The "windows", scales, and bristles of the tropical moth *Rothschildia lebeau* (Lepidoptera: Saturniidae)

Francisco Hernández-Chavarría[1,2*], Alejandro Hernández[3] & Ana Sittenfeld[1,3]

1 Facultad de Microbiología, Universidad de Costa Rica, San José, Costa Rica.
2 Centro de Investigación en Estructuras Microscópicas (CIEMic), Universidad de Costa Rica, San José, Costa Rica.
3 Centro de Investigación en Biología Celular y Molecular (CIBCM), Universidad de Costa Rica, San José, Costa Rica.
* Correspondence author: hchavarr@cariari.ucr.ac.cr

Received 29-VI-2004. Corrected 30-IX-2004. Accepted 06-XII-2004.

Abstract: The common Spanish name of the moth *Rothschildia lebeau* (Saturniidae) is *cuatro ventanas* (four 'windows'), because it exhibits a transparent oval path in each wing. The scales of the colored areas and the bristles from the "window" were analyzed. We developed a simple device to measure transmittance across the "windows" with an spectrophotometer. A square section of "window" was mounted onto a flat black card and placed onto a clamp that hung in the path of the light - beam of the spectrophotometer. Absorbance was measured at 350 and 550 nm, with the "window" positioned perpendicular to the light beam (incidence of 90°); then the measurements were repeated with the "window" moved at an angle of 45°. Each measurement was replicated 5 times. Wing color spots were analyzed with a light dissection microscope (stereoscope) and with scanning electron microscopy. The scales have a minimum of 4 morphological types, 3 of them showed the typical appearance of unspecialized scales described for other butterflies; whereas the fourth has features particular to this species. On the "window" the scales are transformed in hair-like bristles that do not interfere with light, conferring the transparency that characterizes the "windows". However, if the wing is illuminated at an almost grazing-incidence, they reflect the light as a mirror. Two hypothetical functional explanation for the windows are mimicry and interspecies communication. Rev. Biol. Trop. 52(4): 919-926. Epub 2005 Jun 24.

Key words: Scales microstructure, bristles, scanning electron microscopy, moth, interspecies communication, light reflections, *Rothschildia lebeau*.

The Lepidoptera is among the groups of insects that exhibit spectacular color patterns; the majority of these colors correspond to pigments incorporated inside scales or cuticle (Ghiradella 1998). However, the most beautiful tonalities of iridescent color exhibited by some butterflies are generated by light interference in specialized scales (Giradella 1985, Vukusic *et al*. 2000). Some color patterns imitate environmental shapes and colors (crypsis or mimicry) and help avoid predators. Others may be used in intraspecific communication.

An interesting example of mimetic adaptation is the moth *Rothschildia lebeau*, which rests on trees during the day and look like rotten leaves (Janzen 1984). To match different backgrounds, the moth exhibits different color patterns according to predominant seasonal colors; this originally lead to the misclassification of the same species as *R. aroma, R. morana* and *R. forbesi*. These species were later grouped under *R. lebeau* (Janzen 1984). Janzen followed the development of pupae into adults in different weather conditions and obtained color patterns varying from bright orange to chocolate-rust or chocolate, depending on humidity, which is related to the predominant forest color. The work was conducted in Guanacaste, Northwestern Costa Rica, where the common name of the moth is *cuatro ventanas* (four "windows"), due to the presence of an oval transparent patch at each wing (Fig. 1). In this context the patch was called a "window", which is written in quotation marks in order to differentiate it from the section of the scale called a window, according to the nomenclature proposed by Ghiradella (1998).

The purpose of this paper is to describe the fine structure of the "window", bristles and scales of the wings of *R. lebeau*.

MATERIALS AND METHODS

Each "window" of *R. lebeau* appears as a transparent body, meaning that its transmittance would be around 100%; but, when tilted, it reflects incident light: transmittance is reduced and the transparent body acts then as a mirror. To document such a phenomenon we developed a simple device to measure transmittance across the "windows" of *R. lebeau* using a spectrophotometer (Shimatsu 300 UV). A square section of the wing with the "window" was mounted onto a flat black card and placed onto a clamp that hung in the path of the spectrophotometer's light - beam. Absorbance was measured at 350 and 550 nm, with the "window" positioned perpendicular to the light beam (incidence of 90°); then the measurements were repeated with the "window" moved at an angle of 45°. Each measurement was replicated 5 times.

