Eur. J. Entomol. **106**: 619–629, 2009
http://www.eje.cz/scripts/viewabstract.php?abstract=1496
ISSN 1210-5759 (print), 1802-8829 (online)

The eye of the parthenogenetic and minute moth *Ectoedemia argyropeza* (Lepidoptera: Nepticulidae)

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Key words. Nepticuloidea, Microlepidoptera, vision, facets, retinal ultrastructure

Abstract. *Ectoedemia argyropeza* (Zeller, 1839) possesses a compound eye that exhibits features of both apposition and superposition type eyes. Like apposition eyes, the eye of *E. argyropeza* lacks a clear-zone, which in superposition eyes separates the distal dioptric from the proximal light-perceiving structures. On the other hand, a tracheal layer around the proximal ends of the rhabdom as well as a well-developed corneal nipple array on the corneal surfaces are features that *E. argyropeza* shares with the larger moths. Unique, and so far only seen to this extreme degree in any insect, is the hourglass-shape of *E. argyropeza*'s rhabdom, in which two almost equally voluminous regions (one distal, one proximal and formed in both cases by seven rhabdomeres) are connected by a narrow waist-like region of the retinula. An eighth retinula cell, not participating in rhabdom formation, is developed as a basal cell, just above the basement membrane. The eye responds with photomechanical changes to dark/light adaptation, but while the proximal rhabdom moiety slightly expands (as expected) in the dark, the distal rhabdom increases its diameter only upon light-adaptation. Owing to the tandem position of the two rhabdom moities, it is in the light-adapted state that the distally-placed rhabdom is favoured, while the proximal rhabdom plays a more important role at low ambient light levels. With screening pigments withdrawn, tracheal tapetum exposed, and distal rhabdom diameters reduced, the proximal and in the dark enlarged rhabdom is then in a position to capture photons that have entered the eye through not only the ommatidial window above, but other facets as well even in the absence of a clear-zone and superposition optics.

INTRODUCTION

Ectoedemia argyropeza (Zeller, 1839) is an all-female, parthenogenetic species of moth (Fig. 1), whose compound eyes (because of *E. argyropeza*'s phylogenetic position as part of the Neolepidoptera) are expected to be of the clear-zone kind (Yagi & Koyama, 1963). The presence of a pigment-free zone between, on the one hand, the distal dioptric structures of the eye like corneae and cones and, on the other, the proximal light-perceiving elements of the retina like rhabdoms and retinula cells, is one essential requirement for superposition vision to occur (Land, 1981). The other requirement is the ability of the optics of a wide patch of facets to focus a parallel beam of incoming light rays onto one small spot on the retina (Land, 1981; Warrant & McIntyre, 1993). For this latter purpose mirror optics are employed by some decapod crustaceans, but lens cylinders with a radial gradient of refractive index are the rule in insects (Land, 1981). Vision with superposition optics is particularly useful for species in which the objective is not simply to increase sensitivity to light, but to retain a certain amount of resolving power (Warrant & McIntyre, 1996). Thus, it is not surprising to find compound eyes with large clear-zones mainly in species of insects that are nocturnal fliers. For some insect taxa the presence of a clear-zone can be said to be "diagnostic" (Exner, 1891; Yagi & Koyama, 1963; Døving & Miller, 1969; Horridge, 1971; Gokan & Meyer-Rochow, 2000).

For scarab beetles and moths the clear-zone eye is a characteristic feature. Yet, Meyer-Rochow & Gál (2004) on the basis of careful calculations, concluded that a compound eye had to reach a certain size before the clear-zone in concert with the optics of the dioptric structures could lead to an improvement of overall sensitivity. So far support for this conclusion has come only from species of scarab beetles with small body sizes and small eyes (Gokan & Meyer-Rochow, 2000). Caught in the constraints of their phylogenetic background, these beetles had found ways of 're-constructing' their eyes and changing the clear-zone anatomies of their ancestors into something more akin to the apposition eye. The clear-zones were reduced or absent and radial gradients of optical densities in cornea and cone unnecessary. The small species of scarabs had apparently found a way to abandon superposition as their eyes would not have had sufficient space to allow clear-zones to become effective in focusing light rays entering the eye through multiple facets.

However, although small scarab beetles have found a way to cope with their phylogenetic legacy of superposition vision, ways other than those exhibited by the aforementioned beetles are theoretically possible (Horridge, 1971). Towards this end, we decided to examine the eyes

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Fig. 1. Dorsal view of a museum specimen of a female *Ectoedemia argyropeza*. The orange scales on the head are brighter in living specimens. Scale bar: 2 mm (Photo: Marko Mutanen).

of *E. argyropeza*, for body length in this species of moth is rarely little more than 2 mm and, thus, superposition vision with the help of a clear-zone, according to Meyer-Rochow & Gál (2004) ought to be impossible given an isometric reduction of eye size. Still, being a flying insect, *E. argyropeza* must, of course, be able to avoid obstacles, to locate a suitable landing place, to perceive the approach of danger, and to detect ambient brightness fluctuations. In all of these tasks some photoreceptive abilities would clearly be advantageous, which is presumably why *E. argyropeza* individuals possess eyes at all.

The principal objective of this research was, therefore, to examine the structure and ultrastructure of the eye of *E. argyropeza* and to put on record how the eye differed from that of larger moths. Secondly, in order to provide some evidence that the eye was responding to changes in ambient light levels we conducted dark/light adaptation experiments, for photomechanical changes are known to take place in many insect eyes when the latter are exposed to darkness or a bright environment (Autrum, 1981; Meyer-Rochow, 1999).

