Investigation of the Screening Pigment System in the Compound Eye of the Moth *Agrotis segetum* (fam. Noctuidae) by Visible Reflectometry

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The functional properties of the light adaptation system in the superposition eye of the moth *Agrotis segetum* have been investigated by reflection spectroscopy. The spectrum of the reflected light from the tapetum of dark adapted eyes had a peak at about 580 nm corresponding to a spacing between the reflecting layers in the tapetum of 145 nm. During light adaptation of the eye the observed reflectance changes could be explained by light extinction in one screening pigment. The shape of the extinction spectrum of the screening was constant throughout the adaptation process and after a lag phase the optical density of the pigment in the light path increased linearly with time. The screening pigment caused light extinction both by absorption and to some degree also by scattering. The absorption spectrum of the screening pigment had a broad maximum about 590 nm and the scattering efficiency of the pigment particles seemed to be nearly independent of the wavelength.

Introduction

Most insect eyes contain a screening pigment which is localized in both the primary and the secondary pigment cells [1]. Light and dark adaptation is usually caused by considerable motion of the screening pigment within the cells [1, 2].

The most extensive screening pigment migration is found in the eyes of the superposition type [3]. The movement of the screening pigment in moth superposition eyes take place in specialized secondary pigment cells extending along the ommatidial length axis. During dark adaptation the pigment migrates from a dispersed, light adapted state to a distally concentrated and dark adapted state [4−12]. In the dark adapted state light from several ommatidal lenses is able to reach each receptor [13]. Consequently the effective aperture increases during dark adaptation [13, 14]. By means of a tapetum situated in the proximal region of the eye incident light is reflected and a glowing spot or a luminous pseudopupil can be observed on the surface of the eye [1, 15].

Several authors have used the size of the luminous pseudopupil as a measure of the state of the screening pigment system in moths [16, 17]. In these works a direct relationship between the size of the pupil and the position of the screening pigment have been assumed.

In this paper the characteristics of the light reflected from the eye of a noctuide moth will be used to investigate the role of the screening pigment in the process of light adaptation.

Materials and Methods

Animals

Specimens of the turnip moth, *Agrotis segetum* (fam. Noctuidae), were hatched from pupae supplied from The State Plant Pathology Institute, Denmark. The adult animals were kept in darkness at 4 °C before used in the experiments.

Methods

The basic experimental apparatus for measurements of the reflectance from the insect eye is shown in Fig. 1. The whole insect was mounted in a hollow cylinder by paraffin wax, and the mounting system was kept at 15 °C by circulating water from a temperature-regulated bath. The eye was illuminated by near parallel light from a halogen lamp. The illuminated area was adjusted to the same size as that of the eye. The light reflected from the eye was directed
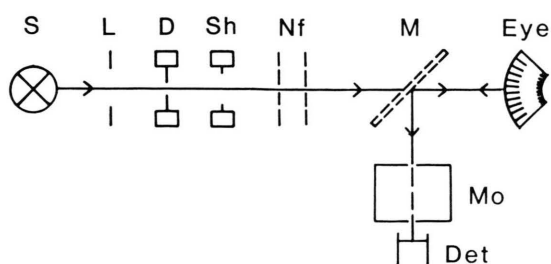
Fig. 1. A schematic drawing of the experimenting apparatus. D: diaphragm; Det: detector; L: lens; M: semitransparent mirror; Mo: monochromator; Nf: neutral density filters; S: light source, Sh: shutter.

by means of a semitransparent mirror perpendicularly to the incident beam into a monochromator, where the spectral composition and the intensity of the reflected light could be analyzed.

The insects were adapted to darkness before each experiments. The light source, which both served as test- and actinic light, was turned on when the fully dark adapted state was reached and the reflectance spectrum was recorded as a function of time after onset of light. The mean of two reflectance spectra, obtained by back and forward scanning over the wavelength range, was used in order to compensate for temporal changes during the scanning. In some cases the intensity of the reflected light at a certain wavelength was continuously recorded during adaptation.

