Microstructures and nanostructures of high Andean *Penaincisalia* lycaenid butterfly scales (Lepidoptera: Lycaenidae): descriptions and interpretations

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Abstract
The scales of one high Andean eumaeine lycaenid butterfly species with pale dorsal coloration and four species with vivid dorsal colour were investigated using field emission scanning electron microscopy. The micro- and nanostructures are illustrated, described, measured, and interpreted. The vivid colours in the species are caused by a pepper-pot nanostructure of *Urania*-type scales. This nanostructure is a three-dimensional lattice within the body of the individual scale. The scales of the non-vivid orange species are lacking this nanostructure and the surfaces of their scales show high microstructure irregularities. This absence of vivid colour may be correlated with thermal regulation. The irregularity of the scale microstructures suggests a heavy environmental pressure on the populations sampled. Previously unknown structural variations of *Urania*-type scales are also described. The existence of closed scale microcell structures, explained as an apomorphic character in the tribe Eumaeini, most probably evolved independently several times. It is hypothesized that scale micro- and nanostructure modifications develop syntopically within a population, which in turn can lead to rapid diversification.

Keywords: Andes, discoloration, Eumaeini, irregularity, Lycaenidae, monomorphy, Penaincisalia, scale microstructure, scale nanostructure, South America, sympatric speciation, Thecloxurina, thermal regulation

Introduction
In temperate regions individual male butterflies belonging to the tribe Polyommatini (Polyommatinae, Lycaenidae) typically have shiny blue wing dorsa while females are generally brown. It has been well demonstrated that this characteristic blue colour is generated by the structure of the wing scales and not by pigments (Vukusic et al. 2000). Entomologists have already noted in the last century that certain polyommatine species do not possess this striking sexual dimorphism and instead have brown males (Tutt 1909, p 158). This phenomenon also occurs in the two other lycaenid subfamilies present in the...
temperate region: Lycaeninae and Theclinae. Some genera in these subfamilies also possess brown species, which in addition show many special, presumably advanced, traits. Interestingly, this phenomenon can also be detected in lycaenines and theclines via different colours: some species have both males and females with uniformly pale or bright orange or red dorsal colours. Pale, non-iridescent orange is most probably produced by pigments, but the vivid copper-orange and red colour typical of many lycaenines and theclines is probably structural (Tilley and Eliot 2002, p 159, 173).

Brown Old World polyommatines have been called “monomorphic” by Mensi et al. (1992), who stressed the loss of sexual dimorphism and the presence of identical wing dorsa in both sexes. Bálint and Johnson (1997) discussed this phenomenon and introduced the term “discoloration”. They pointed out that certain species whose males have vividly coloured wings can have sister taxa which are discolored, indicating an evolutionary change from (presumably) ancestral blue to brown. Thus both Mensi and her co-authors, as well as Bálint and Johnson, suggested that brown monomorphy or discoloration is a derived character. However Kandul et al. (2004) did not find discoloration to be a derived trait in the genus Agrodiaetus Hübner, 1822, and alternative explanations must be sought in this genus.

Mensi et al. (1992) did not present any explanation for the origin of discoloration. Bálint and Johnson (1997) formed the hypothesis that the discoloration is probably connected to thermal regulation of these butterflies living in high altitudes or latitudes, where availability of solar energy is restricted by certain climatic or geographic factors (Bálint and Johnson 1997, p 26–33). This hypothesis was tested experimentally by Biró et al. (2003): it was demonstrated that, in the case of a polyommatine sister species pair, the loss of the vivid colours resulting in discoloration was from the qualitative transformation of scale nanostructures. This transformation influences the thermal regulation of the butterflies. Individuals lacking “pepper-pot”-like (sensu Eliot 1973, p 400) nanostructures in the body of the wing scales are able to absorb more solar energy in the blue and ultra-violet range of the solar spectrum.

Scale pepper-pot nanostructures function as photonic-crystals, generating the vivid colours of the dorsal surface of lycaenid wings (Biró et al. 2003). Photonic crystals are a new class of composites built of a regular, three-dimensional arrangement of two materials with different refractive indices, intensely investigated by physicists and material scientists. Their potential applications range from flat panel displays to optical integrated circuits for high-speed computing. The manufacturing of photonic crystals for visible wavelengths with present-day technology is an extremely challenging and expensive task. It is important, therefore, to understand how structural colours function in the environment and how the butterflies apply them.