MATERIAL AND METHODS

Animals

Ectoedemia argyropeza (Zeller, 1839) is a non-ditrysian moth with leaf-mining larvae that belongs to the family Nepticulidae. It is widely distributed south of the polar circle throughout Europe, excluding the Iberian Peninsula, southern Italy and the Balkans (Johansson et al., 1989). In Finland it is a univoltine, parthenogenetic species found as far north as Rovaniemi, achieving wing spans of 5.0–7.0 mm and reaching a body length of 2 mm or little more (Fig. 1). The host plant of *E. argyropeza* is aspen (*Populus tremula*). Pupae, attached to aspen leaves, overwinter among aspen leaf debris and hatch in Finland between 10 June and 15 July following a winter with at least some frosty nights. The life span of an adult is approximately one week. Feeding during this time does not occur.

For this research cocoons were collected in October by Dr. Marko Mutanen from aspen leaves in Taalintehdas (60°1.2′N; 22°30.7′E) and kept outdoors under a cover of snow until 10 th of January, when they were brought indoors into an environment of 20°C. Dozens of imagines appeared over a period of about 10 days a few days later. That *E. argyropeza* is not always that easy to obtain demonstrated our collections carried out the same way as before, but a year later. Only two individuals hatched from a large amount of pupae.

Scanning and transmission electron microscopy

For observations of the eye by scanning electron microscopy air-dried specimens were used. An Agar High-Resolution Sputter Coater (Agar Scientific Ltd, Stanstedt, England) was used to deposit an approximately 20 nm thick gold-palladium layer over the specimen, which was then examined under a Jeol JSM-6300F Field Emission Scanning Electron Microscope under a slow-scan digital image recording system.

For histological studies severed heads were pre-fixed in 0.1 M phosphate-buffered (pH 7.3) 1% glutaraldehyde solution plus 4% formaldehyde for 21 days. Thereafter the specimens were postfixed in 1% osmiumtetroxide for one hour, dehydrated in a graded series of acetone and embedded in Epon LX 112 (Ladd Research Industries, Vermont, USA) at a temperature of around 65°C for two to three days.

Nighttime dark-adapted specimens were kept in an opaque black tube for two hours (19.30–21.30 h) prior to being killed with chloroform and having their heads fixed in pre-fixative (see above), while daytime, light-adapted specimens were exposed to the light of a fluorescent lamp between 10.15 and 12.15 h, because daytime weather conditions in January, due to the winter season, provided only dim light from a cloudy sky.

Semithin sections for light microscopy were cut with glass knives on a Reichert-Jung Ultracut E microtome. Microscope slides were coated with 1:1 chrome gelatin (1% gelatin plus 0.1% chrome) and the semi-thin sections were stained with a drop of aqueous Toluidine Blue for a few seconds. Ultrathin sections were cut with diamond knives, picked up on 100 mesh pioloform-coated copper grids, and stained for 30 and 5 min with uranyl acetate and lead citrate, respectively. Observations were carried out under a Philips CM100 transmission electron microscope and images were captured digitally with a Morada