The different color spots of the wings of *R. lebeau* were analyzed whith a light dissection microscope (stereoscope) and with scanning electron microscopy (SEM) in order to describe the pattern of scales and bristles on the "windows" and around them. For stereoscopic analysis the wings were mounted directly. For SEM analysis, sections of each color spot of the wing were cut and mounted directly on aluminum studs, without fixing or dehydration. They were covered with gold (20 nm thick) by using an ion sputter cover (Eiko IB-3) and observed through a SEM (Hitachi S-570).

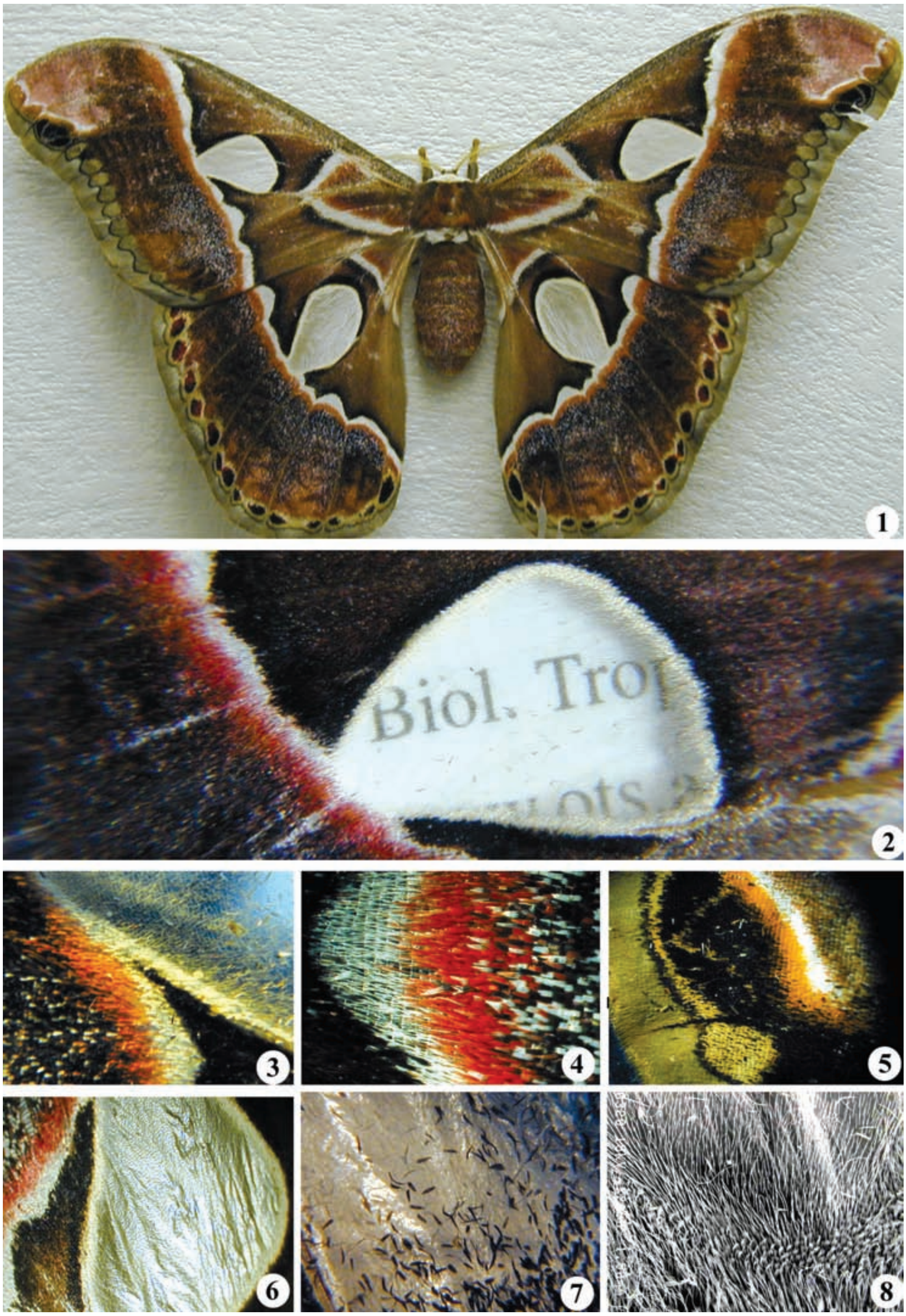
RESULTS

Macroscopically the color of *R. lebeau* varies from chocolate to a dark chocolate-rust; however, under the stereoscope different colors and designs arose. For example, the "windows" are edged by white (Fig. 2) or yellow scales (Fig. 3) and near the lighter band there is a red and white spot (Fig. 4). Additionally, the fringes of the forewing and hind-wing have a marginal design of yellow spots with black lines (Figs. 1 and 5).