In the present paper we have examined samples representing the lesser-known New World “hyperdiverse” sensu Wilson (2003) tribe Eumaeini of the theclines (Pierce et al. 2002, p 760). This tribe, which dominates the Neotropical lycaenid fauna, displays both the orange and brown type of sexual monomorphy. We investigated the following questions: (1) Does the vivid eumaeine dorsal colour of the species examined originate through Urania-type (pepper-pot) scale structures typical for polyommatines? (2) Is eumaeine orange coloration pigmental or structural? (3) Are there any special eumaeine scale microstructures? And, (4) what is the probable explanation for the appearance of orange discoloration and monomorphy in the Andes?

Eliot (1973) documented the scales of one Neotropical eumaeine species. Later, Tilley and Eliot (2002) investigated four more. In this paper, we present data on five more species, raising the number of investigated and documented eumaeine species to 10. This number represents less than 1% of the total eumaeine fauna, estimated to be 1250–1300
(Robbins 2004, p.xxvii). Thus, any interpretation of our results must be treated as preliminary, as with those of Tilley and Eliot (2002, p 175).

Material and methods

The five species we selected represent two genera of the diverse Penaincisalia genus group (Bálint and Wojtusiak 2001, p 382–383): (1) Penaincisalia Johnson, 1990 (type species: Thecla culminicola Staudinger, 1894, by original designation) and (2) Thecloxurina Johnson, 1992 (type species: Thecla loxurina Felder and Felder, 1865, by original designation). From the Pan-Andean genus Penaincisalia (see Johnson 1990; 1992, p 159–167), the group of an orange monomorphic species, P. aurulenta Johnson, 1990, with its dimorphic orange-purple (presumably) sister-species P. perezi Bálint, 2001, and the blue-violet P. culminicola were selected (see Figure 1A–C). A dimorphic species-pair, representing another closely related but phenotypically divergent lineage, was chosen from the Pan-Andean genus Thecloxurina (Johnson 1992, p 5–21; Bálint and Wojtusiak 2003): the blue T. loxurina and the violet-orange T. atymna (Hewitson, 1870) (see Figure 1D–F). The latter species tends toward orange monomorphy as its violet-hued male displays dorsally wide orange areas on both of its wings (the extent of which vary individually, see Figure 1E, F); the female is dorsally orange.

We have studied scales from one male individual of each species, as females are very rare in collections (Bálint and Wojtusiak 2003, p 364). All the species we have examined are figured to show their dorsal colours (Figure 1) and are tabulated according to their collecting data (Table I). All individuals originate from a longer series of samples which were collected recently in the wild (Bálint 1997; Bálint and Wojtusiak 2003) and deposited in the collections of the Hungarian Natural History Museum. Individuals selected as sources of wing samples are databased and labelled accordingly have been deposited in the Research Institute for Technical Physics and Material Science.

Wing fragments of those individuals studied were taken from the right hind wing of each specimen. Field emission scanning electron microscopy (FESEM; type: LEO 15040 XB) was used to study the micro- and nanostructure of the scales. In order to avoid charging effects, samples were thinly gold sputtered for FESEM examination. The whole wing fraction was examined to determine the micro- and nanostructure of the scales. At lower magnifications in larger observation windows the most characteristic scale shapes were identified, followed by high-resolution investigation.

We investigate and describe the scales following Tilley and Eliot (2002). The new aspect we introduce concerns the cross ribs when they are viewed from a lateral aspect. The term “straight cross rib” refers to the case where the rib linking longitudinal ridges is straight, without any concavity. The term “curved cross rib” is used when the rib connecting longitudinal ridges has a concave saddle-like shape. The term “v-shaped cross rib” is used when the rib connecting longitudinal ridges is v-shaped, concavely angling between the ridges.

Scale surface structures are measured in micrometres (µm): (a) for length, a line drawn between the attachment to wing membrane and scale termen, and (b) for width, a line drawn between the parallel margins at the middle. A length/width calculation (“length/width” figure) is derived from these measurements. Scale internal (pepper-pot) structures are less than 1 µm long, and hence are termed “nanostructures”, and so precise measures are not given.

For descriptive text we follow Tilley and Eliot (2002), who applied the terminology of Mason (1926), Eliot (1973) and Downey and Allyn (1975). The capitalized colour names are taken from Maerz and Paul (1950), but the names record subjective impressions of the dorsal colour of specimens examined in daylight and are not meant to be a quantitative
assessment. In every case, we first give the colour name and a general indication to the shape of the scales; we then characterize the microstructures that have been found.