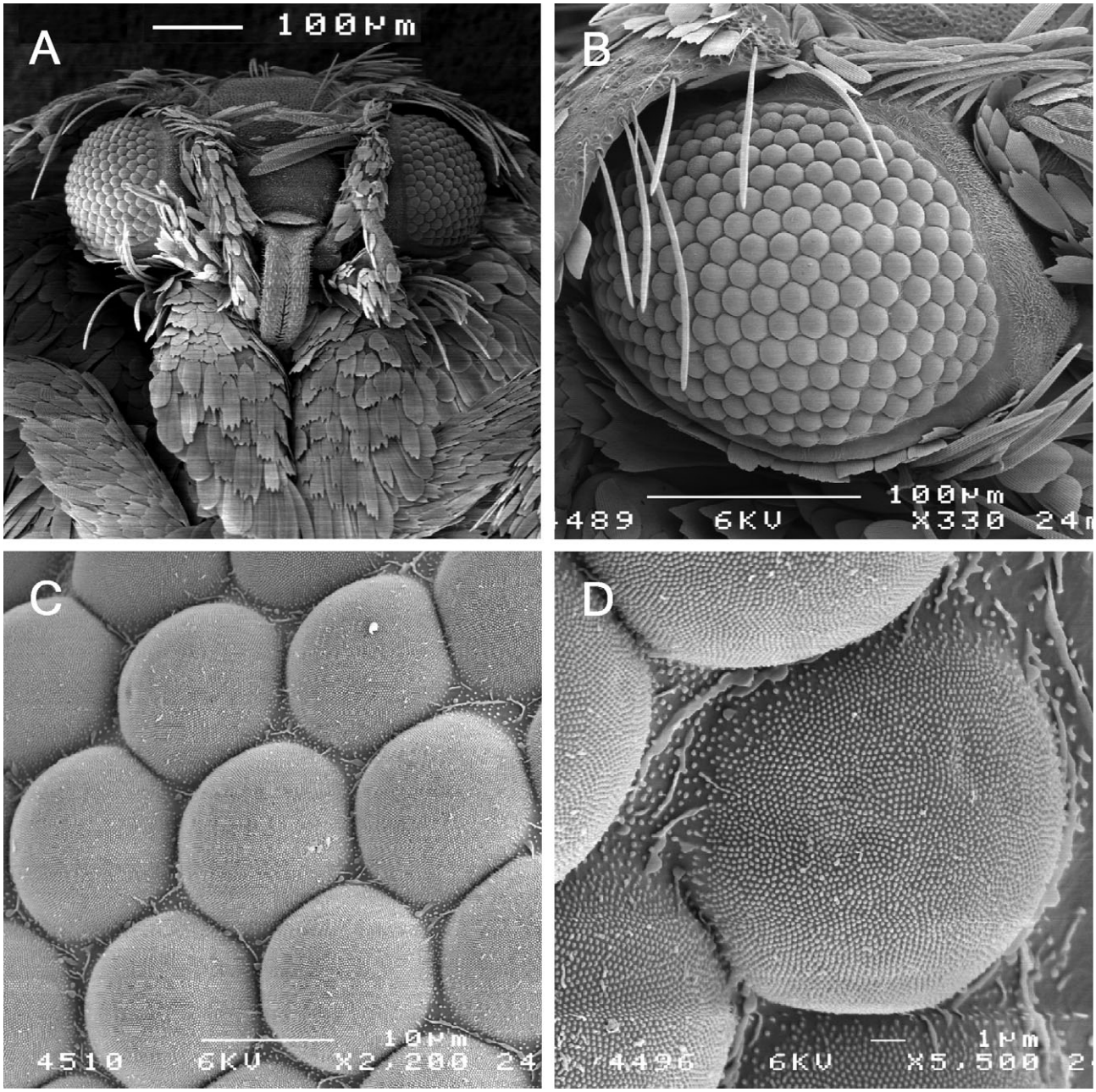

Fig. 2. Scanning electron micrographs of a critical-point dried specimen showing external features of the *E. argyropeza* eye at different magnifications (see scale bar in each photo). A – the two compound eyes are in a position to look downward; B – the size of the facets is constant throughout the eye; no interfacetal hairs are developed; C – the facets are developed as regular hexagons and their outer surface is covered by corneal facets; D – an individual facet, showing the regular corneal nipple array and the corneal bulge and more widely-spaced nipples in the interfacetal spaces.

CCD camera (Olympus GmbH, Münster, Germany) and Olympus iTEM software.

Statistical analysis

Analyses of the micrographs involved "ImageJ" open-source software (Rasband 1997–2008 ImageJ 1.40.). Where comparisons between two data populations had to be carried out, the independent sample t-test for normally-distributed data was used and Wilcoxon's rank sum test (a.k.a. Mann-Whitney U test) was used for data that were not normally distributed (Ranta et al., 2005). For statistical analyses open-source software R-2.8.0 for Windows was used.

RESULTS

Ectoedemia argyropeza has two laterally-positioned compound eyes, one on either side of the head (Fig. 2A). Each eye is lemon-shaped (Fig. 2B) and made up of 224 ± 11.5 ommatidia (n = 3). In dorso-ventral direction the eye extends across 206.4 ± 18 µm and in anterior-posterior direction it is 152.3 ± 13.5 µm wide (n = 4). These figures correspond to approximately 20 and 16 rows of facets, respectively. Both eyes peer downward and cannot be seen from above.