Results

In Fig. 2A the spectral composition of the reflected light from the eye of *Agrotis* is shown at different times during the light adaptation process. The reflection spectrum from the dark adapted eye had a broad peak around 580 nm. As the eye gradually approached the light adapted state, the reflectance was substantially reduced in the visible region and a rather abrupt change in reflectance from the eye in the wavelength range from 700 to 750 nm became apparent. In Fig. 2B these reflection spectra were converted into absorption like spectra by means of a $\ln(I_0/I_r)$-transformation where I_0 is the intensity of the light source and I_r is the intensity of the reflected light. In Fig. 3 differences between such absorption spectra are shown and it is seen that they all have a similar shape. These differences in absorbances are due to temporal changes within the eye during the adaptation process.
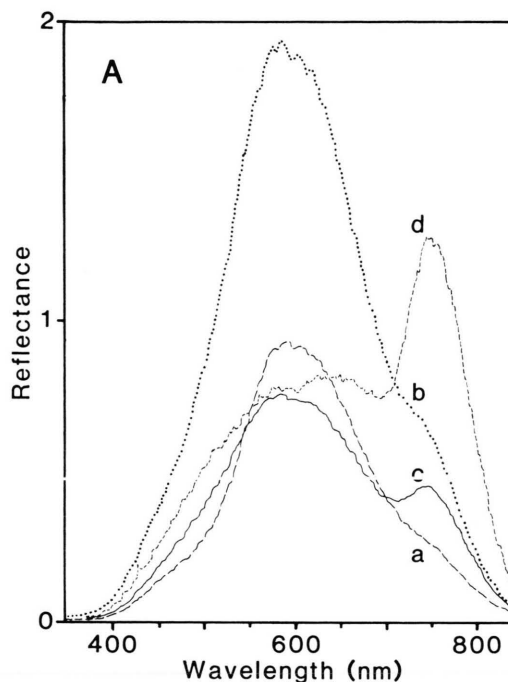

Fig. 2A. Uncorrected recordings of reflection spectra from the eye of *Agrotis segetum*. The curves were recorded at different times after the onset of dim white light in the order a–d. Scaling of the intensity axis relative to curve a: ×3.33 for curve b and c, ×10 for curve d.
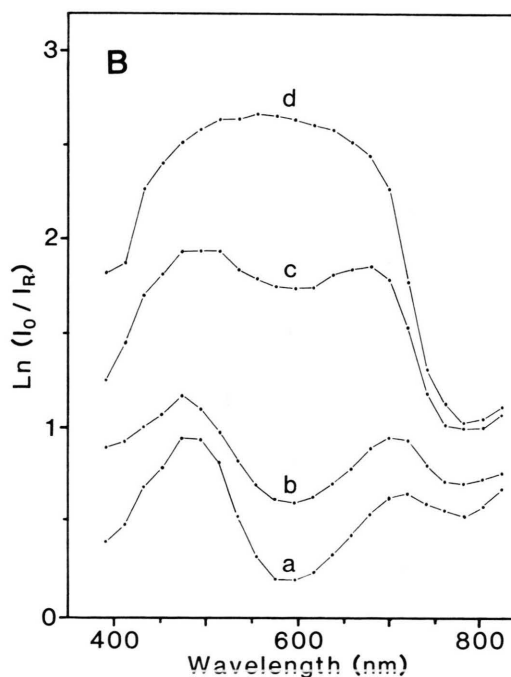

Fig. 2B. The reflection spectra from Fig. 2A transformed into absorption like spectra. The ordering of the curves is as in 2A.
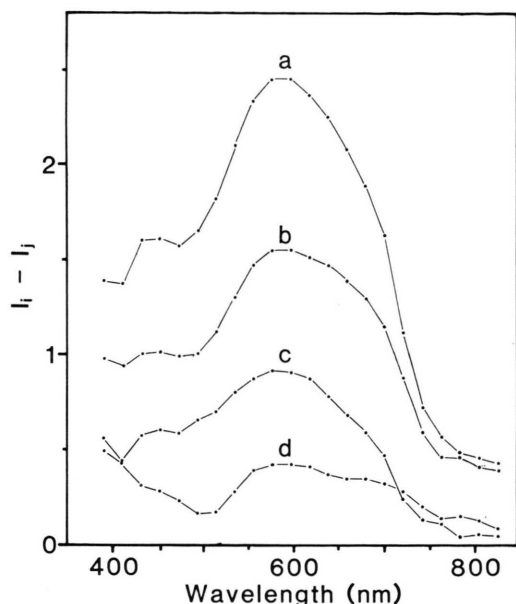
Fig. 3. Differences between absorption curves from Fig. 2B. From each curve in Fig. 2B the initial absorption curve was subtracted.