Results

*Penaincisalia aurulenta*

(Figures 1A, 2)

The dorsal colour is Burnt Orange without iridescence (Figure 1A). The scales are tile- or shovel-shaped with a toothed anterior margin. The length/width ratio of tile-shaped scales is 3/1 (120/40 μm). The shovel-shaped scales are shorter and wider (length/width = 2/1, 80/40 μm). The dentation of the tile-shaped scales is deeper (Figure 2A). We could not detect any qualitative difference between the microstructures of the two types. The longitudinal ridges are
parallel and low, with flutes. The space (=length of the microcells) between the ridges is approximately 1 µm. Cross ribs are straight, their intervals are slightly irregular and less than 0.5 µm. The microcells are oblong or rectangular and widely open (Figure 2B, C). The nanostructures are irregularly fragmented and sparse or lost with the trabeculae, and the reverse surface of the scale can be clearly seen at higher magnification (Figure 2D).

**Penaincisalia perezi**
(Figures 1B, 3)

The dorsal colour is vivid Magenta Orange with Ecclesiastic Purple iridescence (Figure 1B). The scales are tile- or shovel-shaped with a uniformly toothed anterior margin. The length/width ratio of the tile-shaped scales is 3/1 (150/50 µm); the shovel-shaped scales are shorter, the length/width ratio is 2/1 (100/50 µm). The dentation of the tile-shaped scales is more pronounced while the dentation of the shovel-shaped scales is far less prominent (Figure 3A). We found three types of scale microstructures, which suggest a transformation series (see Discussion):

1. The longitudinal ridges are low, showing flutes on the longitudinal ridges, the cross ribs are straight. The space between the cross ribs is approximately 0.5 µm; but highly variable, there are larger and smaller ratios. The widely open microcells are rectangular with fragmented nanostructures, the trabeculae and the lower scale membrane can be clearly seen (Figure 2B).
2. The longitudinal ridges are moderate with flutes, which with curved cross ribs form rectangular-shaped deeper-laying microcells. Spaces between cross ribs are

| Distribution | Collection data | Reference | Database reference of wing sample |
|--------------|-----------------|-----------|-----------------------------------|
| *Penaincisalia aurulenta* Johnson, 1990 | Peru, dept. Ancash, PN Huascarán, Llanganuco, 3800 m, 9–13 February 1995, leg. Zs. Bálint | Bálint 1997 | 2003_1 |
| *Penaincisalia perezi* Bálint, 2001 | Peru, dept. Ancash, PN Huascarán, Quebrada Demanda, 4500 m, 10 February 1995, leg. Zs. Bálint | Bálint 1997 | 2003_2 |
| *Penincisalia culminicola* (Staudinger, 1894) | Peru, dept. Ancash, PN Huascarán, Llanganuco, 3800 m, 9–13 February 1995, leg. Zs. Bálint | Bálint 1997 | 2003_3 |
| *Thecloxurina loxurina* (Felder and Felder, 1865) | Ecuador, prov. Morona-Santiago, 9 de Octubre/Macas, 1700 m, August 2001, leg. Wojtusiak | Bálint and Wojtusiak 2003 | 2003_11 |
| *Thecloxurina atymna* (Hewitson, 1870) | Ecuador, prov. Tungurahua, Tungurahua volcano, Baños-Pondoa road, 3400 m, 17–20 January 2002, leg. Wojtusiak and Garlacz | Bálint and Wojtusiak 2003 | 2003_12 |
3. The longitudinal ridges with flutes are high and the cross ribs are v-shaped and form rectangular, very narrow, almost completely closed microcells with a single circular opening at the bottom. The spaces between the cross ribs are less than 0.1 µm; their sequence is regular. Pepper-pot nanostructures can be seen beneath the openings (Figure 3D).

**Penaincisalia culminicola**
(Figures 1C, 4)

The dorsal colour is iridescent Lobelia Blue (Figure 1C). The scales are uniformly tile-shaped with moderately toothed apical margins, with length/width ratio 3/2 (90/60 µm) (Figure 4A). We could detect two kinds of scale microstructures.