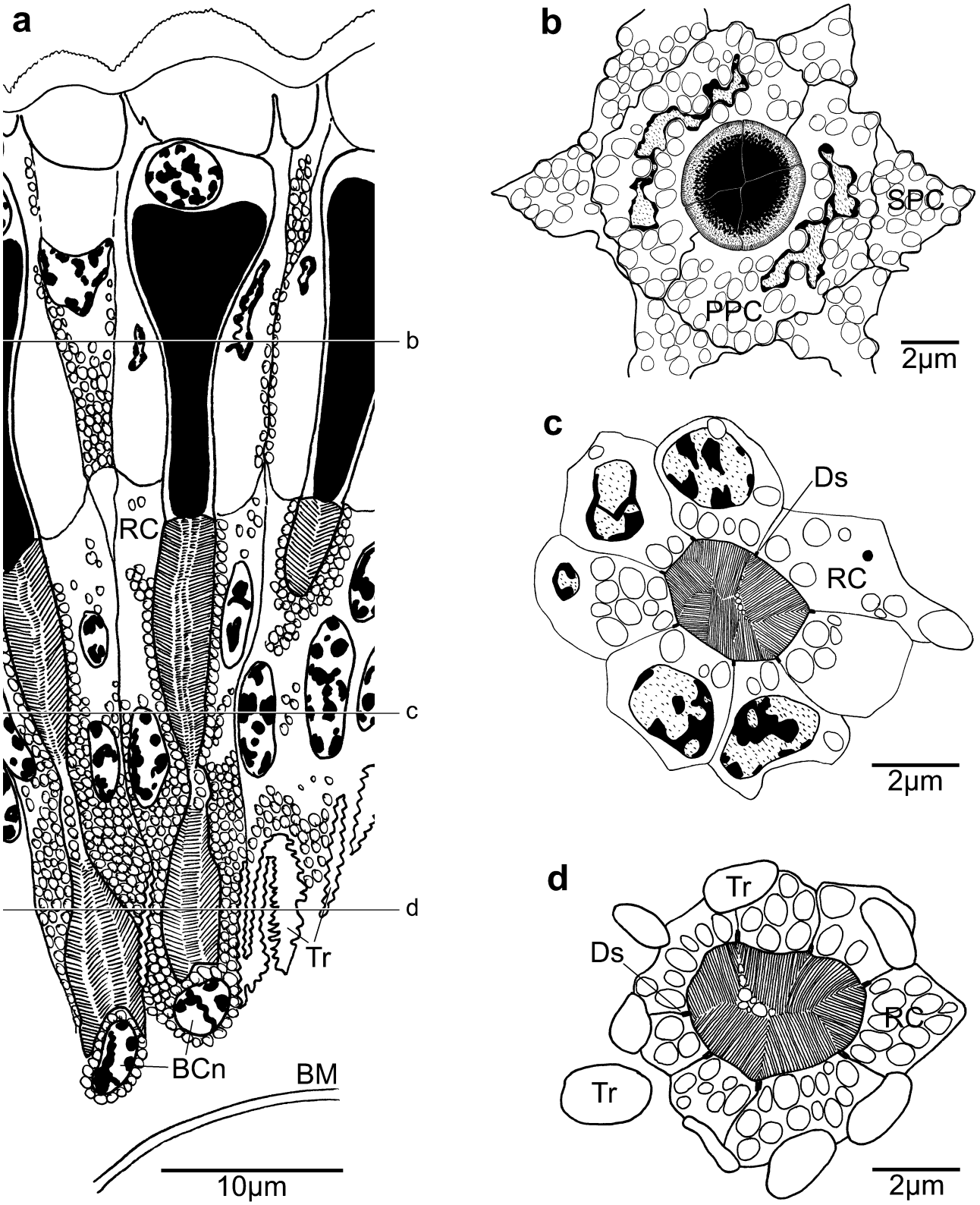

Fig. 3. Semi-schematic illustration of (A) a longitudinal and (B–D) three transverse sections across the planes indicated in (A). A – pigment granules (small circles) in the retinula cells (RC) line the rhabdom and are present in primary (PPC) and secondary (SPC) pigment cells. Ovoid mottled structures near the narrow waist of the rhabdom are retinula cell nuclei. The nucleus of the basal cell (BCn) is seen near the proximal end of the rhabdom above the basement membrane (BM). Tracheoles (Tr) are evident around the proximal rhabdom. B – transverse section showing the cone being surrounded by two primary pigment cells (PPC) with their nuclei and 6 secondary pigment cells (SPC). C – transverse section at the level of the distal rhabdom. Seven retinula cells (RC) contribute their rhabdomeres to the rhabdom. Desmosomes (Ds) connect adjacent retinula cells to each other. The microvilli of the rhabdom are oriented in three different directions offset to one another by 120 degrees. D – transverse section at the level of the proximal rhabdom. The surrounding tracheoles form a *tapetum lucidum*.

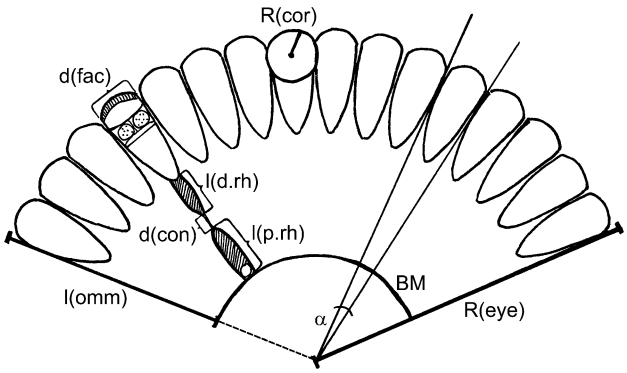

Fig. 4. Schematic drawing of a longitudinal section of an eye of *E. argyropeza*, giving information on some of the structures measured routinely. BM denotes the basement membrane and the conical structures symbolize the dioptric apparatuses. R(cor) – corneal outer radius of curvature; R(eye) – eye radius of curvature; α – interommatidial angle; d(fac) – facet diameter; l(omm) – ommatidial length; l(d.rh) – length of distal rhabdom; l(p.rh) – length of proximal rhabdom; d(con) distance between distal and proximal rhabdom.

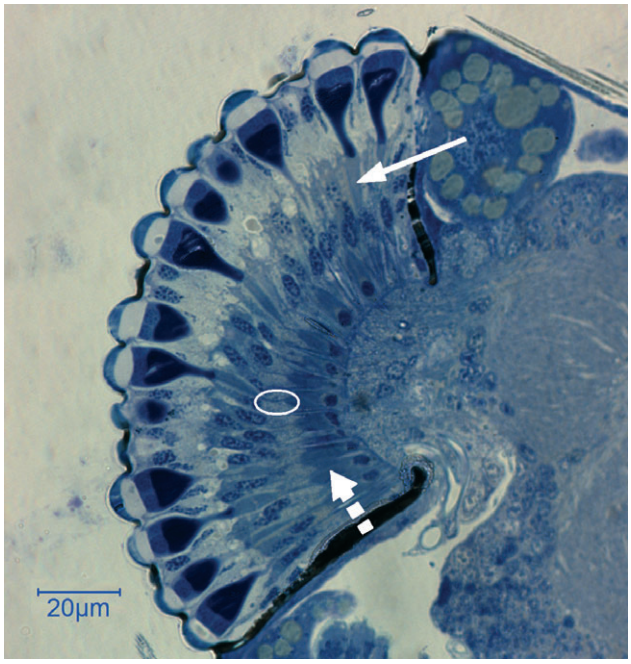

Fig. 5. Light micrograph of longitudinal section through the eye of *E. argyropeza*, showing hourglass-shape of rhabdom with distal layer (white solid arrow) and proximal layer (white broken arrow). The filled white oval in the centre indicates the narrow, waist-like region of the rhabdom. Cornea and cone layer (the latter with tapering proximal ends contacting the distal rhabdoms) are clearly visible.

According to the terminology introduced for facet morphologies by Deane (1932), those of *E. argyropeza* are favate rather than uvate, hexagonal in outline with strongly bulging external surfaces (Fig. 2C), that provide the ommatidial corneae with an outer radius of curvature of 5.40 ± 0.68 µm. Interfacetal hairs, common in many insects, were not seen, but innumerous, regularly-spaced corneal nipples were present on the outer surface of each facet in densities of around 30 per µm^2 (Fig. 2D) Facet diameters (measured as corner to corner distances) were 13.8 ± 1.2 µm and exhibited no statistically significant differences across the eye.

Light micrographs of transverse and longitudinal sections of the eye (semi-schematically shown in Fig. 3) revealed that gross-anatomically the eye of *E. argyropeza* possessed all the typical structural elements known also from other insect compound eyes (Fig. 4). However, some of the structural components exhibited modifications likely related to the small size of the eye (Fig. 5). Individual ommatidia, measured from corneal surface to basement membrane, had an average length of 61.3 ± 3.8 µm, irrespective of whether the eye was dark or light-adapted. Toluidine-Blue treatment of the sections through the eye (Figs 4, 5) revealed that the corneae consisted of two distinct horizontal layers: an intensively blue-staining distal one of 2.55 ± 0.5 µm thickness and a non-staining proximal one of 4.74 ± 1.4 thickness. Judging by the uniform and structurally homogenous appearance of both layers, we do not expect a radial gradient of refractive index to be developed.

Crystalline cones, of the eucone type (defined by Grenacher, 1879), were formed by the characteristic 4 cells of Semper, whose nuclei just below the cornea were clearly visible at the distal ends of the cone cells. Two primary pigment cells enveloped each cone and six secondary pigment cells, placed even further peripherally, created a sleeve that insulated the ommatidium from its six immediate neighbours (Fig. 3B).