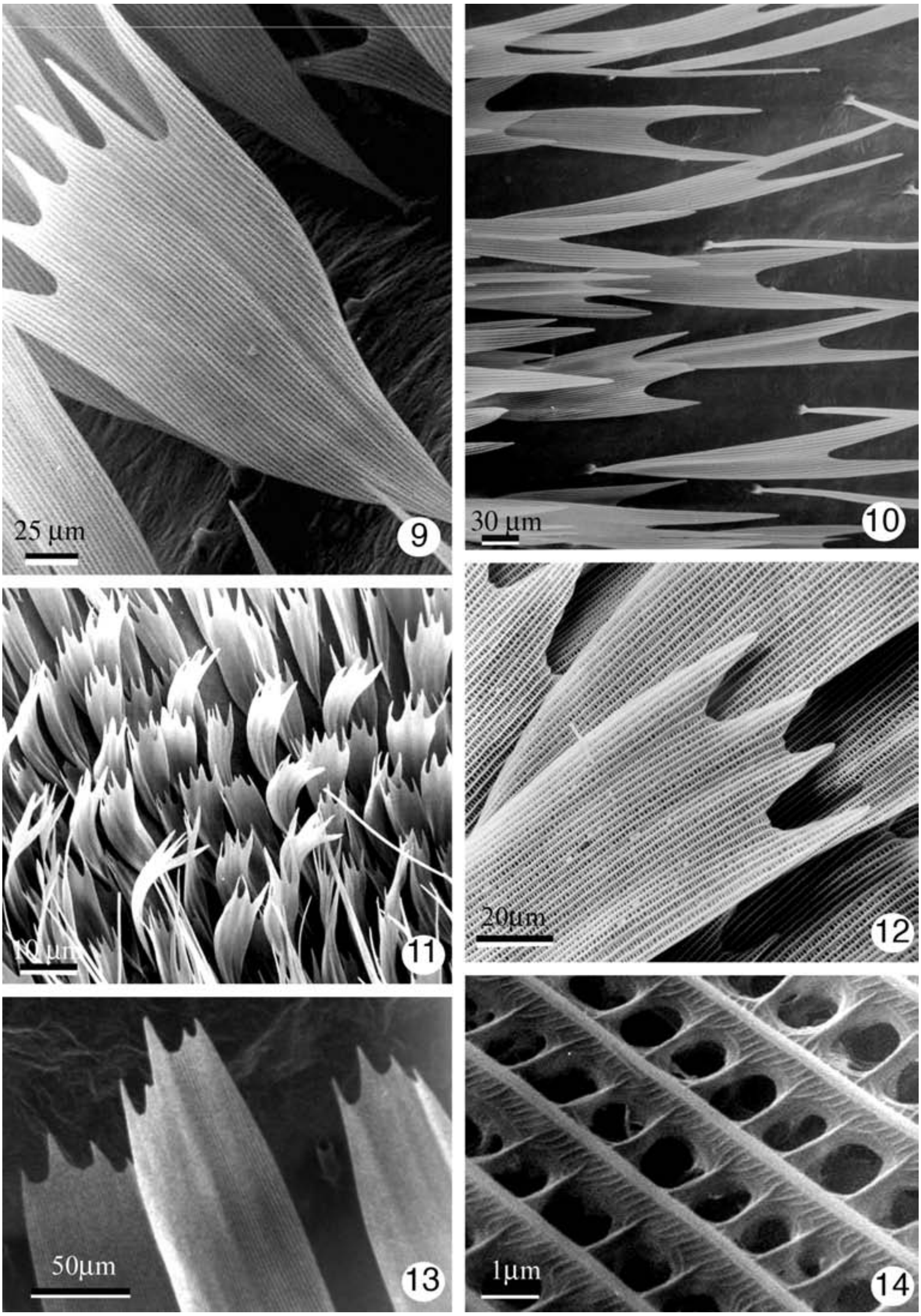
The transparency of the "window" is evident in Fig. 2: one can read through it. However, if the "window" is observed through the stereoscope with a grazing-incident light, it appears as brilliant as a mirror, reflecting light (Fig. 6). This observation was corroborated by analyzing wing fragments with "windows" through a spectrophotometer with a light wavelength of 550 µm (blue to green light). When the "window" was perpendicular to the light beam, the absorbance was 0.974±0.017, and when the wing was tilted around 45° the absorbance changed to 1.127±0.043. However, at 350 µm (ultraviolet light), both measurements were 0.803±0.068 and 0.858±0.022, respectively. These data imply that the absorbance was altered when the "window" was illuminated at 90° and 45° using visible light but was not affected by ultraviolet light.