1. The longitudinal ridges with flutes are low and they merge with straight cross ribs having widely open rectangular microcells. The spaces between cross ribs are 0.5 and 1 µm, and irregular. In the microcells are irregular pepper-pot-like layers, and the trabeculae and the scale membrane can be clearly seen (Figure 4B), but there are some areas which show more regularity (Figure 4C).
2. The longitudinal ridges are high, showing flutes, and they merge with closely set v-shaped cross ribs forming rectangular, narrow and almost completely closed microcells. The space between the cross ribs is less than 0.3 μm; their intervals are slightly irregular. Almost every microcell has a single circular nanoscaled opening at the base. Beneath the opening, pepper-pot nanostructures can be clearly seen (Figure 4D).

_Thecloxurina loxurina_
(Figures 1D, 5)

The dorsal colour is iridescent Lyons Blue (Figure 1D). The scales are tile- or shovel-shaped with slight anterior dentation (Figure 5A) or are not dentate (Figure 5B). The dentate tile-shaped scales are long with a length/width ratio of 3/1 (150/50 μm); the shovel-shaped scales are wider with a length/width ratio of 5/3 (100/60 μm). The non-dentate scales are intermediate with a length/width ratio of 2/1 (120/60 μm). The slightly toothed scales possess moderate longitudinal ridges with flutes. They merge with curved cross ribs with rectangular shaped microcells. The space between the cross ribs is approximately 0.5 μm, but slightly irregular. The microcells are widely open, nanostructured material fragments and the trabeculae, plus the reverse surface of the scale, can be clearly seen (Figure 5C). There are identically microstructured scales with pepper-pot nanostructures.
The square-ended and non-dentate scales possess high longitudinal ridges with very closely set v-shaped cross ribs (Figure 5E). They form rectangular, but very narrow and almost completely closed, microcells. The spaces between the cross ribs are less than 0.3 \( \mu m \) wide; their sequence is regular. The microcells have a single circular nanostructure opening below their base. Pepper-pot nanostructures can be seen beneath the openings (Figure 5F).

**Thecloxurina atymna**

(Figures 1E, F, 6)

The dorsal colours are Pansil Violet and Burnt Orange (Figure 1E, F). The scales are tile- or shovel-shaped (Figure 6A). The tile-shaped scales are long with a length/width ratio of 3.5/1 (140/40 \( \mu m \)). The tile-shaped scales are deeply dentate. The less dentate shovel-shaped scales are intermediate with a length/width ratio of 3/1 (90/60 \( \mu m \)). The microstructures of the scales are identical: the parallel longitudinal ridges are low, showing flutes, and form, with straight cross ribs, rectangular microcells (Figure 6B). The ridges and the ribs are strong, and sometimes with highly irregular interval sequences; the microcells are either open, or partly to completely closed (Figure 6C–F). Pepper-pot-like nanostructure fragments can be seen deeply within, but there are also entirely empty regions.
The violet structural colour of the basal and median area of the wing dorsa is produced by the pepper-pot nanostructures still intact in particular areas (Figure 6G, H).

Discussion

Scale shapes and structural colours

Results suggest that the shape and the microstructure of the scales have no influence in generating the structural colours of the *Penaincisalia* and *Thecloxurina* lycaenids. Scales with
Figure 6. Scanning electron micrographs of *Thecloxurina atymna* scale microstructures. (A) Tile- or shovel-shaped scales, deep dentation; (B) low longitudinal ridges with flutes, straight cross ribs, and rectangular microcells; (C–F) strong ridges and ribs in slight disorder, microcells open or closed; (G, H) wispy material in microcells.
qualitatively and quantitatively different shapes and microstructures produced similar structural colours. The colours are generated by the pepper-pot nanostructures; their presence or absence determine colour generation. This is verified by the fact that differently microstructured scales with similarly nanostructured pepper-pot material in the body of the scale generate the same structural colour (cf. Figure 5D and 5F).

It has been demonstrated that the pepper-pot nanostructured microcells in the *Urania*-type scales are responsible for producing the different kinds of vivid colours (Vukusic et al. 2000). We could detect scales with pepper-pot nanostructures or their fragments in all the species we have examined. *P. culminicola* and *T. loxurina* reflect blue, *P. perezi* reflects purple and *T. atymna* reflects violet colour; however, the Burnt Orange of *P. aurulenta* is not vivid. The scales of *P. aurulenta* do not generate iridescence, although the scale microcells contain deep lying wispy material of pepper-pot nanostructure fragments. This fragmented nanostructure cannot function as photonic crystals because there is no three-dimensionally ordered or quasi-ordered structure with characteristic dimensions in the suitable range for visible wavelengths. Photonic crystal-type systems (Joannopoulos et al. 1995) are compositions built of two different materials with different refractive indexes arranged in a three-dimensional order similar to that shown in Figure 7. This simple cubic arrangement may not give a complete photonic band gap, i.e. a spectral range in which the transmission of the composite is zero and its reflectance is 100%. This arrangement is used only to illustrate the three-dimensionality of the materials with refractive indexes \( n_1 \) and \( n_2 \), where \( n_1 \neq n_2 \). In the case of butterfly scales, \( n_1 = 1 \) corresponds to air (void) and \( n_2 = 1.5 \) corresponds to chitin (full).

