Structural Differences in the Tracheal Tapetum of Diurnal Butterflies

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The three-dimensional structure of the tracheal tapetum lucidum, its reflection properties and the resulting eye glow hue were studied in members of the diurnal butterfly families Pieridae, Nymphalidae, Satyridae and Lycaenidae. Two main groups of tapeta can be distinguished by structural and physiological differences. Whereas in pierids the main tracheal trunk at the bottom of the rhabdom bifurcates into two side branches before bifurcating again more distally, the tracheal trunk in the members of the family Nymphalidae, Satyridae and Lycaenidae investigated first divides into four side branches. A second bifurcation shortly after the first results in eight subbranches which are regularly arranged between adjoining receptor cells. The broad banded reflection colour from incidently illuminated tapetal structures (at the level of the first bifurcation) varies between and within families but does not change significantly within the same eye. Whereas in nymphalids, satyrids and lycaenids the eye glow hue corresponds with the colour of the tapetal reflection, in pierids it is dominated by the coloured receptor screening pigment.

Introduction

The phenomenon of eye glow is seen in a variety of animals, and is generally thought to be related to a reflecting surface found in the eye, the tapetum lucidum. In vertebrates three general types are known. A cellular tapetum, derived from modified choroidal cells is found in carnivores. The ungulates have a collagenous, acellular layer between the retina and choroid, while some fish and reptiles exhibit a retinal tapetum consisting of plates or clusters of guanine crystals in the pigment epithelium of the retina. Eye glow can also be observed in invertebrates. The only structure that could be responsible for this eye glow is the tracheal tapetum at the end of the rhabdom [1 – 3]. This structure is found in some diurnal butterfly species with apposition eyes [2, 3] as well as in nocturnal flying moths (*e. g.* [1, 4]). The hue of this eye glow varies between species. It has been suggested that the eye glow hue is determined by the tapetal structures [2]. In this study a comparison was made of the fine structure of the tracheal system in different diurnal butterfly families. In addition the observations were made on the colour of reflection from the tapetum itself to test Miller and Bernard's [2] theory of eye glow hue.

Materials and Methods

Members of the most common European diurnal lepidopteran families, Pieridae, Nymphalidae, Satyridae and Lycaenidae, were collected locally. Freshly caught animals were used immediately for the following treatments. The structure of the ommatidial tracheal system was investigated in the *light microscope* in several 1 μm longitudinal and cross section series (toluidine blue stained Araldite sections) from different eye regions. The fine structure of the tapetum and the pigmentation of the receptor cells were investigated by routine *electron microscopical techniques* [5, 6]. For the determination of *eye glow hue*, the intact butterfly was mounted on top of a goniometer and one of its compound eyes illuminated orthodromically via a half mirror. The *tapetal reflection colour* was determined from orthodromically illuminated eye slices, cut at the level of the first bifurcation [3].

Observations

The tracheal tapetum as found in butterflies shows anatomical characteristics. Whereas in nocturnal moths the tracheal branche entering the ommatidium divides into numerous fine tracheoles just above the basement membrane, to form the tracheal bush [4], in most of the diurnal butterflies the tracheal trunk branches into a few subbranches at the proximal end of the rhabdom.

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Two main tracheal branching patterns can be distinguished in diurnal butterflies (with the exception of the members of the family Papilionidae which do not have a tapetal system, Demoll [7], Miller and Bernard [2]. In members of the family Pieridae *(Pieris brassicae, P. napi, P. rapae, Colias australis Gonepteryx rhamni)* the main trachea entering the ommatidium divides at the proximal end of the rhabdom into two branches. At this point a dorsoventrally arranged septum is built, on which the chitinous helically arranged taenidiae lie. These taenidial platelets may serve to reinforce the trachea against possible collapse in the region of bifurcation. There are altogether 20 – 30 such taenidial platelets on each side of the septum.

After the bifurcation the two tracheal branches run distally, being uniformly distributed over the eye. Ordinary taenidial threads furnish the trachea in this region. Within the proximal third of the retina a second bifurcation takes place. The four resulting subbranches lie peripherally between the receptor cells 5/3, 3/6, 7/4 and 4/8 [5]. Further distally they branch into small tracheoles which extend to the periphery of the retina [3] (Fig. 1 A).

In the eyes that were studied from members of the families Nymphalidae, Satyridae and Lycaenidae the tracheal trunk divides at the bottom of each ommatidium into four occasionally side branches. At the center of the point of division a cross shaped septum emerges, on top of which the end of the rhabdom sits. The four tracheal branches always straddle the rhabdom and bifurcate a second time shortly after. Each of the eight resulting subbranches follows the ommatidium distally, positioned between adjoining receptor cells. In contrast to the peripherally arranged subbranches in pierids, they are located close to the rhabdom. The 20 – 30 helically arranged taenidial threads which furnish the tracheal trunk continue into the four side branches. Their pitch (15°), comparable to those in pierids, remains almost constant through the retina. As in pierids the taenidiae change from threads to platelets which touch the septal walls at the first tracheal bifurcation. The platelets extend across no more than one third of the tracheal branch and cover no more than 45% of its area. Due to this and due to the helical arrangement of the platelets the oxidative ventilation in this region remains functional (Fig. 1 B).

