*, turns from green to red just before pupation as a result of the production of ommochrome in the epidermis. The early larva of the swallowtail butterfly, *Papilio demodocus*, is brown with a white band at the center, whereas the late larva is green with purple markings and a white lateral stripe.

In adult hemimetabolous insects, color change is often associated with ageing and maturation. Adult male *Schistocerca* change from pink to yellow as they mature. The pink is produced by ommochromes in the epidermis which decrease in amount as the insect gets older, and the yellow is due to β-carotene, which increases with age. Color changes related to sexual maturation also occur in some Odonata.

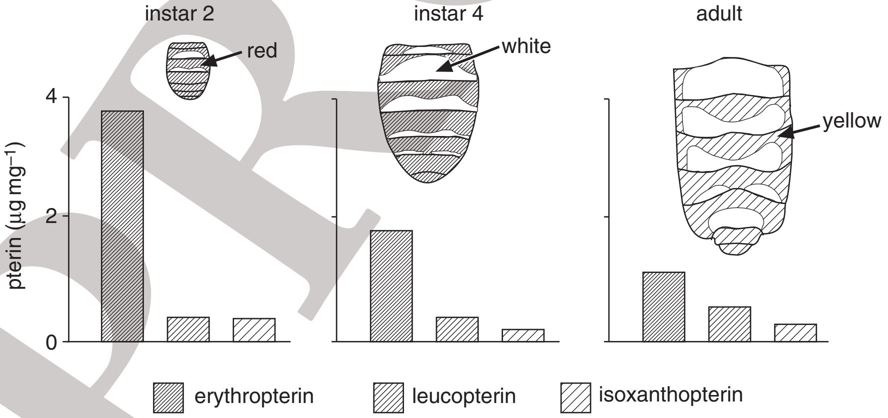


Figure 25.9 Ontogenetic changes in pigmentation in *Dysdercus nigrofasciatus*. Underside of the abdomen of second- and fourth-instar larvae and adult male. The change from red to yellow is accompanied by a reduction in the relative amount of erythropterin present (expressed as μg of pterin per mg body weight). The white areas are due to uric acid in the epidermal cells (data from Melber and Schmidt, 1994).

These changes are controlled by changes in hormone levels associated with molting and sexual maturation, but the effects may be very different in different species. The changes in larval *Cerura* are initiated by a low level of ecdysteroid in the hemolymph, leading to the metabolism of 3-hydroxykynurenine to an ommochrome. Perhaps the commitment peak which occurs before pupation is normally responsible for the change (Section 15.4.2). Juvenile hormone leads to the accumulation of xanthommatin in *Bombyx* caterpillars, but prevents it in *Manduca*. Juvenile hormone, concerned in sexual maturation in adult *Schistocerca*, also regulates the accumulation of carotenoids which make the insects yellow as they become sexually mature. The hormone bursicon, which controls sclerotization, probably also regulates darkening occurring immediately after ecdysis.

Homochromy The colors of several insect species change to match the predominant color of the background. This phenomenon is called homochromy. The changes may involve the basic color of the insect or may involve a general darkening. For example, larvae of the grasshopper *Gastrimargus* tend to assume a color more or less matching the background when reared throughout their lives on that background (Fig. 25.10). Insects