A clear-zone, similar to that known from larger moths, was absent and the long, fused rhabdoms were seen to extend from the basement membrane to the proximal tip of the crystalline cone. However, rhabdoms were not rod- or column-like, but possessed an hourglass shape with distal and proximal parts connected by a thin, threadlike "waist" devoid of microvilli. Seven regular rhabdomere-bearing retinula cells (cells with a centrally-pointing fringe of microvilli) were developed in each ommatidium and one rhabdomereless basal retinula cell was also present. The nuclei of the seven regular retinula cells measured ca. 7 µm in length and 2.5 µm in width and occurred mainly around the proximal end of the waist-region of the rhabdom. The prominent and spherical nucleus of the basal cell, measuring 2.5 µm in diameter, was consistently located below the proximal rhabdom and just above the basement membrane (Fig. 3C).

Tracheoles ran in an axial direction from the basement membrane to partially surround the proximal rhabdom up to its "waist region", creating in this way a kind of poorly developed *tapetum lucidum*. The interommatidial angle was found to be 8.5 ± 1.2°. When this angle was multiplied by 16 (viz., the number of facets in an anterior direction across the eye), the result was 136° for the angle of curvature of the eye. This value fits pictures of the longitudinal sections of the eye surprisingly well.

Ultrastructural examinations of sections of the eye of *L. argyropeza* supplemented earlier observations on corneal

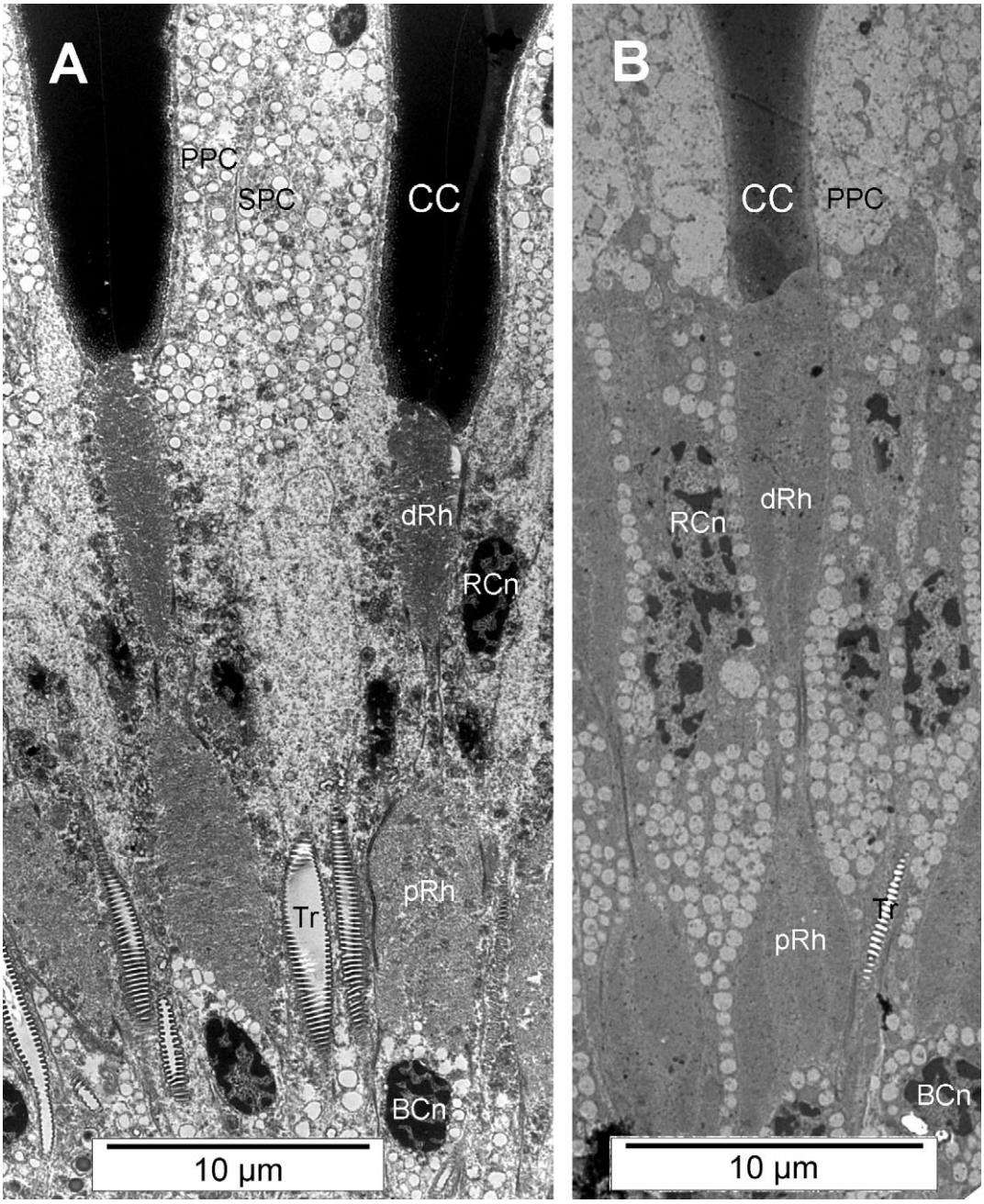

Fig. 6. Transmission electron micrographs of longitudinal sections through *E. argyropeza*'s ommatidia in dark- (A) and light-adapted (B) states. In DA eyes the screening pigment granules (here the small hollow spheres) of primary and secondary pigment cells (PPC, SPC) occupy distal places between adjacent crystalline cones (CC). Some pigment grains are also visible in the basal cells around their nuclei (BCn), but none are lining the rhabdoms or occlude the tracheoles (Tr). In the LA eye, screening pigment granules are spread along the entire length of the rhabdom and above the tracheoles. The retinula cell nuclei (RCn) appear to have migrated distally, but most obvious is the greater volume of the distal rhabdom (dRh) when compared with the DA eye and the narrower end of the crystalline cone (CC).