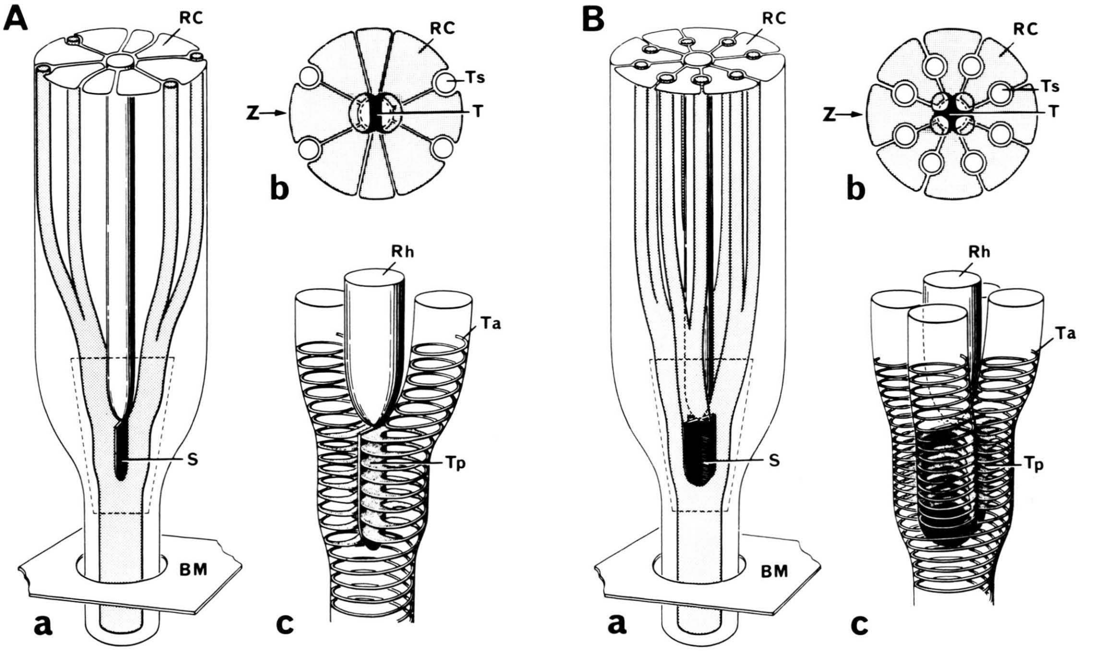

Fig. 1. Schematic diagram of the ommatidial tracheal system as seen in pierids (**A a**) and members of the families Nymphalidae, Satyridae and Lycaenidae (**B a**). (**b**) Cross section of the tracheal tapetum (**T**), superimposing receptor cells 1 – 8 (**RC**) and the tracheal subbranches (**Ts**) ; **z** indicates the horizontal axis of the eye. (**c**) Shows a detailed view (see inset in **a**) of the tracheal tapetum at the proximal end of the rhabdom (**Rh**). A septum (**S**) occurs between the dividing branches of the tracheal trunk upon which the taenidia (**Ta**) form platelets (**Tp**).