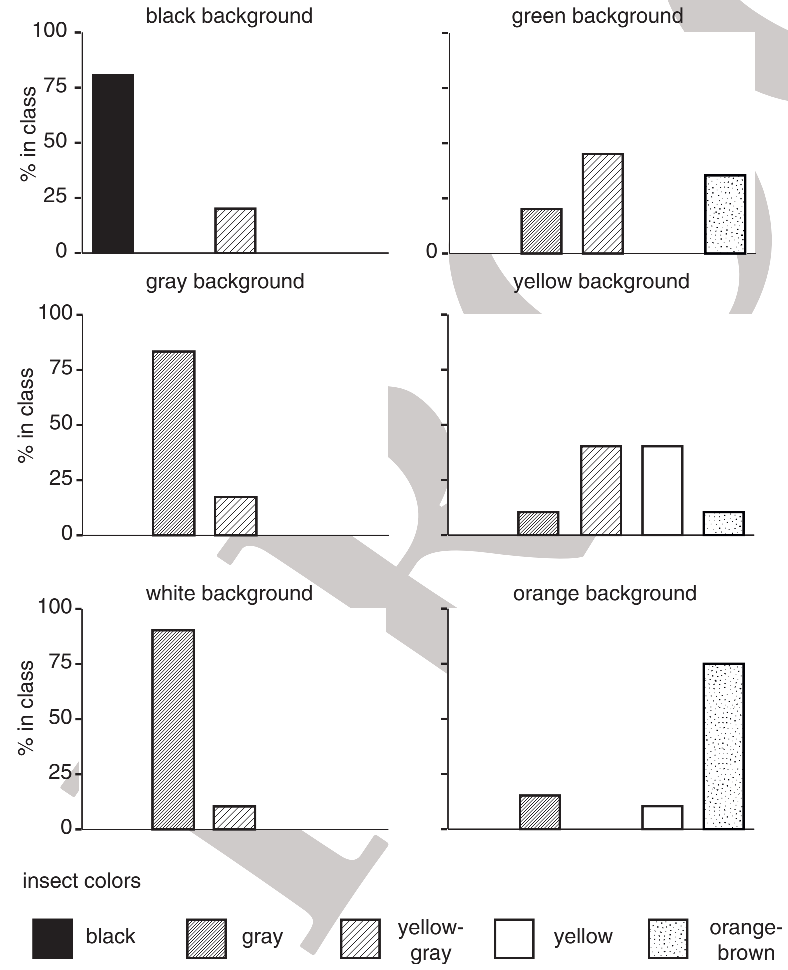


Figure 25.10 Homochromy in the grasshopper *Gastrimargus*. Each graph shows the ground color of insects in the final larval stage after rearing from the first stage in containers with different colored backgrounds (after Rowell, 1970).

reared on black become black, those reared on white are pale gray. On a green background, however, most insects develop a yellowish coloration. The differences are produced by different amounts of black pigment, possibly melanin, in the cuticle and a dark ommochrome in the epidermis, together with yellow and orange pigments in the epidermis. The darkening of the stick insect *Carausius* that occurs on a dark background is also due to the accumulation of two ommochromes, xanthommatin and ommin. In this insect, changes occur at any time during the course of a stadium, but in most other examples the change is first seen at a molt. Ommochrome deposition begins after about five days on the new background, and the changes in other insects generally require a similar period of exposure. Caterpillars, too, may exhibit some degree of homochromy. Larvae of the hawk moth *Laothoe populi* may be almost white or yellow-green, depending on the plants on which they are reared, and the pupae of some butterflies that are not concealed in a cocoon or in the soil may be green or dark or pale brown, according to their surroundings.

An extreme example of homochromy occurs in African grasshoppers after bush fires. Many species become black within a few days of the fire, the change occurring in adults as well as larvae, but only in bright sunlight. In diffuse light the change is much less marked.

All these changes depend on visual stimulation, although the details may vary from species to species. In *Carausius* darkening results from weak stimulation, or the absence of stimulation of the lower parts of the compound eye, such as would occur when the insect is on a dark surface. In *Gastrimargus* darkening occurs if a high incident light intensity is associated with a low reflectance background, and changes in the yellow and orange pigments are dependent on the wavelength of light. Homochromy in caterpillars and lepidopteran pupae is also determined by the wavelength of light and the contrasts in intensity reaching the different stemmata.

Visual inputs affect the activity of neurosecretory cells in the central nervous system. Homochromy in pupae of the peacock butterfly, *Inachis io*, is produced by changes in the relative amounts of melanin and lutein (a carotenoid) in the cuticle. The accumulation of both pigments is controlled by a single neuropeptide, which is widely distributed in the central nervous system before being released into the hemolymph. A low hormone titer immediately before pupation stimulates melanization, a higher titer results in increased incorporation of lutein into the pupal cuticle. In the grasshopper *Oedipoda miniata*, the dark-color-inducing neurohormone (DCIN) has been identified as (His[7])-corazonin.

The stink bug *Nezara viridula* (Hemiptera) changes from green to brown during diapause when overwintering under leaf litter or bark, and changes back to green again in spring, presumably to aid camouflage; these changes are induced in part by day length; temperature can also have an effect in some species.

Other factors affecting color Temperature is important in pigment development and there is a general tendency for insects reared at very high temperatures to be pale, and those developing at low temperatures to be dark. Humidity affects the color of many Orthoptera. Green forms are more likely to occur under humid conditions, and brown forms under dry conditions.

Crowding influences color in some insects, the most extreme examples being locusts. Locust larvae reared in isolation are green or fawn, while rearing in crowds produces yellow and black individuals. The colors and patterns change as the degree of crowding alters; under high-density conditions, locusts consume toxic host plants, and predators learn the aposematic yellow–black coloration as a predictor of toxicity. The larvae of some Lepidoptera, such as *Plusia* and the armyworms, undergo comparable changes, some occurring in the course of a stadium, but the most marked alterations occur only at molting.

25.5.3 Color polymorphism

Insects of many orders exhibit a green–brown polymorphism, tending to be green at the wetter times of year and brown when the vegetation is dry. The two forms are genetically determined, but homochromy that develops when the insect moves to a new background may be superimposed on this. In green morphs the production of ommochromes in the epidermis is largely or completely inhibited; if it occurred, the green would be obscured.

In some insects there are marked differences in color between successive generations, correlated with seasonal changes in the environment. Such seasonal polyphenism and its physiological regulation is discussed in Section 15.5. Extreme cases of color polymorphism occur in some butterflies in relation to mimicry (Section 25.6.1).

25.6 Significance of color

Insect pigments are often the end products of metabolic processes and may have evolved originally as forms of storage excretion. Pterins, for example, may be derived from purines, such as uric acid. Similarly, melanin production might be a method of disposing of toxic phenols ingested or arising from metabolism and it may be significant that melanin in the cuticle is often produced above metabolically active tissue such as muscle. Tryptophan in high concentrations reduces the rate of development of *Drosophila* and *Oryzaephilus* and it is noteworthy that ommochrome production follows the appearance of unusually high levels of tryptophan in the tissues. However, it is clear that storage excretion is not the sole, or even primary, function of pigments in most insects. For example, most insects excrete most of the end product of the biologically active hydrogenated pterins and do not store them. *Pieris*, on the other hand, synthesizes much larger amounts than would result from normal metabolism and accumulates them in the wings, where they contribute to the color.