nipples made by SEM in as much as heights and base diameters of the nipples could be determined accurately as 0.125 ± 0.017 µm and 0.10 ± 0.015 µm, respectively. Although the coloration of the screening pigment granules had been removed during the fixation process, the electron micrographs clearly revealed the outlines of the screening pigment granules in primary, secondary, and retinula cells and allowed observations to be made on their diameters and their positions under dark- and light adapted conditions (Fig. 6). With an average diameter of 0.76 ± 0.12 µm, the screening pigment granules of the primary pigment cells are significantly larger than those of both the secondary and the retinula cells (0.65 ± 0.15 and 0.66 ± 0.10 µm, respectively). Finally, details of the microvillar orientations in distal and proximal rhabdoms and the region of the "waist" could be gleaned from the electron micrographs and showed that at all levels micro-

TABLE 1. Measured values of key parameters of the eye of *E. argyropeza*, based on data obtained from at least three dark- and three light-adapted individuals.

	Unit	DA and LA	DA	LA
Ommatidial length	µm	61.27 ± 3.80		
Interommatidial angle	deg	8.51 ± 1.17		
Eye radius of curvature	µm	79.37 ± 7.31		
Corneal radius of curvature	µm	5.40 ± 0.68		
Corneal outer layer width	µm	2.55 ± 0.49		
Corneal inner layer width	µm	4.74 ± 1.41		
Microvillus diameter	nm	58.46 ± 7.17		
Pigment granule diameter				
(a) primary pigment cells	µm	0.76 ± 0.12		
(b) secondary pigment cells	µm	0.65 ± 0.15		
(c) retinula cells	µm	0.66 ± 0.10		
(d) basal cells	µm	0.54 ± 0.09		
Distal rhabdom diameter	µm		2.43 ± 0.06	3.15 ± 0.47
Proximal rhabdom diameter	µm		3.76 ± 0.92	3.82 ± 0.62
Distal rhabdom length	µm		10.09 ± 1.14	13.52 ± 1.38
Proximal rhabdom length	µm		17.81 ± 0.86	15.45 ± 1.62
Distal rhabdom occupation ratio	%		7.35 ± 3.06	12.91 ± 3.91
Proximal rhabdom occupation ratio	%		53.76 ± 5.41	30.36 ± 8.41

villi of individual rhabdoms ran in predominantly 3 directions offset to one another by 120°.

At the level of the distal rhabdom seven desmosomes could be seen connecting the seven retinula cells of an ommatidium to one another, so that collectively they could form the characteristic hourglass shaped centrally fused rhabdom. The rhabdom occupation ratio [originally defined by Eguchi (1982) in comparative retinal studies of diurnal and nocturnal Lepidoptera] of approx. 7–13% at this distal level was statistically significantly less than that measurable further below in the proximal rhabdom region (approx. 30–54%). Large, mottled, ovoid retinula cell nuclei occupied the lower waist region between distal and proximal rhabdom. Measuring on average 58 nm in cross section, microvillar diameters did not differ between distal and proximal rhabdom regions. The retinula cell axons of individual ommatidia surrounded the basal cell and its nucleus and then penetrated the basement membrane in the form of distinct bundles that ended in the lamina.

Dark/light adaptational changes (Fig. 6) involved the shapes of the crystalline cones, the positions of the screening pigment granules, and the dimensions of the rhabdoms. In the dark adapted (DA) eyes screening pigment granules of both primary and secondary pigment cells were mainly withdrawn to distal positions between the cones, while those of the retinula cells had moved proximally towards the basement membrane, exposing the tracheolar tapetum. Compared with the wider tip diameters of the cones in dark-adapted eyes, the proximal ends of the crystalline cones were considerably more elongated and thinner in the light-adapted (LA) state. Rhabdom occupation ratios were greater and diameters of the distal rhabdoms wider in the light-adapted rather than dark-adapted eye, but the opposite held true for the dark adapted eye. In the latter rhabdom occupation ratios had increased from ca. 30% in the LA state to almost 54%. Whether or not a circadian rhythm governed the observed photomechanical changes was not studied, but given the almost continuous daylight during the northern Finnish summer, immediate responses to ambient light level changes would appear to be more important than any possible circadian changes. An overview of the major morphometric data, assembled from a minimum of three individual moths for each item, is provided in Table 1.

DISCUSSION

Butterflies and moths can generally be distinguished quite easily from each other on account of their morphology and behaviour; differences between them are even manifest in their eye structures (Yagi & Koyama, 1963). Butterflies rely on apposition optics and their eyes lack the clear-zone, whereas moths usually possess eyes of the superposition type, for which the presence of a clear-zone is essential (Struwe, 1973; Eguchi, 1982; Meyer-Rochow & Gál, 2004; Lau & Meyer-Rochow, 2007; Lau et al., 2007; Meyer-Rochow & Lau, 2008).

Although *E. argyropeza* is a moth and thus expected to use superposition optics for image formation, its eye lacks the clear-zone. Structurally its eye therefore resembles an atypical kind of apposition eye. Assuming ancestral species of *E. argyropeza* did possess the clear-zone, it is not too difficult to imagine that the hourglass shaped rhabdom in *E. argyropeza* was the result of a shrinking clear-zone, forcing together a distal retinal element (the current distal rhabdom) and the proximal retina. Although the hourglass shape of the *E. argyropeza* retina is so far a unique anatomical finding for any insect species, a somewhat similar rhabdom anatomy with an apparent (but wider) "waist" has also been found in the nocturnal sphingid moth *Cechenena lineosa* by Eguchi (1982). In *C. lineosa*, however, the distal rhabdom is formed by only

two retinula cells and not all seven as in *E. argyropeza*. A distal rhabdom, confluent with the more voluminous proximal rhabdom by a narrow microvillus-bearing retinula cell strand, was described for the eyes of the pyralid moths *Ephestia kuehniella* (Fischer & Horstmann, 1971; Horridge & Giddings, 1971) and *Amylois transitella* (Bernard et al., 1984) and distally-placed retinal cell bodies, albeit usually without rhabdomeres, are not uncommon in insect clear-zone eyes generally (Horridge, 1975). Why then this seemingly "compressed" inner structure of the *E. argyropeza* eye, in which a proper clear-zone has been abolished and a tiered rhabdom has been installed instead?

