The use of colour is ubiquitous in nature. Evolution has produced complex visual organs, as well as systems that cause a variety of colourful optical effects. Vision is considered a fundamental sense in a vast majority of animals. The struggle for survival has induced the creation of a large diversity of systems to manipulate environmental light (pigments, nanostructures). Butterfly wings have perhaps the most diverse coloration in the animal kingdom. As described in Chapter 1, the colour of an intact butterfly wing is however a complex optical phenomenon.

In many butterfly species, dorsal and ventral sides of the wing can have very different patterns. When observed with a microscope, wings resemble pointillistic paintings where each point is formed by a coloured scale. How to study the fine structure and optical properties of single scales is treated in Chapter 2.

Scales are on both sides of the wing substrate arranged in partially overlapping rows of cover and ground scales. Incident light thus is reflected and transmitted by five layers. Chapter 3 shows how the stack of five layers determines wing coloration. We have investigated a number of accessible species of the butterfly family Pieridae. A rather high wing reflectance, up to 70%, is due to the superposition of effectively reflecting single scales. But, why are there two overlapping scales and not more? We have investigated intact, denuded and semi-denuded wings of the Small White, *Pieris rapae rapae*. Experimentally measured reflectance and transmittance spectra were interpreted with a theoretical model for a pile of reflecting plates. We concluded that the actual system of two superimposed scales on both wing sides is close to optimal. By adding more layers (scales), the reflectance does not increase substantially; but the weight does. However, some of the shiniest butterflies like *Morpho aega* or *Morpho cypris* have on their dorsal wings only one layer of blue scales, which scatter light coherently, causing iridescence. The reflectance is very high, and two overlapping scales can produce other effects, to the detriment of the iridescence (e.g. in *Morpho peleides* and *Morpho deidamia*).

Although the architecture of a scale depends upon the species, general characteristics can be recognized. A scale generally consists of two laminae, the one facing the viewer (abwing) and the one facing the wing proper (adwing), which are connected by trabeculae, also called pillars. The lower lamina is rather flat and unstructured, but the upper lamina is typically formed by densely spaced

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**Summary and general discussion**

The use of colour is ubiquitous in nature. Evolution has produced complex visual organs, as well as systems that cause a variety of colourful optical effects. Vision is considered a fundamental sense in a vast majority of animals. The struggle for survival has induced the creation of a large diversity of systems to manipulate environmental light (pigments, nanostructures). Butterfly wings have perhaps the most diverse coloration in the animal kingdom. As described in Chapter 1, the colour of an intact butterfly wing is however a complex optical phenomenon.

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ridges, connected by crossribs. The scales of the family Pieridae have an unusual feature. The ridges and crossribs are adorned with beads. When incident light is incoherently scattered by the scale structures a white colour results, unless there is pigment that absorbs in a given wavelength range.

After having explored the macroscopical effect of the scale distribution on the wing, we have studied the anatomical and optical properties of single scales. Chapter 4 presents measurements on the wings of two subspecies of _P. rapae_. Spectrophotometric analyses of the wings of the European subspecies, _P. r. rapae_, show virtually no dependence on sex; the wings of both sexes strongly absorb ultraviolet light. However, the Japanese subspecies, _P. r. crucivora_, displays a definite sexual dichromatism. As in the European case, dorsal wings of males absorb UV light, but those of females do not. Examination of the microscopical structure of single scales, from different areas in dorsal and ventral wings, reveals anatomical differences in male and female scales. Beads appear to be absent in female dorsal scales. Thus, by comparing the structure and optical properties of scales, we concluded that the scale pigment is concentrated in the beads. Furthermore, they appeared to have a dual role in butterfly coloration. They store the pigment and also enhance the scattering of light outside the pigment absorption range. This spectral range is rather wide for the scales of _Pieris rapae_, as its pigment, presumably leucopterin, absorbs only ultraviolet light. For other coloured species of Pieridae, the spectral range narrows, since their beads contain pigments that also absorb in the blue, green or yellow wavelengths, causing yellow, orange or red scales.

Scales of pierids have another peculiarity; the distribution of beads, and consequently of the pigment, is not uniform throughout the scale. Chapter 5 demonstrates that instead, the granule density increases gradually from the base of the scale to the tip. Spectrophotometric analyses show also a gradient of the scale reflectance, proportional to the bead density. Extreme gradients can be artificially produced in scales by extraction of pigment with aqueous ammonia. Reflectance spectra of different areas of those scales permitted to quantify the relative contribution of the two scale laminae to the scale reflectance. Experiments with manipulated overlapping scales were performed, which allowed to elucidate a functional reason for the scale gradient. By having a non-uniform distribution of beads, with beads being high in the area not covered by neighbouring scales (the base), incident light is effectively reflected.

Pigmentary coloration, also called chemical coloration, usually produces (in butterflies) yellow to red colours, that is, the material reflects is in the long-wavelength part of the visible spectrum. Besides pigmentary coloration, another way of generating colour is to produce ordered nanostructures, which because of their regularity create coherent light scattering. This physical or structural coloration usually produces ultraviolet, blue or green colours. As illustrated in
Chapter 6, the scale colour is either determined by its pigmentation or its structural organization, or by both. We have compared the spatial reflectance profiles of various species. We found that scales of white pierids (Delias nigrina and Pieris rapae) scatter light randomly, in close agreement with Lambert’s cosine law, which can be well understood from the randomly distributed beads. Since pierid scales with different colours do not differ in bead distribution but only differ in the type of pigment stored in the granules, white, yellow, orange or red scales will have a similar scattering profile. The Morpho butterfly scales, on the other hand, have elaborated ridges that form a system of multilayers. The system causes constructive interference of blue wavelengths (normal illumination), and so blue light is specularly reflected. The organization of the multilayers in the spaced ridges causes also diffraction. When the multilayers are not localized in the ridges but in the body of the scale, between the upper and lower laminae, the reflected light is not diffracted. Instead, light is backscattered in a narrow spatial angle. That is the case for the green-yellow scales of the moth, Urania fulgens. The purple scales in the dorsal wing tips of the pierid male Colotis regina act similarly as the Morpho scale in the blue, due to multilayers in the ridges. The scattering in the red occurs as in the Pieris case, because the scales have ridges with multilayers and contain beads with pigment that does not absorb in the red wavelength range.

This thesis has elucidated the optical and structural mechanisms that determine the wing coloration of notably the pierid butterflies, but also the optical characteristics of some other species with differently structured wing scales have been clarified. The macroscopic features of butterfly wings are now beginning to be understood from the microscopic level. Many butterfly species have spectral properties that require further studies, for instance the pigmentation of nymphalids and the colours produced by the photonic crystals occurring in the wing scales of lycaenids and papilionids.