As demonstrated earlier by Biró et al. (2003), comparing measured structural data and mathematical modelling, the pepper-pot structure exhibits photonic crystal-type behaviour. The scale structures of *P. aurulenta* resemble those of *P. perezi* and *T. atymna* but there is a notable difference: the presence of vivid violet or purple coloration in the latter two species.

Figure 7. Illustration showing the principle of regular, three-dimensional arrangement of two materials with different indexes of refraction, \( n_1 \) and \( n_2 \).

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We speculate that the origin of the vivid wing dorsal colour is the result of remaining and still functioning pepper-pot nanostructures lying in the body of the longitudinal ridges and in some individual scales. Transmission electron microscopy (TEM) investigation (Eliot 2002, p 165, Figure 6G) shows that in certain species the ridges themselves may have a pepper-pot-type structure. Of course, due to the low fraction of scale surface covered by ridges, if there is no pepper-pot structure all over the scale surface, the coloration given only by the ridges can be very weak. An enhancement of the colour may be expected when viewing the scale from shallow angles with respect to the plane of the wing. Viewed from the lateral aspect at 90° to the longitudinal ridges, one may see a somewhat enhanced colour. But only a weak colour is expected when viewed from the anterior or posterior aspect in parallel with the ridges. Such a change of colour was observed on P. perezi and T. atymna wing dorsa. The colour enhancement when viewing the wing at a shallow angle from lateral aspects is attributed to the increase in the relative fraction of the visible surface, which is covered by the ridges possessing the pepper-pot structure.

The coppery orange colour, orange discoloration and monomorphy

We are of the opinion that the pale orange coloration of T. atymna, P. aurulenta, and P. perezi originates from pigments, probably carotin, and is formed basically in the same way as the orange of many Old World Lycaenini or Theclini. The origin of the coppery shade of these colours has not been understood hitherto (Tilley and Eliot 2002, p 173); it was also not discussed by Vukusic et al. (2000).

We have found that the orange species examined by Tilley and Eliot (2002, listed in Table I) share the following traits: (1) low longitudinal ridges with side flutes, (2) straight cross ribs and (3) open microcells with deeply laying internal structures. We are of the opinion that the Urania-type scales with the combinations of these three morphological traits give rise to the iridescent orange (=coppery) colour. More samples have to be investigated to further evaluate this theory.

P. aurulenta, P. perezi, and T. atymna inhabit high altitudes very close or at the equator where solar energy reaches the surface of the Earth most powerfully. These genera have not developed brown discoloration and monomorphy, despite having the genetic potential to do so, since eumaeine genera displaying brown monomorphy are known not only in the northern but also in the southern American continent. Melanin absorbs solar energy with high efficiency in visual spectra and infrared. However, brown monomorphy is unlikely to be an economical response for environmental pressures at higher altitudes, near the Equator for Penaincisalia and Thecloxurina. We suspect that carotin, the orange pigment of the wings in high altitude, also plays a role in thermal regulation of these butterflies, but probably in a different way than we have demonstrated for Polyommatus (Biró et al. 2003).

The scale microstructure with v-shaped cross ribs

Tilley and Eliot (2002) described a wide array of lycanid butterfly scale microstructures and discussed their importance for phylogenetic inference. One of the most interesting results they published was the discovery of Morpho-type microstructures occurring together with the Urania-type microstructures in certain lycaenid groups as hitherto it was known that only scales representing the latter type are present in Lycaenidae. Tilley and Eliot also pointed out that there are structures intermediate between the two main types, and one of
the microstructure types with intermediate morphology was reported to occur among the Eumaeini lycaenids.