The described organization, reminiscent of an apposition eye, is in good agreement with predictions made by Meyer-Rochow & Gál (2004), who claimed that insects below a certain minimum size would not benefit from superposition optics and would probably have to use some kind of apposition optics instead. The same authors further stated that in compound eyes with an eye radius smaller than 250 µm, the presence of a clear-zone would be counterproductive as it would lead to worsened acuity without a concomitant enhancement of absolute light sensitivity. The radius of the eye of *E. argyropeza* is 80 µm and therefore well below the limit predicted by Meyer-Rochow & Gál (2004) for superposition vision to be effective.

While absolute sensitivity is generally higher in superposition eyes, acuity improvements are more easily achieved in apposition eyes. Decreasing facet sizes, a narrowing of the interommatidial angle, an increase in the number of ommatidia and thinner rhabdoms can all lead to improved resolving power (Land, 1981). Looking at *E. argyropeza* we find facet sizes that are small (ca. 14 µm), yet not quite as small as those of some other insects of comparable minute body sizes, e.g., the minute moth *Leucoptera coffeella* (facet sizes of 10 µm: Meyer-Rochow & Stringer, 1993), the Antarctic midge *Belgica antarctica* (facet sizes of 11 µm: Meyer-Rochow & Reid, 1994), and the psocopteran *Psyllipsocus ramburi* with minimum facet sizes of 10 µm (Meyer-Rochow & Mishra, 2007), the latter three with facet sizes close to the minimum functional diameter of arthropod eyes (Barlow, 1952).

Interommatidial angles of 8.51° in *E. argyropeza*, too, are not as small as those known from other moths (3° in *Ephestia kuehniella*: Fischer & Horstmann, 1971; 3.82° in males and 4.13° in females of *Acentria ephemerella*: Lau et al., 2007; 2.80° in males and 3.20° in females of *Operophtera brumata*: Meyer-Rochow & Lau, 2008). Thus, given that in the small eyes of *E. argyropeza* no possibility existed to improve absolute sensitivity with the help of a clear-zone, acuity had to be sacrificed through a relative enlargement of facets and an increase in interommatidial angle to allow more light to enter a single facet. The strongly convex bulge of the ommatidial corneae in *E. argyropeza* is yet another modification from the typical moth superposition eye to maximize the amount of light entering an ommatidium, for larger insects with clear-zone eyes often have facets that are far less convex and often even nearly flat on the outside (Yagi & Koyama, 1963; Meyer-Rochow, 1975; Welsch, 1977; Anton-Erxleben & Langer, 1988).

Facet sizes and ommatidial array in *E. argyropeza* are very regular, which suggests that vision in this insect is not completely unimportant, since irregular retinal mosaics lead to image degradations (French et al., 1977). The well-developed corneal nipple cover of the facet surface is thought to function as an anti-reflectance coating, enhancing the usage of the available light by preventing photon loss by reflection (Miller, 1979). Corneal nipples of the kind seen on the eye of *E. argyropeza*, representing 50–200 nm high nano-protuberances, are classified as "type II" and according to Bernhard et al. (1970) particularly effective with light of shorter wavelengths. Corneal nipples measuring more than 200 nm in height are classified as "type III" and known to interact best with light of longer wavelengths. The nipples on the surface of the eye of the predominantly diurnally-active *E. argyropeza* (Finnish summers do not have dark nights) could therefore be used in separating UV from green wavelengths, possibly assisting the insects in visually detecting suitable host trees to deposit their eggs on.

Our observation of rhabdom microvilli running predominantly in three directions, offset to one another by 120 degrees, according to Kirschfeld (1972) should allow this tiny moth to unambiguously distinguish the e-vector of ambient light. An ability such as that could be extremely useful, if we consider the difficulties the small moth must have to locate its host trees. However, fact is that in insects possessing polarization vision, there are three POL-units with three different e-vector directions preferred. In a given POL-unit there are two subunits with orthogonal e-vector (microvilli) directions. Therefore, although correct in principle, Kirschfeld's (1972) explanation simplifies what is really required to allow polarization vision to occur. The whole subject is treated extensively in part III of Horváth & Varjú (2004), which makes it clear that it would be crucial to our understanding of polarization sensitivity in *E. argyropeza* to know whether it possesses UV and green or perhaps even additional colour receptors in its eye. Being small and poor fliers, *E. argyropeza* has to "ride" on air currents that would transport the insect above the canopy of trees. Since *E. argyropeza* is known to be monophagous, feeding exclusively on aspen as a larva and being highly specific when it comes to deposit its eggs (Johansson et al., 1989), it, therefore, must locate the correct host tree amongst other trees.

The trembling green poplar leaves induce oscillating polarization signals and due to the Umow effect, the degree of linear polarization of leaf-reflected light is minimal in the green part of the spectrum, and maximal in the UV/blue and red spectral ranges (Horváth, 1995; Horváth et al., 2002; Horváth & Varjú, 2004; Hegedüs et al., 2006). Looking downward, the trembling, shiny aspen leaves with their waxy upper surfaces would provide an unmistakable flickering polarization signal (M. Horváth, pers. comm.), especially since pyralid and other moths

have been shown to be maximally sensitive in the UV, blue, and green spectral ranges (Hamdorf, 1979; Bernard et al., 1984; Meinecke & Langer, 1984). Once the insect has found and approached the aspen tree, other senses to choose the most suitable egg-laying site on aspen leaf petioles (Johansson et al., 1989) may take over. It is interesting to note that mines of *E. argyropeza* caterpillars are found exclusively in the topmost leaves of the aspen tree, but almost never in leaves less than one metre from the ground (M. Mutanen, pers. comm.).