In most cases, the colors of present-day insects are ecologically important. Color is most frequently involved as a defense against vertebrate predators and is also important in intraspecific recognition. It also has important consequences for body temperature, and color changes may contribute to thermoregulation (Chapter 19).

25.6.1 Predator avoidance

In many species, color and color patterns have evolved as part of a strategy to avoid predation. The color patterns may function in a variety of ways in different insects: for concealment (crypsis), to startle a predator (deimatic behavior), to deflect attack from the most vulnerable parts of the body or to advertise harmfulness or distastefulness (aposematism).

Crypsis Color often helps to conceal insects from predators. Many insects are known to select backgrounds on which they are least conspicuous. Often, homochromy is associated with some appropriate body form and behavior as in stick insects and many mantids and grasshoppers which may be leaf-like or twig-shaped according to the backgrounds on which they normally rest. Protection may also be afforded by obliterative shading. Objects are made conspicuous by the different light intensities which they reflect as a result of their form. A solid object usually looks lighter on the upper side and darker beneath because of the effect of shadows, but by appropriate coloring this effect can be eliminated. If an object is shaded in such a way that when viewed in normal lighting conditions all parts of the body reflect the same amount of light, it loses its solid appearance. Such countershading is well-known in caterpillars, where the surface toward the light is most heavily pigmented and the side normally in shadow has least pigment. To be successful in concealing the insect, this type of pigmentation must be combined with appropriate behavior patterns; if the larva were to rest with the heavily pigmented side away from the light it would become more, not less, conspicuous. Color may also afford protection if the arrangement of colors breaks up the body form. Such disruptive coloration is typically most efficient when some of the color components match the background and others contrast strongly with it. The color pattern should introduce high-contrast internal boundaries on the body surface, which surpass the salience of the outline. Disruptive coloration occurs in some moths which rest on tree trunks.

Deimatic behavior Some insect species have colored wings, or other parts of the body, which are normally concealed, but are suddenly displayed when a potential predator approaches. This behavior, sometimes associated with the production of a sound, has been shown, in a few cases, to startle the predator. It is known as deimatic behavior. In many insects exhibiting this behavior, the hindwing is deep red or black. It is normally concealed beneath the forewing, but is revealed by a sudden partial opening of the wings. This occurs in a number of stick insects and mantids, and in moths that normally rest on vegetation. Moths in the family Arctiidae have bright red or yellow abdomens, often with black markings. When disturbed, the abdomen is displayed by opening the wings. The deimatic display of some mantids involves the front legs, which have conspicuous marks on the inside. *Galepsus*, for example, displays the insides of the fore femora and coxae, which are orange, at the same time exposing a dark mark in the ventral surface of the prothorax.

Some Lepidoptera have a pattern of scales on the hindwing forming an eyespot which is displayed when the insect is threatened. One of the best-known examples is the peacock butterfly, *Inachis io*. It has one eyespot on the upper surface of each wing. These eyespots are primarily black, yellow and blue, surrounded by dark red. The butterfly rests with its wings held up over its back, the upper surfaces of the forewings juxtaposed so that the eyespots are concealed. If the insect is disturbed by visual or tactile stimuli it lowers the wings so that the eyespots on the forewings are displayed and then protracts the forewings to expose the hindwing eyespots. At the same time the insect makes a hissing sound with a timbal on the forewing (Section 26.3.3). The forewings are then retracted and partly raised and the sequence of movements repeated, sometimes for several minutes. While displaying, the body is tilted so that the wings are fully exposed to the source of stimulation and at the same time the insect turns so as to put the stimulus behind it. This eyespot display causes flight behavior in at least some birds. Some mantids also have a large eyespot on the hind wing. It remains controversial whether these eyespots obtain their startling function by resembling eyes, or whether their highly contrasting patterns present a more general aposematic pattern (see below) or induce neophobia.

Deflection marks Small eyespots, often present on the underside of the hindwings of butterflies, appear to deflect the attention of birds away from the head of the insect. There is no sharp distinction between eyespots used for deimatic behavior and those concerned with deflection. In general, deflecting spots are probably smaller than those used in intimidation, but it is possible that some may serve either function, depending on the nature and experience of the predator. Some butterfly species (in the families Lycaenidae, Riodinidae and Nymphalidae) have appendages on the hind wings that look like antennae or legs, paired with eyespot patterns, creating the illusion of the insect's head (Fig. 25.11). Observations from predator-induced wing damage indicate that these "false heads" are indeed efficient in deflecting vertebrate attacks to less vulnerable body parts, giving the butterfly a chance to escape.