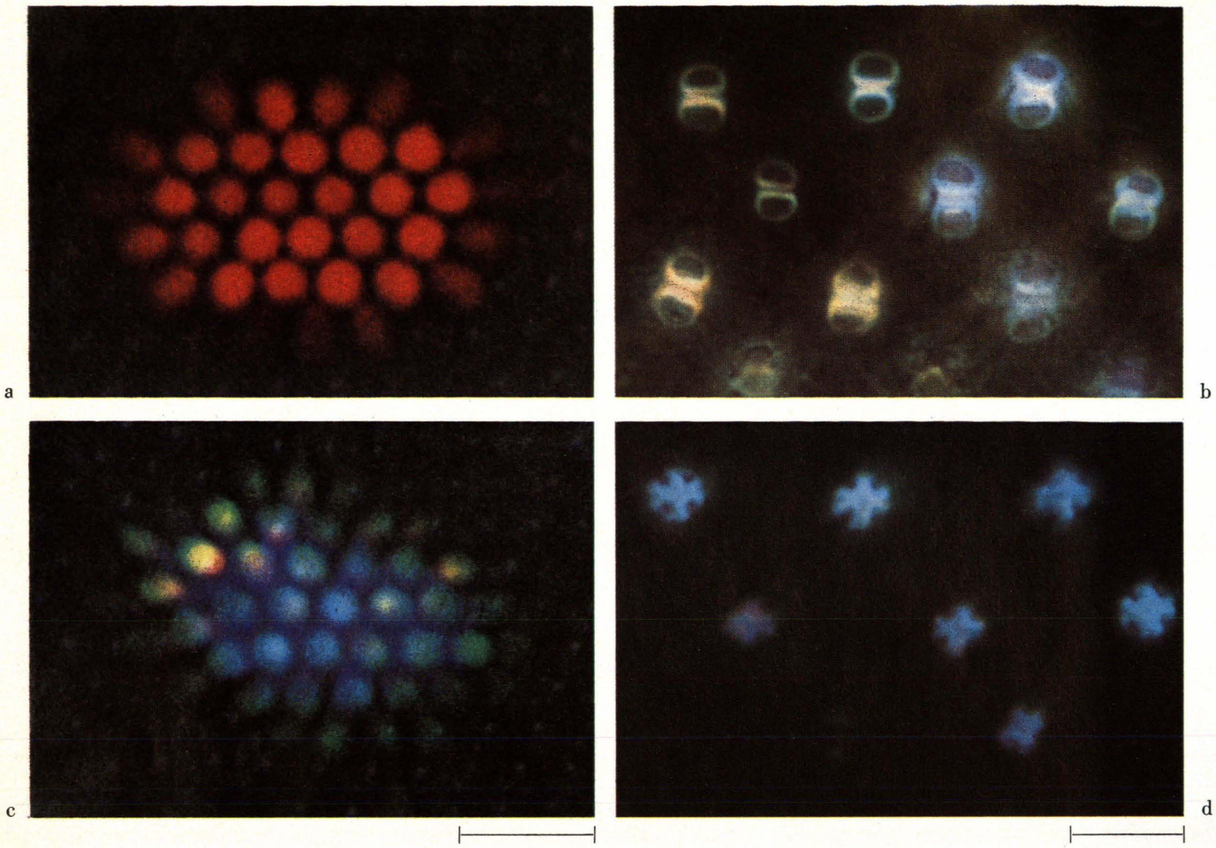

Fig. 2. Light micrographs of the eye glow hue in intact eyes; (**a**) of *Pieris rapae* (Pieridae), (**b**) of *Lysandra coridon* (Lycaenidae). The corresponding tapetal reflection is shown in (**b**) for *P. rapae* and in (**d**) for *L. coridon.* The pictures (**b, d**) were taken from incidentally illuminated tissue slices at the level of the first tracheal bifurcation. Scale **a, c** 50 μm; **b, d** 10 μm.

As in pierids the septa and tracheal branches are regularly distributed over the eye. In addition thickness and spacing of the taenidiae at various retinal levels, remains constant within one species, but varies within and between families. The functional significance of the differences in the structure of the tracheal system between families remains unclear.

To test whether the tapetal structures determine the eye glow hue, as Miller and Bernard [2] suggested, the reflecting colours of the tracheal tapetum, determined from incidentaly illuminated tissue slices, were compared with the eye glow hue observed in intact animals (Fig. 2). The colour of the tapetal reflection and the eye glow correspond in members of the families Nymphalidae, Satyridae and Lycaenidae, but not in members of the family Pieridae (Table I). The eye glow hue in the pierids *Colias australis* and *Gonepteryx rhamni* is red over

Table I. Eye glow hue and tapetal reflection colours in some common diurnal butterflies.

Observed species	Eye glow hue ventral/dorsal	Tapetal reflection colour
Nymphalidae		
Aglais urticae	*orange*-red	orange
Vanessa attalanta	orange	yellow-*orange*
Inachis io	orange	orange
Argynnis paphia	orange-*red*	*orange*-red
Satyridae		
Aphantopus hyperantus	turquoise	turquoise
Coenonympha arcania	turquoise	turquoise
Maniola jurtina	*green*-yellow	*green*-yellow
Lycaenidae		
Lysandra cordion	light blue	blue
Pieridae		
Pieris brassicae	red/turquoise	turquoise-green-yellow
P. napi	red/turquoise	
P. rapae	red/turquoise	
Colias australis	red	*green*-yellow
Gonepteryx rhamni	red	*green*-yellow

the entire eye, whereas the reflection colour of the tapetal structures is turquoise to yellow-green. The same phenomenon is seen in ventral and medial eye regions of the species *Pieris brassicae*, *P. napi*, and *P. rapae*. Histological and physiological observations now demonstrate that in the nymphalids, satyrids and lycaenids studied, eye glow hue is mainly determined by structural differences in the reflecting properties of the tracheal system (differences in the tracheal diameter and in the periodic alteration of refractive indicies, chitin and air); for the pierids however, the eye glow hue in most eye regions is determined, by coloured receptor cell screening pigments [3].

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