If the moths were to select leaves with the smallest amount of cellulose fibres in them, they would choose the youngest leaves, but if they were most interested in leaves containing the least amount of toxic compounds, they ought to choose older leaves, for young aspen leaves (and those of other trees as well: Liu et al., 1998) are particularly rich in phenolic glycosides (Palo, 1984). The fact that predominantly the topmost, young leaves are attacked by the larvae despite their greater toxicity suggests that the insect's approach to deposit eggs on the leaves is visually-guided, i.e., scanning the canopy from above, and does not involve a chemically-guided search for the least toxic leaves.

Possessing a distal and a proximal rhabdom of nearly the same dimensions, but different responsiveness to dark-light-adaptations, is a feature unique amongst insects and worthy of a longer discussion. Some of the reported dark-light photomechanical changes like the distal migration of screening pigments into spaces between the cones or the proximal pigment translocations to positions below the rhabdoms are phenomena known since Exner (1891) and of widespread occurrence in insects with superposition eyes (Mazokhin-Porshnyakov, 1969; Autrum, 1981; Meyer-Rochow, 1999). Dark-light adaptational changes of the diameters of the rhabdoms (unlike screening pigment migrations: Mazokhin-Porshnyakov, 1969) are more commonly observed in apposition rather than superposition eyes (Meyer-Rochow, 1999), but they must be of particular significance in *E. argyropeza* on account of the tandem position of distal and proximal rhabdoms

What we see in the eye of *E. argyropeza*, prompted by its phylogenetic legacy of a superposition eye and its modified function as an apposition type of eye, appears to be a blend of both photoreceptor types. The larger rhabdom occupation ratio of the proximal rhabdom (54% under DA versus 30% in the LA state), the presence of a tracheolar shield around the proximal rhabdom, and the withdrawal of the screening pigment to below the tracheolar layer under dark-adapted conditions are what is to be expected from a typical superposition, clear-zone eye and commonly encountered in larger moths (Höglund, 1966; Welsch, 1977; Anton-Erxleben & Langer, 1988; Meyer-Rochow & Lau, 2008). Yet, the clear-zone in *E. argyropeza* is absent and superposition cannot effectively work.

What does allow the proximal rhabdom to receive a greater light signal in the dark-adapted state is the exposure of the proximal tracheolar layer in combination with the absence of screening pigments around the distal rhabdom, the distal rhabdom's diminished size in the dark and the screening pigment-free "waist region" of the rhabdom. Even if focusing a parallel beam of light by superposition optics is not possible in the eye of *E. argyropeza*, crossing-over of light rays from one ommatidium to another and scattering of light inside the eye can also lead to a certain improvement in absolute sensitivity (Horridge et al., 1972). The increase in diameter of the distal rhabdom under light-adapted conditions and its rhabdom occupation ratio increase from about 7% to nearly 13% in the light underscores the roles of the two rhabdom moieties in the eye of *E. argyropeza*. Lying distal, i.e., on top of the proximal rhabdom (the usual place of the retinula cell bodies and their nuclei in other moths), the distal rhabdom has acquired the role of an apposition eye rhabdom. But because of its position, it cannot help but filter the incoming light and, thus (when enlarged during light-adaptation) reduce amount and possibly spectral composition of the light before it reaches the proximal rhabdom.

There are two reasons that made it possible for this rhabdom to be established in a distal location and to achieve a kind of apposition vision with it. The first is related to the position of the retinula cell bodies, which, in larger moths (and thus moths ancestral to *E. argyropeza*) possessing a clear-zone, were always located just below the crystalline cones on the distal side of the clear-zone. Therefore, phylogenetically a clear-zone never separated cone tips and retinula cell bodies in moth eyes (unlike, for instance, in the superposition eyes of rock lobsters, which therefore possess analogous rather than homologous clear-zones: Meyer-Rochow & Tiang, 1984).

The second reason relates to the plasticity of rhabdom formation by the retinula cells. Although larger moths never produce a rhabdom in the distal cell body region and instead allow the retinula cells to elongate and collectively form a clear-zone on whose proximal side far from the cone ends rhabdom microvilli develop, retinula cells retain the ability to generate rhabdom microvilli in any place along their entire length. This explains why in some moths like *E. kuehniella* and *Amyelois transitella* we find a thin strand of microvilli all the way from distal to proximal retina (Fischer & Horstmann, 1971; Bernard et al., 1984) or a distal rhabdom generated by just two retinula cells as in *C. lineosa* (Eguchi, 1982).

What the eye of *E. argyropeza*, therefore, demonstrates is how evolution coped with the phylogenetic legacy of a superposition eye in a species that could no longer use the original structure. Large moths have eyes with wide clear-zones and no distal rhabdoms while very small moths developed substantial distal rhabdoms at the expense of the clear-zone; medium-sized moths like *E. kuehniella* and *A. transitella* exhibit eye anatomies of an intermediate nature. The combination of characters and adaptations linking superposition with apposition in *E. argyropeza* is beyond doubt the consequence of eye miniaturization, but to what extent these first results on the structure and ultrastructure of a minute compound eye can be generalized and applied to other small species, will

hopefully be revealed by future studies on photoreceptors of additional species of minute insects.

ACKNOWLEDGMENTS. We are most grateful to M. Mutanen (Eläinmuseo, University of Oulu, Finland) for collecting the moths. Further thanks go to S. Mänttäri for practical advice and encouragement, K. Anttila for helping with the statistical evaluations, T. Uusitalo and P. Huhtala for advice on ultramicrotomy and scanning electron microscopy, respectively, and R. Sormunen and T. Piispanen of the Biocenter Oulu (Tissue Imaging Unit) for the transmission electron microscopical preparations. This research was supported by the DFG through a grant to VBM-R.

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Received May 21, 2009; revised and accepted July 14, 2009