Aposematic coloration Many insects are distasteful by virtue of chemicals they produce themselves, or that are sequestered from their food;

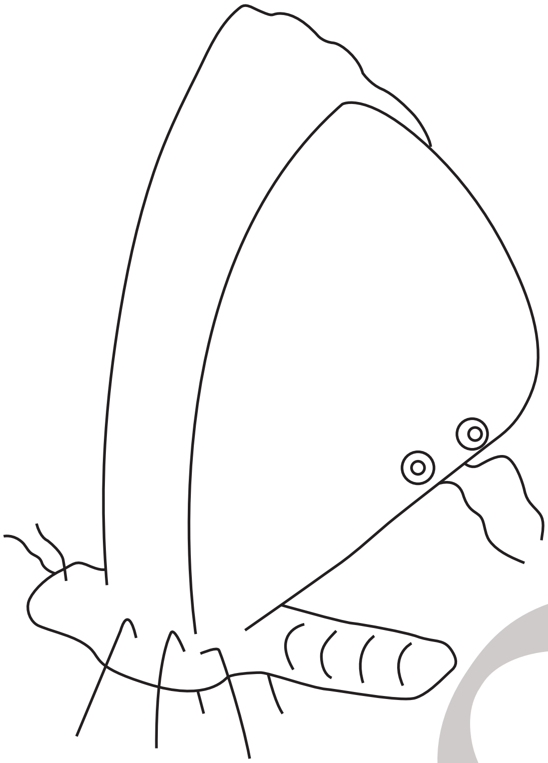


Figure 25.11 Deflection marks on the hind wing of a lycaenid butterfly. Two eyespots paired with false "antennae" generate the impression of a head, deflecting predator attacks away from body parts where a bite might be fatal (figure 3 from Stevens, 2005).

other insects sting (Section 27.9). Such insects are commonly brightly colored, and are usually red or yellow combined with black. Such coloration is a signal to predators that the potential prey is distasteful and should be avoided. It is called aposematic coloration. In ladybird beetles, for example, the degree of carotenoid-based redness in the elytra of individuals correlates with alkaloid content. For such coloration to be effective, the predator must exhibit an innate or a learned avoidance response. Aposematic species occur in many orders of insects (Table 25.2). Development of aposematic coloration is dependent upon population density in some species of locust and grasshopper.

Mimicry Predators learn to avoid distasteful insects with distinctive colors. If the color patterns of some species are similar to each other, learning to avoid one species because it is distasteful also produces an avoidance of the other. Resemblance of one species to another is called mimicry. Mimicry takes two forms, Müllerian and Batesian. Species exhibiting Müllerian mimicry are all distasteful. Here, the advantage to the insects is that predation on any one species is reduced. For example, many social wasp species – such as *Vespa* and *Vespula*, which all have a sting – have the same basic black and yellow pattern; if a predator learns to avoid one species, it is likely to avoid others with a similar appearance. Sometimes the mimics have different lifestyles. Cotton stainer bugs of the genus *Dysdercus* usually have similar red and black coloration and all the species are distasteful. The predaceous reduviid bug *Phonoctonus* lives with *Dysdercus* and preys on them. It, too, is unpalatable to predators, and is a color mimic of *Dysdercus* so that avoidance of one leads to avoidance of both species. Müllerian mimicry is also common among Lepidoptera, with the genus *Heliconius* having been especially well studied. The adaptive polymorphism in *H. erato* is orchestrated by a precise temporal and spatial expression pattern of the cinnabar and vermilion genes, coding for enzymes in ommochrome production.

In Batesian mimicry, only one of a pair of species is distasteful, the other is not. They are called the "model" and "mimic," respectively. Here, the palatable species gains some advantage from a resemblance to a distasteful species. In this type of mimicry it is essential that the mimic is uncommon relative to the model. If this were not the case a predator might learn to associate a particular pattern with palatability rather than distastefulness. This limits the numbers or distribution of a mimetic form, but the limit may be circumvented by the mimic becoming polymorphic, with each of its morphs resembling a different distasteful species. The best-known example of such polymorphism is that of the female *Papilio dardanus* (Lepidoptera), which has

Table 25.2 **Aposematic insects; examples from different orders**

Order and species	Stage	Color	Basis of unpalatability
Orthoptera			
Zonocerus variegatus	Adult	B/Y and R markings	Pyrrolizidine alkaloids
Hemiptera			
Aphis nerii	All	Bright Y	Cardiac glycosides
Oncopeltus fasciatus	Adult	Y/B spots	Cardiac glycosides
Coleoptera			
Coccinella septemfasciata	Adult	R/B spots	Alkaloids
Tetraopes oregonensis	Adult	R	Cardiac glycosides
Hymenoptera			
Vespula vulgaris	Adult	Y/B stripes	Sting
Lepidoptera			
Tyria jacobaeae	Larva	B/Y stripes	Pyrrolizidine alkaloids
Tyria jacobaeae	Adult	B/R marks	Pyrrolizidine alkaloids
Battus philenor	Larva	R/B spots	Aristolochic acids
Danaus plexippus	Larva	W/B and Y stripes	Cardiac glycosides
Zygaena filipendula	Adult	B/R spots	Cyanogenic glycosides

Note:
B, black; Y, yellow; R, red; W, white.

a large number of mimetic forms mimicking a series of quite different-looking butterflies. It appears that alleles of a single gene explain all the natural variants of this species.