Tilley and Eliot (2002, p 158) described and figured the peculiar *Urania*-type scales of two eumaeines, *Siderus tephraeus* (Geyer, 1837) and *Eumaeus minijas* (Hübner, 1809), noting that the scales of these species differ significantly from the norm. *S. tephraeus* exhibits closely situated v-shaped cross ribs and the microcells are closed, having only a single quadrant-shaped opening at the abyss at their lowest points. Beneath the openings pepper-pot structures are visible (Tilley and Eliot 2002, Figure 4f, g). The *E. minijas* scale microstructure is similar but the cross ribs are even more closely situated, almost attached ("satin" type *sensu* Ghirandella 1984), and there is no opening at the lowest point; however, the pepper-pot structures are present (Tilley and Eliot 2002, Figure 5a, b). We found scale microstructures with v-shaped cross ribs, identical to the type described from *S. tephraeus* in the studied species *P. culminicola*, *P. perezi*, and *T. loxurina* (Figures 3D, 4D, 5D). We did not find v-shaped cross ribs in *P. aurulenta* and *T. atymna*, a matter we consider to be a derivative loss due to adaptation (see below).

This microstructure was figured by Eliot (1973), but since its significance had not been recognized, the structure has not been widely described or interpreted. The neotropical *Panthiades bitias* (Cramer, 1775) was examined by Eliot, and a scale microstructure with high longitudinal ridges and closely situated v-shaped cross ribs was figured (Eliot 1973, Plate 1, Figure 6). The scale microstructure figured was from the "visual patch" considered by Eliot as androconia (Eliot 1973, p 460). Similarly, but more interestingly, an almost identical scale microstructure has been figured for *Creon cleobis* (Godart, 1824) also by Eliot (1973, Plate 2, Figure 9). This species, a representative of the lycaenid subtribe Iolaiti, typifies the Palaeotropics. The sample was taken from the ventral forewing androconial patch situated along the anal margin. Thus, according to Eliot’s data, this structure should typify thecline androconial scales (Eliot 1973, p 400–401). Our results suggest that these scales probably play a role in generating special visual signals as none of the scales we studied could function in pheromone diffusion, as there was neither "scent pad” nor "scent patch” *sensu* Robbins (1991) in the hind wing area from where we took the samples.

This kind of *Urania*-type microstructure, which corresponds to the *Morpho*-type scale in *Eumaeus* Hübner, 1809, is lacking in certain species of various pan-Neotropical genera but has been shown in the case of *Arawacus meliboeus* (Fabricius, 1793) and *Atlides halesus* (Cramer, 1775) by Tilley and Eliot (2002, Figures 5c, d). We found this kind of structure in our supplementary eumaeine samples representing large and vividly coloured species in moderately diverse genera (with ca 25 or fewer species) distributed all over the Neotropical region (*Denivia* Johnson, 1992, *Pseudolycaena* Wallengren, 1859, and *Theritas* Hübner, 1819). We could trace the structure repeatedly in additional *Eumaeus* species, plus in the genera *Argentostriatus* Johnson and Kroenlein, 1993, *Cyanophrys* Clench, 1961, and *Micandra* Schatz, 1888 which are also diverse pan-Neotropical clusters of eumaeines. The more closely investigated *C. remus* (Hewitson, 1868) possesses the same transformation series in dorsal scale microstructures as was revealed by *P. perezi* and *Th. atymna*, plus highly interesting novel structures producing non-vivid structural colour in its ventrum (Zs. Bálint et al., unpublished).

Tilley and Eliot suggested that in Lycaenidae the *Urania*-type scales appeared first. If this is true the scale type with v-shaped cross ribs and almost closed microcells should be a derived character. Consequently, it cannot define monophyly as we presumed, since a wide array of species obviously representing distinctly related genera also possess this trait, whilst
many others do not. Thus, it is plausible to suggest that the trait under discussion is a character that has arisen independently multiple times within the eumaeine lineage. In the light of this hypothesis, *Eumaeus* fits well with its “satin” microstructure plus with its numerous unique characteristics raising many systematic questions for over a century (Scudder 1877; Tilley and Eliot 2002, p 158). The *E. minijas* scales are highly specialized *Urania*-type scales with the advanced traits of entirely closed microcells, almost attached cross ribs and hidden pepper-pot structures, which are the remnant of previous differently formed and functioning microcells. We can see different grades of the transformation from open to closed microcells in eumaeine lycaenids: there are larger quadrant microcell openings in *S. tephraeus* while the microcells are almost closed in *T. loxurina*. The microcell openings of *P. culminicola* appear intermediate in this regard. Note that *P. perezi* gives an almost complete transformation series from “common” *Urania*-type open microcells with straight cross ribs to the specialized closed microcells with v-shaped cross ribs (Figure 3B–D). The appearance and function of this wide array of scale transmutation is not yet understood.