Fig. 5. Logarithmic representation of the decrease in reflectance from the eye of *Agrotis* in response to dim monochromatic light. The curves have been recorded at different times in the same animal. Stimulus wavelength: a = 480 nm, b = 600 nm and c = 375 nm.
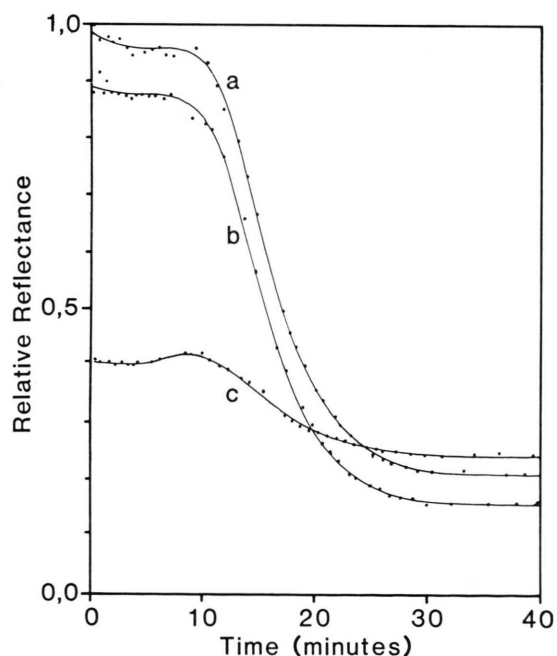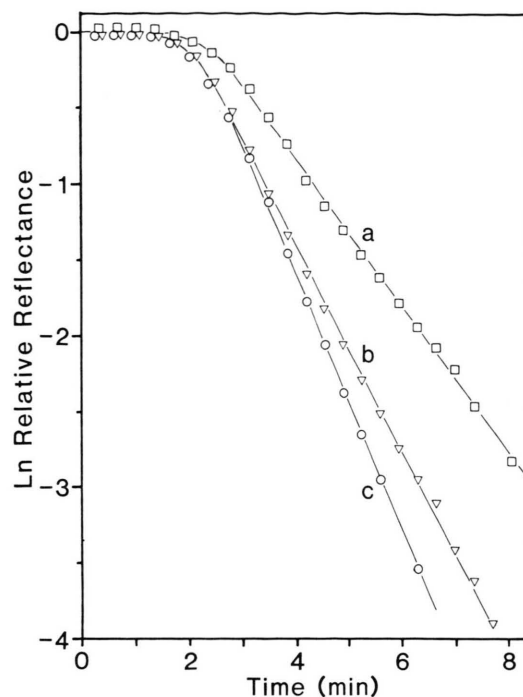
Fig. 4. The time course of light adaptation in response to dim white light. The reflectance from the eye have been recorded simultaneously at three different wavelengths: a = 650 nm, b = 550 nm and c = 750 nm.

In Fig. 4 the temporal changes in the reflectance at 750 nm, 650 nm and 550 nm were measured after the onset of white light to a dark adapted eye. After a latency period of about 10 min the intensities of the reflected light at these wavelengths decreased in an exponential way with the same rate constants and approached the steady state levels.

In the experiments shown in Fig. 5 monochromatic light stimuli were applied and the reflectance was recorded continuously in each case during light adaptation. Also in this case the decay of the reflected light was exponential after a latency period of 2 min. The efficiencies of the stimulating light were somewhat different in the three cases.

Discussion

The anatomical structures which constitutes the insect eye may grossly be divided into static and dynamic ones. It was the dynamic or mobile parts of the specimen which were responsible for the temporal changes in the reflectance. The surface of an insect

eye, which is a static structure, is known to be anti-reflecting in moths [15]. The dynamic reflectance changes must therefore be caused by time-dependent processes inside the eye itself. The amount of screening pigment in the light path will of course vary with the degree of adaptation.
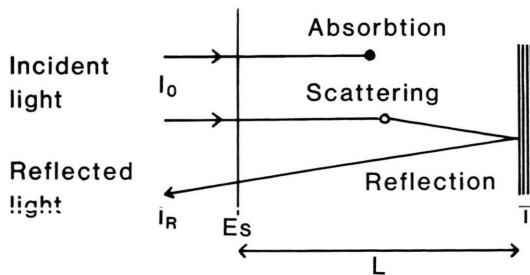

Fig. 6 A. Simplified description of the light attenuation processes in the insect eye. Es: eye surface; T: tapetum; L: optical path.
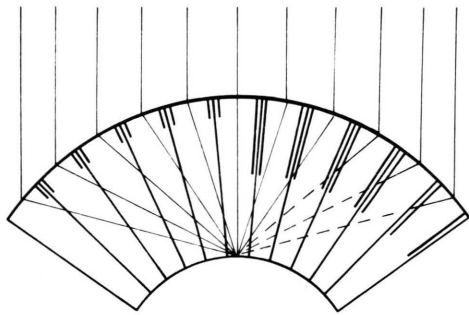

Fig. 6 B. A schematic description of the light propagation in the eye of the superposition type. Incident, parallel light will be refracted at each ommatidial crystal cone so that the light will be focused on only a few receptors. In the left half of the eye the screening pigment is in a dark adapted position which allows light from several ommatidia to reach one receptor. At the right side of the eye light from only one or a few ommatidia will reach the receptor, since the screening pigment is in a light adapted position.