Because the unpalatability of an individual may be affected by the nature of the food it eats, mimicry may vary temporally and spatially. For example, the viceroy butterfly (*Limenitis archippus*) and queen butterfly (*Danaus gilippus*) of North America are both distasteful and normally exhibit Müllerian mimicry. However, sometimes larvae of the queen butterfly feed on plants that are so low in the cardenolides sequestered by the insect that the resulting adults are not distasteful. It may be supposed that the palatable queen butterflies now depend for protection on their resemblance to other members of the species that have sequestered cardenolides, and on unpalatable viceroy butterflies. A special case of Batesian (deceptive) mimicry occurs where some insects mimic the visual appearance of their own predators, such as jumping spiders.

25.6.2 Intraspecific recognition

Color is important in intraspecific recognition in many diurnally active insects, and its role is most fully understood in damselflies and dragonflies (Odonata), and in butterflies. It often has two principal functions in male behavior: the recognition of females and the recognition of conspecific males; females also select males by coloration.

In some dragonflies, male color is important in defending a territory against other males. For example, the dorsal side of the abdomen of male *Plathemis lydia* is blue. If a second male enters a territory, the resident male faces the intruder and displays the color by raising the abdomen toward the vertical. This has an inhibiting effect on the intruding male. In the presence of a female, the abdomen is depressed so that the blue is not visible as the male approaches her. The females of many species change color as they become sexually mature and this is associated with a change in male behavior toward them. Among the damselflies, the females of some species are dimorphic, with one of the morphs resembling the male, and there are distinct differences in the behavior of males toward these two morphs.

In butterflies the general color of a female may be more important than the details of pattern in attracting males, although size and movement are also important. For example, males of the African butterfly, *Hypolimnas misippus*, are attracted by the female's red-brown wing color; the black and white markings sometimes present on the forewings are unimportant, although white on the hindwing may have an inhibitory effect. Males of *Colias eurytheme* are attracted by the yellow underside of the female hindwing, which is exposed when the female is at rest. In this species the male reflects ultraviolet from the upper side of its wings. This signal is used by the female in interspecific mate recognition, and it also inhibits attraction by other males, reducing the likelihood of further male intervention when a male is copulating. Such female mate recognition can have an important role in reproductive isolation between species. Strength of pigmentation or structurally based ultraviolet iridescence can depend on an individual's ability to acquire resources, and therefore be indicative of phenotypic or genotypic mate quality. In some social wasps, facial patterns differ between members of the nest, and colony members can remember the individual appearance of a nest-mate.

25.7 Light production

Intrinsic luminescence, i.e., light produced by the insects, is known to occur in various Coleoptera, primarily in the families Lampyridae (fireflies), Elateridae (click beetles) and Phengodidae (railroad worms), as well as in a few Collembola, such as *Onychiurus armatus*, in the homopteran *Fulgora lanternaria*, some larval Diptera of the families Platyuridae and Bolitophilidae.

The light-producing organs occur in various parts of the body. The collembolan *Onychiurus* emits a general glow from the whole body. In most beetles the light organs are relatively compact, and are often on the ventral surface of the abdomen. In male *Photuris* (Coleoptera) there is a pair of light organs in the ventral region of each of the sixth and seventh abdominal segments. In the female the organs are smaller and often only occur in one segment. The larvae have a pair of small light organs in segment eight, but these are lost at metamorphosis when the adult structures form. Larvae and females of railroad worms (Phengodidae, Coleoptera) have 11 pairs of dorso-lateral light organs on the thorax and abdomen and another on the head. In *Fulgora* (Hemiptera) the light organ is in the head. The light organs are generally derived from the fat body, but in the glow worm fly *Arachnocampa* (Diptera) they are formed from the enlarged distal ends of the Malpighian tubules.

25.7.1 Structure of light-producing organs in Coleoptera

Each light organ of an adult firefly consists of a number of large cells, the photocytes, which lie just beneath the epidermis and are backed by several layers of cells called the dorsal layer cells (Fig. 25.12).