**Adaptation, discoloration and speciation**

One of the possible applications of the vivid colours amongst butterflies is that they produce visual signals during courtship in a visually complicated environment (e.g. Silberglied 1984; Sweeney et al. 2003). This theory does not explain why many lycaenid females are also vividly coloured dorsally and how the courtship of monomorphic species works. Moreover, the butterflies we studied live in relatively simple visual biotopes: (1) at the edge of boreal forest canopy, (2) in the upper canopy, or (3) in the montaneous tundra or steppes. In these environments, structural colours may function differently and their loss may indicate important evolutionary changes.

A possible explanation was presented by Biró et al. (2003): scale microstructures are working as solar panels and play a role in the thermal regulation of individual butterflies. This was tested in a polyommatine lycaenid sister-species pair, and it was experimentally demonstrated that the representative of the discolored monomorphic brown species living in an environment with less insulation per hour absorbed 1.5 times more solar energy than its dimorphic blue relative inhabiting a warmer biotope. The results of this experiment may also explain why females are dorsally brown in most of the temperate species: they need more energy to maintain the optimal body temperature to develop fertile, viable eggs. We concluded that the putative loss of blue or green iridescence on the wings of male polyommatine lycaenid individuals we examined was the result of adaptation to an environment differing with respect to the task of absorbing energy compared with that of the parental stock. Consequently, the phenomenon may serve as an indicator of an ecosystem where populations of a species are forced to obtain energy in more efficient ways. Thus, discoloration (and monomorphy, which can be considered an end point along the spectrum) might be expected to occur at the geographical or spatial limits of the species involved.

The discoloured (and monomorphic) orange species *P. aurulenta* inhabits orale biotopes in very high altitudes above 4000 m (Lamas and Pérez 1983, p 35–36; Bálint 1997, p 11–12). This was the most common *Penaincisalia* between 4100 and 4600 m in a deep glacial valley. The other congeneric dimorphic species we investigated, *P. culminicola* and *P. perezi*, concentrated in large numbers at lower elevations (Bálint 1997, p 12; 2001, p 171). The genus *Penaincisalia*, according to the available data (Johnson 1990, 1992), typifies the
oreal biota of the high Andes as it extends from central Ecuador to north-eastern Chile and north-western Argentina—an area accommodating many dimorphic species. Obviously, *P. aurulenta* is one of the *Penaincisalia* taxa penetrating into a high-altitude niche, extreme for the genus. Most of the internal layers producing the pepper-pot nanostructures in the *P. aurulenta* scale microcells are not present, and hence its scales do not function as a photonic band gap material. Consequently, the bodies of the scales can absorb more energy, exactly in the manner we have demonstrated for *Polyommatus marcidus* (Lederer, 1862) (see Biró et al. 2003). This is well supported by the similarity of the scale structures of *P. marcidus* and *P. aurulenta* (see for *P. marcidus*: Biró et al. 2003, Figure 2c; *P. aurulenta*: Figure 2C). Therefore we presume that the loss of the pepper-pot layers in this eumaeine lycaenid is also adaptive and is the product of thermal regulation by the individual butterflies.

The curiosity of the orange *Penaincisalia* species is also emphasized by the irregularity we have detected in their scale microstructures. If we compare with *P. perezi* (Figure 3A) and *P. culminicola* (Figure 4C) the distance between the cross ribs (=the width of microcells) of *P. aurulenta* (Figure 2C) we see a lower degree of irregularity in *P. culminicola* and *P. perezi*. The distance between the cross ribs (=the width of the microcells) in *P. aurulenta* has a dramatic effect on its nanostructures. Populations exposed to heavy environmental pressure display irregularity in their structures which can easily lead to structural asymmetry, and is often reflected by populations under stress (Moller 1999). *P. aurulenta* inhabits a particularly barren high-altitude habitat most hostile for the species in genus *Penaincisalia*.