The following simplified description of the light propagation in this insect eye is put forward: The incident light of intensity I_o, which is nearly totally transmitting the eye surface, may either be scattered or absorbed by pigments inside the eye (see Fig. 6). At the back of the eye there exists a reflecting structure with reflectance $R(\lambda)$, which may vary with the

wavelength λ. The intensity of the reflected light from this system will be:

$$I_R = I_o \cdot e^{-\varepsilon 2l} \cdot R(\lambda),$$

where ε is the extinction of the medium and $2l$ is the length of the light path, which is twice the distance from the surface of the eye to the reflecting tapetum. The extinction may further be divided into one part due to scattering (τ) and another due to absorption (ε'): $\varepsilon = \tau + \varepsilon'$.

The logarithm of the ratio between the incident and the reflected light will be:

$$A = \ln (I_o/I_R) = \varepsilon \cdot 2l - \ln R(\lambda).$$

If the reflector is a static structure, then the differences between the calculated absorbances (A) due to kinetic changes will be:

$$\Delta A = \Delta\varepsilon \cdot 2l.$$

Such differences, which were presented in Fig. 3, have all the same shape. This is typical of a case when only one pigment is present in the light path in varying concentrations.

This pigment starts to absorb at 750 nm because there is a sudden increase in these extinction differences given in Fig. 3 when the wavelength is lowered from this value. Maximum absorbance is reached for 590 nm. Below 750 nm there is an almost wavelength independent extinction which is typical of scattering at particles that are large compared to the wavelength of the light (larger than $1/10$ of the wavelength, Mie scattering). The same particles are both scattering and absorbing, since the changes in the visible and the infrared region of the extinction differences, within experimental error limits, are in proportion to one another.

The importance of the visual pigments in the light attenuation processes within the eye seems ruled out from the above considerations since the presence of only one pigment could explain the observed reflectance changes. However, it can only be stated from the above conclusions that the main light attenuation is caused by one screening pigment. Furthermore, in the dark adapted eye of the superposition type, the main light propagation is outside the rhabdom threads, since light on one receptor cell is collected from several ommatidia (see Fig. 6).

If one made, on the other hand, the hypothesis that light transmission were mediated by rhabdomeric waveguide structures in the *Agrotis* eye, the

position of the absorption edge in the extinction spectra in Fig. 3 would depend upon the degree of light adaptation. In this case the efficiency of the light penetration depends upon the difference between the indices of refraction of the inside and outside of the waveguide which will change in response to a redistribution of screening pigment. The results are therefore in contradiction to this hypothesis since the position of the absorption edge towards the infrared region is independent of the degree of adaptation.

The reflectance from a dark adapted eye, in which case the influence of the screening pigment to the overall reflectance is minimal, has a maximum at about 580 nm (see Fig. 2B, curve a). It is well established that the tapetum may act as a high reflectance structure [15]. This property is obtained by a stacking of layers of different index of refraction, and maximum reflectance is obtained when the thickness of each layer is one quarter of the wavelength. This implies that the mean thickness of each reflecting layer in the tapetum is approximately 145 nm. The width of the reflection peak is indicative of the difference between the indices of refractions of the two neighbouring layers and the number of such layers in the tapetum.

These results are also somewhat in contradiction to the pseudopupil concept. If the function of the screening pigment were similar to that of an ordinary iris only intensity changes and no spectral changes would occur during dark adaptation. However, these results seem to indicate that light intensity regulation is obtained by simple light extinction in one screening pigment which can move into the light path.

The Beer law expression for the intensity of the reflected light introduced earlier to explain the spectral changes during the adaptation process of the eye can also be expanded to account for the time dependence of the reflected light. This expression indeed describes the time dependence of the reflected light correctly when it is simply assumed that the concentration of the screening pigment in the light path increases linearly with time. This indicates that the particles during the adaptation move at a constant speed within the pigment cells. A random, diffusion controlled particle motion is not consistent with this case where the particle position changes linearly with time. A linear motion of the screening pigment during the adaptation process is more in line with the electrophoretic theory of granula motion [18] or a microtubuli controlled particle motion [19].

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