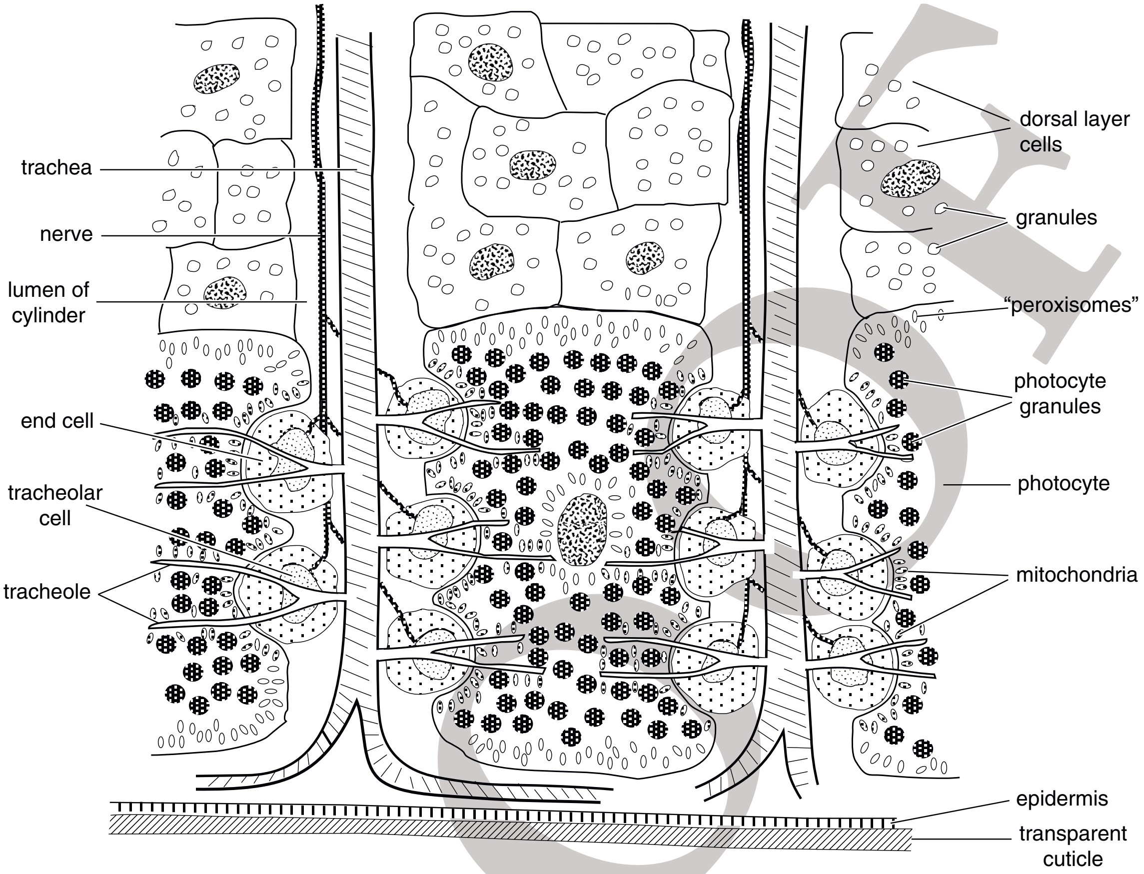


Figure 25.12 Diagrammatic section through part of the light organ on the ventral side of an adult *Photuris*. The tracheoles pass between the photocytes, but do not penetrate into the cells (based on Smith, 1963).

The cuticle overlying the light organ is transparent. The photocytes form a series of cylinders at right angles to the cuticle, with tracheae and nerves running through the core of each cylinder. Each trachea gives off branches at right angles and as these enter the region of the photocytes they break up into tracheoles, which run between the photocytes parallel with the cuticle. The tracheoles are spaced 10–15 μm apart, and as the photocytes are only about 10 μm thick the diffusion path for oxygen is short. The origin of the tracheoles is enclosed within a large tracheal end cell, the inner membrane of which is complexly folded where it bounds the tracheolar cell (Section 17.1.2). In some species the end cells are only poorly developed. The neurons entering the photocyte cylinder end as spatulate terminal processes between the plasma membranes of the end cell and the tracheolar cell within which the tracheoles arise. In adult *Pteroptyx* (Coleoptera) and some other genera, nerve endings occur on the photocytes as well as on the tracheal end cells.

The photocytes are packed with photocyte granules, each containing a cavity connecting with the outside cytoplasm via a neck. It is presumed that the reactants involved in light production are housed in these granules. Smaller granules also occur dorsally and ventrally. Mitochondria are sparsely distributed except where the cell adjoins the end cells and tracheoles. The dorsal layer cells also contain granules, generally understood to comprise urate crystals, which form a reflecting layer at the back of the light-producing region which directs the luminescence more efficiently in the outward direction. It is estimated that the two lanterns of *Photinus* together contain about 15 000 photocytes, forming some 6000 cylinders, each with 80–100 end cells. The lanterns of larval fireflies contain the same elements, but their organization is simpler. The tracheal system is diffuse and there are no tracheal end cells. Nerve endings occur on the photocytes, and are not separated from them by the tracheal end cells as in adult *Photuris*.

25.7.2 Mechanism of light production

Light is produced in organelles called peroxisomes, noted as centers for enzymatic oxidation reactions. A two-stage reaction occurs within these peroxisomes. First, adenylation of substrate luciferin (which is dependent on the presence of magnesium-ATP) occurs under the catalytic action of luciferase. The subsequent oxygenation of luciferyl adenylate by molecular oxygen results in the emission of light and the production of oxyluciferin (Fig. 25.13).

The reaction is very efficient, some 98% of the energy involved being released as light. Furthermore, in fireflies the metabolic cost of the light-flash production is low. It increases metabolic rates by approximately 40%, compared to 60% for walking activity.

In many insects the light produced by the bioluminescence organs is yellow-green in color, extending over a relatively narrow band of wavelengths, 520–650 nm in *Photinus* and *Lampyris* (Coleoptera). In larval and adult female railroad worms (Coleoptera) such as *Phrixothrix*, the light organs on the thorax and abdomen produce green to orange light, depending on the species, in the range 530–590 nm. That on the head produces red light with peak emission at 620 nm, but extending from about 580 nm to over 700 nm. The light produced by *Arachnocampa* (Diptera) is blue-green, that of *Fulgora* (Hemiptera) white. In fireflies, despite noticeable spectral differences in light production, the luciferin substrate is identical and their DNA similarity is 70—99%. The observed differences in spectral output are caused by single amino acid substitutions; namely, with variations in the luciferase active site that controls the conformation of the bound substrate.

