Chapter 1

General introduction

Many plants display brightly colored flowers to provide a visual signal to potential pollinators (Faegri and Van der Pijl 1979). The color of flowers is often studied in conjunction with their pollination system, providing valuable insight into the complex nature of plant-pollinator interactions. An important finding is that flower color is often optimized to attract a specific pollinator (Kevan and Baker 1983, Chittka and Menzel 1992, Dyer et al. 2012, Papiorek et al. 2015). For example, bee-pollinated flowers are very rarely red, presumably because bees have minimal spectral sensitivity in the long wavelength range. In contrast, bird vision is often sensitive up into the long wavelength range, and indeed many bird-pollinated flowers are red (Lunau et al. 2011, Shrestha et al. 2013). Despite considerable knowledge of the functional aspects of flower coloration, the optical mechanism leading to flower coloration, i.e. the complex interaction of light with the petal’s inner components, has so far received much less attention.

Flower coloration is generally due to incoherent light scattering by irregularly structured petal components that contain pigments. Pigments modulate the reflected light by absorbing in a specific wavelength range, and the backscattered light in the complementary wavelength thus determines the hue of the flower. For example, the yellow color of many flowers is often due to blue-absorbing pigments, such as carotenoids. The efficiency of the pigmentary filtering depends on the concentration and localization of the pigment. Flowers with a high concentration of floral pigments will reflect light of a higher spectral purity (saturation) than flowers with a low concentration of pigment. When the pigment is deposited on one side of the flower, only the pigmented side will have a distinct color and the unpigmented side often has a much paler color.

Generally, backscattering of light occurs in a medium when the incident light propagates through structures with different refractive indices. In the case of flowers these can be vacuoles, for example (Fig. 1). When the scattering components are randomly structured, the resulting coloration is angle-independent, that is, the light is diffusely reflected and the visual signal is
similar in a wide angular space. Light that is neither absorbed nor reflected will be transmitted through the flower. The proportion of backscattered and transmitted light depends on the structure and thickness of the flower. For example, a thick flower that contains many, densely packed cell structures will scatter more light than a very thin flower that contains only few cell structures.

In addition to pigmentary coloration, structural coloration can occur when structures are regularly ordered with a periodicity in the sub-micrometer range, i.e. in the order of the light wavelength. Typical for structural coloration is that the color changes with the angle of observation or illumination, a phenomenon called angle-dependent coloration or iridescence (Srinivasarao 1999, Kinoshita 2008). Structural coloration and iridescence are found in many animals, for example in beetles, butterflies and birds (Vukusic and Sambles 2003, Seago et al. 2009, Stavenga et al. 2010, Wilts et al. 2014), where it is often considered to have a signaling function, e.g. to attract a partner.

Structural coloration and iridescence were also reported to occur in flowers with specific surface structures (Whitney et al. 2009, Vignolini et al. 2012). Whitney et al. (2009) suggested that the cuticular ridges found on the petals of many flowers act as a diffraction grating, yielding an iridescence signal that acts as a cue to pollinators. The glossy appearance of many buttercup flowers was described to be due to a very smooth and thin upper epidermis that together with an underlying air layer acts as a multilayer (Vignolini et al. 2012). The visual signals of surface structures were, however, studied independently of the visual signal due to diffuse pigmentary coloration, leaving the contribution of structural coloration and iridescence to the overall visual signal unknown.
A considerable amount of work on both pigmentary and structural coloration of plants was done by Dr. David Lee, who provided an elegant overview on the different aspects of leaf and flower coloration in his book *Nature's palette, the science of plant color* (Lee 2007). Lee extensively described the great diversity by which flowers achieve coloration, including many examples of different surface structures and pigment localizations. Yet, a quantitative analysis of how the different components of the flowers contribute to the overall visual signal of flowers remains unknown. For example, the dependence of the efficiency of the pigmentary filtering on different pigment localizations and the functional importance of the differences in the amount of scattering have never been examined.

The aim of this thesis is twofold: to provide an in-depth overview of the different optical mechanisms of flower coloration, and to study the functional significance of different optical mechanisms and plant reproductive strategies. The chapters of this thesis can subsequently be divided into two sections: the first section, comprising chapters two to five, treats the various optical mechanisms of flower coloration, and the second section, comprising chapters six to nine, discusses the biological implications of flower coloration and plant reproductive strategies.

**Chapter 2** provides an extensive study on the pigmentation and light backscattering of the flowers of *Nolana paradoxa*, in which the pigments are deposited asymmetrically, i.e. only in the upper epidermal layer. Measured reflectance and transmittance spectra are interpreted using anatomical data and a modeling approach, where the flowers are considered as a stack of layers, each layer with different reflection and absorption characteristics.

**Chapter 3** provides an overview of the common coloration principles of flowers. It was found that flowers greatly differ in thickness as well as pigment concentration and localization, yet the amplitude of the reflectance spectra (i.e. the fraction of the reflected light, which determines the flower’s brightness) is rather constant. The model developed in chapter 2 is applied to study the effect of different scattering parameters, thickness and pigment localizations on the visual signal of flowers. The resulting different visual signals are interpreted using two well-established insect vision models.

**Chapter 4** extends the optical and anatomical approaches of the previous chapters in a detailed study of the origin of the simultaneously glossy and matte flowers of buttercups (*Ranunculus* spp.). It was found that the coloration of glossy buttercup flowers is due to a unique combination of a very thin, pigmented upper epidermis, which acts as a thin film reflector, and an underlying starch layer that effectively backscatters incident light. It was furthermore found
that the contribution of the gloss to the visual signal is surprisingly small, but that the gloss might enhance light reflection to the center of the flowers, thereby increasing the temperature of the reproductive organs.

The contribution of structural coloration to the overall coloration of flowers is continued in Chapter 5. In this chapter, the surfaces of flowers of 50 plant species were studied using light and scanning electron microscopy. An imaging scatterometer was used to examine the spectral and spatial distribution of the reflected light. The contribution of the surface reflection relative to the reflection of the whole petal was examined by measuring both replicas of flower surfaces and intact flowers. It was found that flat and smooth surfaces reflect light directionally, conically shaped surfaces reflect light diffusely and striated surfaces can act as a diffraction grating, creating an iridescence signal. However, the contribution of iridescence to the overall petal reflectance is minimal and therefore the visual signal of flowers is virtually solely due to pigmentary coloration.

The second section of this thesis focuses on the biological implications of flower coloration and plant reproductive strategies. The first chapter of this section, Chapter 6, continues with the role of iridescent signaling of flowers. Specifically the biological relevance of floral iridescence relative to the overall visual signal of flowers is discussed.

Chapter 7 studies the role of flower color in a large plant community in the context of plant-pollinator signaling. Using an exhaustive, previously published database, the degree of competition for pollinators by plants in a Dutch nature reserve was determined. It was found that pollinator-competing plants exhibit more spectral dissimilarity than non-competing plants.

Chapter 8 discusses the fate of reproductive structures of clonally propagating plants. Because sexual traits in clonal plants have lost their significance, they are released from selection and generally decay. This chapter provides an overview of sexual trait decay in clonal plants and discusses different evolutionary mechanisms behind the observed trait decay.

Finally, in the synthesis of Chapter 9, the role of spectacular visual signals in plant-pollinator signaling is debated. Fluorescence and polarization patterns of flowers were previously suggested to act as a cue in plant-pollinator signaling. Similar as floral iridescence, the visual signal of these mechanisms is, however, negligible compared to the overall visual signal, due to pigmentary coloration.
References