The genus *Thecloxurina*, according to available data (Johnson 1992; Bálint and Wojtusiak 2003), is typically found in the montane cloud forest ecotone from the Venezuelan Cordillera de Mérida through the Colombian, Ecuadorian, Peruvian, and Bolivian Andes to north-western Argentina at altitudes from 800 to 4000 m. The two *Thecloxurina* species we studied live in the upper canopy or in the edge of the cloud forest. *T. atymna* inhabits high altitude mountain “paramo” regions close to the equator. The species is typically found in the upper belt of the montane cloud forest ecotone belonging to the boreal biome. Although the blue dimorphic *T. loxurina* and the orange-violet *T. atymna* generally appear regionally sympatric and synchronic, in the field their distributions only partly overlap. It was recorded by Fassl (1911, p 29) that *T. atymna* replaces *T. loxurina* at higher elevations. He also noted that *T. atymna* never occurs below 2800 m. Recent field studies in transects aimed at studying vertical butterfly diversity support Fassl’s observations (Bálint and Wojtusiak 2003, p 367, 375). Therefore we suggest the dull orange of the male *atymna*, as well as the deeper orange of *atymna* female, are also adaptive. Most of the internal layers producing the pepper-pot nanostructures in the microcells are lost in *T. atymna*. Hence, the scale bodies cannot work as photonic band gap materials. The structures are also similar to those in *P. aurulenta* and *P. marcidus* (see above). And the irregularity in the microstructures of *T. atymna* is very striking: there are “H”, “Y” and “X” letter-like formations of the cross ribs; in addition, there are microcells completely or partly closed (Figure 6C–H). If we compare the *T. atymna* cross ribs with that of *T. loxurina*, the irregularity and asymmetry is even more marked: the *T. loxurina* microcell widths are almost regular; there are no cross ribs in juxtaposed or fused positions (Figure 5E, F).

Most lepidopterists presume that the high Andean butterfly fauna, including eumaeine species we studied, are of relatively recent origin among the South American fauna (Descimon 1986; Viloria 2003). Indeed, their sclerotized genital structures differ from their closest relatives only by quantitative characters, suggesting that *Penaincisalia* and *Thecloxurina* divergence is quite recent (Hall et al. 2005). Their qualitatively identical
ventral wing patterns also support this view, as it is generally accepted by lepidopterists that wing pattern is subject to rapid adaptation. Another feature in support of their recent divergence is their restricted distributions. The high-altitude habitats they occupy are younger and far more recent compared to those of the surrounding biota (Shapiro 1991, p. 211). However, some aspects of their presence are hard to explain, especially if we consider that five Penaincisalia species inhabit the Cordillera Blanca (Bálint 2001, p. 172), which was certainly heavily glaciated 50,000–10,000 years ago. It is similarly hard to explain the occurrence of five Thecloxurina species in the Cordillera Oriental of Colombia (Bálint and Wojtusiak 2003). The available geohistorical time is difficult to reconcile with biotic events if we want to explain such a diverse fauna arising from allopatric or parapatric speciation (cf. Viloria 2003).

A rapid diversification across an elevational gradient seems to be far more plausible. Doebeli and Dieckmann (2003) convincingly demonstrated that diversification along environmental gradients is a probable explanation for syntopic speciation if the gradient is steep enough. The heavily glaciated Cordillera Blanca with its Penaincisalia fauna, and the Equatorial high Andes with their diversified Thecloxurina lineage, support this theory. We believe that populations of lycaenid butterflies respond quickly to environmental pressures by wing scale structure modifications. These influence the lives of the individual butterflies, as thermal regulation is involved, which is the key to their activity: behaviour, habitat preference, reproduction, and survival. Clusters of individuals with different scale nanostructures producing different dorsal colours absorb solar energy differently. If they are syntopic and synchronic, as are the three Penaincisalia in Peru and the two Thecloxurina in Ecuador that we studied, they need to occupy different ecological microniches, a situation that may result in rapid diversification.

In the future it will be fundamental to test this scenario via phylogenetic analysis, field studies and experiments, especially in the context of other recent investigations in butterflies, such as an analysis of large nymphaids displaying chemical dorsal coloration on their wing surfaces, that appear to have a different mechanism for speciation in the Andes (Willmott et al. 2001).

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Dedication

The present paper is dedicated to the memory of John Nevill Eliot (died in 2003), who elaborated the higher classification of Lycaenidae (Eliot 1973). J. N. Eliot was a friendly colleague of Zs. Bálint, who visited him on 16 August 2000 at his home. During that visit J. N. Eliot showed him a manuscript that he had produced at that very advanced age in
his life. The work was impressive, and was actually another significant step in lycaenid systematics. This paper was his last opus: Tilley and Eliot 2002. An offprint of this paper reached Zs. Bálint on 26 August 2002 with the dedication: “Zsolt: Best wishes. John”.

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