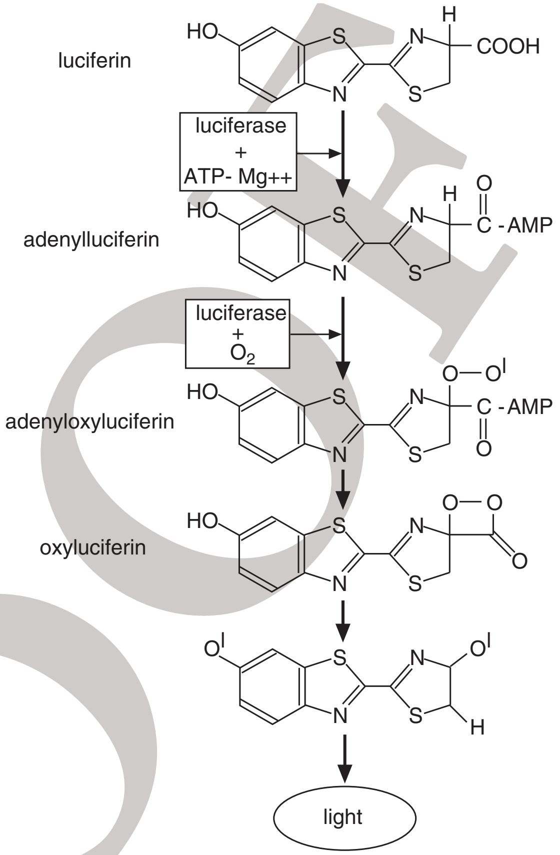


Figure 25.13 Basic reactions involved in light production from luciferin.

25.7.3 Control of light production

The light organs of adult *Photuris* (Coleoptera) are innervated by three and four dorsal unpaired median (DUM) cells in the last two abdominal ganglia, respectively, which release octopamine. Like other DUM cells (Section 10.3.2), the axons from these cells divide to send symmetrical branches to the lanterns on each side. In most adult fireflies the axons terminate on the tracheal end cells, but in larvae, where there are no end cells, they innervate the photocytes directly. The lantern end cells, nerve endings and tracheolar cells form the "end organ" complexes. These, together with the enclosed tracheal channels, play a dominant role in the control of light production since they are only observed in species exhibiting flashing lanterns. No flashing lantern is observed *Photuris* larva, but there is a slowly rising and falling light production capacity. Peroxisomes and mitochondria are also present in the larva but are unsegregated and no specializations are observed in the tracheal cell compartments.

In adult *Photuris*, light production appears to be regulated by the availability of oxygen. As the DUM neurons terminate on the tracheal end cells it is presumed that neural activity causes a change in these cells, facilitating the flow of oxygen to the photocytes. Flash duration in adult fireflies is generally very brief, a few hundred milliseconds, with flashes following each other at regular intervals. This implies that the oxygen supply to the photocytes is closely regulated. It is believed that hydrogen peroxide plays a role in this regulation: The peroxisome oxidases use oxygen arriving at open mitochondria to create hydrogen peroxide that builds up explosively due to the shutdown of the catalase. This completes the oxidation reaction and triggers the flash. The precise temporal control of firefly flashing is understood to be regulated by nitric oxide (NO). NO synthase is localized near synaptic terminals within the firefly lantern and measurements indicate that externally added NO stimulates bioluminescence production while the addition of NO scavengers inhibits light production. Furthermore, NO is known to control respiration by photocyte mitochondria reversibly. The proposed mechanism comprises neural stimulation resulting in the transient release of NO that diffuses into the periphery of adjacent photocytes. This inhibits mitochondrial respiration and permits oxygen to diffuse into the photocyte, which holds the bioluminescence reactants.

Species of fireflies of the genus *Pteroptyx* (Coleoptera), notably in Southeast Asia, form male groups in which the individual insects flash in synchrony. The males of these species, in isolation, can flash regularly with almost constant intervals between flashes. If an individual detects a flash within a critical period of having produced its own flash, it immediately flashes again. As the individuals produce flashes at regular intervals, this resetting rapidly results in synchronous flashing by all the insects.

25.7.4 Significance of light production

Sexual communication is the principal role for self-luminescence in most luminous insects. Light signals are used in two basic ways in Lampyridae (Coleoptera). In some species, such as *Lampyris*, the female is sedentary and attracts the male to herself; in other species, such as *Photuris* and *Photinus*, one sex, usually the male, flies around flashing in a specific manner. Flash duration and the interval between flashes are often characteristic for the species (Fig. 25.14) and flashing is associated with distinct flight patterns. For instance, male *Photinus pyralis* produce a flash lasting about 500 ms at six-second intervals. During the flash the male climbs steeply and then hovers for about two seconds. If a female flashes 1.5–2.5 seconds after the male flash, he flies toward her and flashes again three seconds later. He does not respond to flashes occurring after different time intervals. Repeated flashing sequences bring the male to the female. Precise timing requires a very well-defined time marker and flashes begin or end (sometimes both) sharply; these transients probably provide the

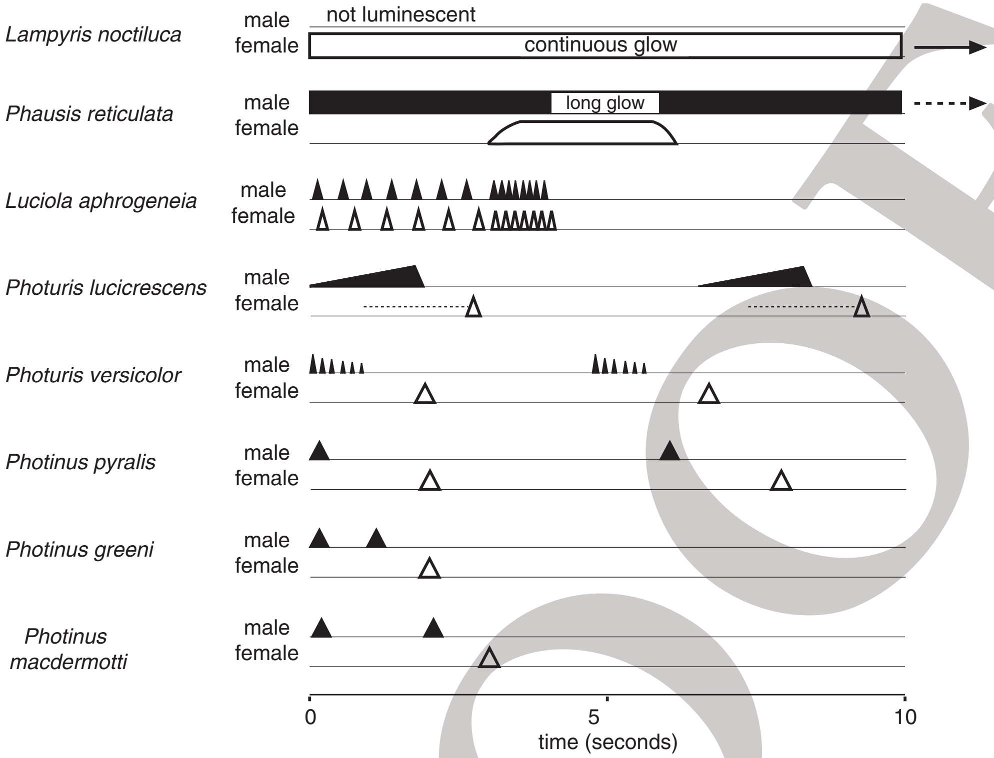


Figure 25.14 Flash patterns of different fireflies (Lampyridae). The height of each symbol represents the intensity of the flash; the shape shows intensity rising to a maximum and then declining. The dotted line in female *Photuris lucicrescens* shows the interval during which the female flash may occur (based on Case, 1984).

temporal signals. Females of *Pteroptyx* are attracted by the flashes of the male group.

After mating, the females of some *Photuris* species change their behavior so their patterning of flashing comes to resemble that of females of other species. In this way they lure males of those species on which they then feed. The luminescence of *Arachnocampa* (Diptera) larvae also serves as a lure, attracting small insects into networks of glutinous silk threads on which they become trapped. The larvae then eat them.

In railroad worms (Coleoptera) the lateral light-producing organs may be suddenly illuminated if the insect is attacked, and they possibly have a defensive function. It is suggested that the red head light provides these insects with illumination, presumably when they are searching for the millipedes on which they prey. The relatively long wavelengths emitted by this organ may not be visible to most other insect predators, but the eyes of the railroad worms probably can detect them.

The sensitivity of the dark-adapted compound eyes of fireflies varies diurnally, as it does in at least some other insects. It increases rapidly by about four orders of magnitude at a time approximately corresponding with the time at which the insects normally flash.

Summary

- There is a spectacular variety of insect color patterns, such as in the butterflies, dragonflies and beetles. The ways in which butterflies, for example, generate iridescence by physical structures shows nature's nanotechnology at its best. The way in which pigment patterns are generated by the interaction of morphogens and response thresholds in flies and butterflies has become a model system in evolutionary developmental biology.
- Insects use visual color signals in a large variety of behavioral contexts, such as the recognition of mates and conspecific competitors, but also to deter predators – and signals are not always honest, such as in butterflies displaying eyespots and harmless flies mimicking the body coloration of wasps.
- Some color signals are constitutively displayed, whereas in other species they are only displayed when necessary, either to display to potential mates or to deter avian predators with a sudden display of an eye-like pattern.
- Some species operating in dim light or darkness generate their own light, mostly for purposes of mate attraction, but also to deter predators or indeed to attract prey.
- In many other cases, insect body coloration serves not to attract attention, but the opposite – to blend in with the substrate to minimize the chances of detection by predators. Some insects can change color by remarkable physiological and morphological mechanisms when necessary.

Recommended reading

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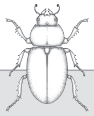

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