The stereoscope showed than the "windows" covered by thin bristles do not interrupt the path of light (Fig. 7). This observation was confirmed through the SEM (Fig. 8). The "window" edge presents a row of two or three teeth scales.

The difference between cover and ground scales was not evident; however, this moth has at least four morphological scale types; and each had a narrow pedicel and a flat main body with a serrated end (Fig. 9). The broader area of the wings had predominantly brown tones with yellow and red spots. The scales of these



Figs. 1-8. "Window" structure. Fig. 1. *Rothschildia lebeau* with its four transparent areas, called "windows". In Fig. 2, a "window" was photographed over print to make transparency evident. Figs. 3 to 5 enlarge segments of the wing, displaying different color patterns, such as yellow and red (Fig. 3), white and red (Fig. 4), and black lines on yellow areas (Fig. 5). The transparency of the "window" disappears when is photographed with a grazing- illumination, because it reflects light as a mirror (Fig. 6). At the stereoscope scales around the "window" and the fine bristles on its surface are visible (Fig. 7). This is even more clear with scanning electron microscopy (Fig. 8).



Figs. 9 to 14. Scanning electron micrographs of scales of *Rothschildia lebeau*. Fig. 9. General appearance of an unspecialized scale with 6 teeth at its serrated end. Fig. 10: Scales with 2 and 3 teeth. The scales with 4 teeth remember the "Batman" cartoon character logotype (Fig. 11); one of them is showed at higher magnification in figure 12. Scales with an irregular teeth pattern are shown in Fig. 13. Fig. 14: High magnification of a four-teeth scale, composed by longitudinal ridges, transversally connected by cross-ribs that form an open window; the ridges are also ornamented by orthogonally micro-ribs.

colored areas had the typical appearance of unspecialized scales; they are around 300 μm long by 120 μm wide at the toothed border. Around the "window" there was a yellow or white line with scales of only two or three teeth, some of them projecting the toothed-end to the "window" (Fig. 10). The average width of these scales was 80 μm. A second morphological scale type was represented on the clear brown area near the "window" or on the red spots. These scales showed four picks; the central picks higher than the lateral picks (Figs. 11 and 12). A third type of scale appeared on the dark brown areas. These scales had irregular patterns of 3 to 5 teeth (Figs. 9 and 13).

The fine structure of these scales, independent of their tooth pattern, showed a top surface with a lattice of parallel longitudinal ridges. The ridges are transversally connected by cross-ribs, forming square concave windows and partially obliterated with only a central round hole (Fig. 14). The border of the window is ornamented with micro-ribs, which were more evident when the wing was tilted around 30° in the SEM (Figs. 15 and 16).

A fourth morphological type of scale was observed at the light brown area underneath the forewings, near their edges. These scales had a single pointed end (Fig. 17) and their fine structure was characterized by ridges with a prominent system of ridge-lamellae (Fig. 18). The distance between ridges ranged from 1.4 to 1.8 μm; but the cross-ribs measured around 1 μm. For this reason, they do not connect to the adjacent ridge (Inset of figure 18). Additionally, they delimited square windows with a small central oval hole. Their micro-ribs appear in only the side of the ridge that is not touched by the cross-ribs (Fig. 18).

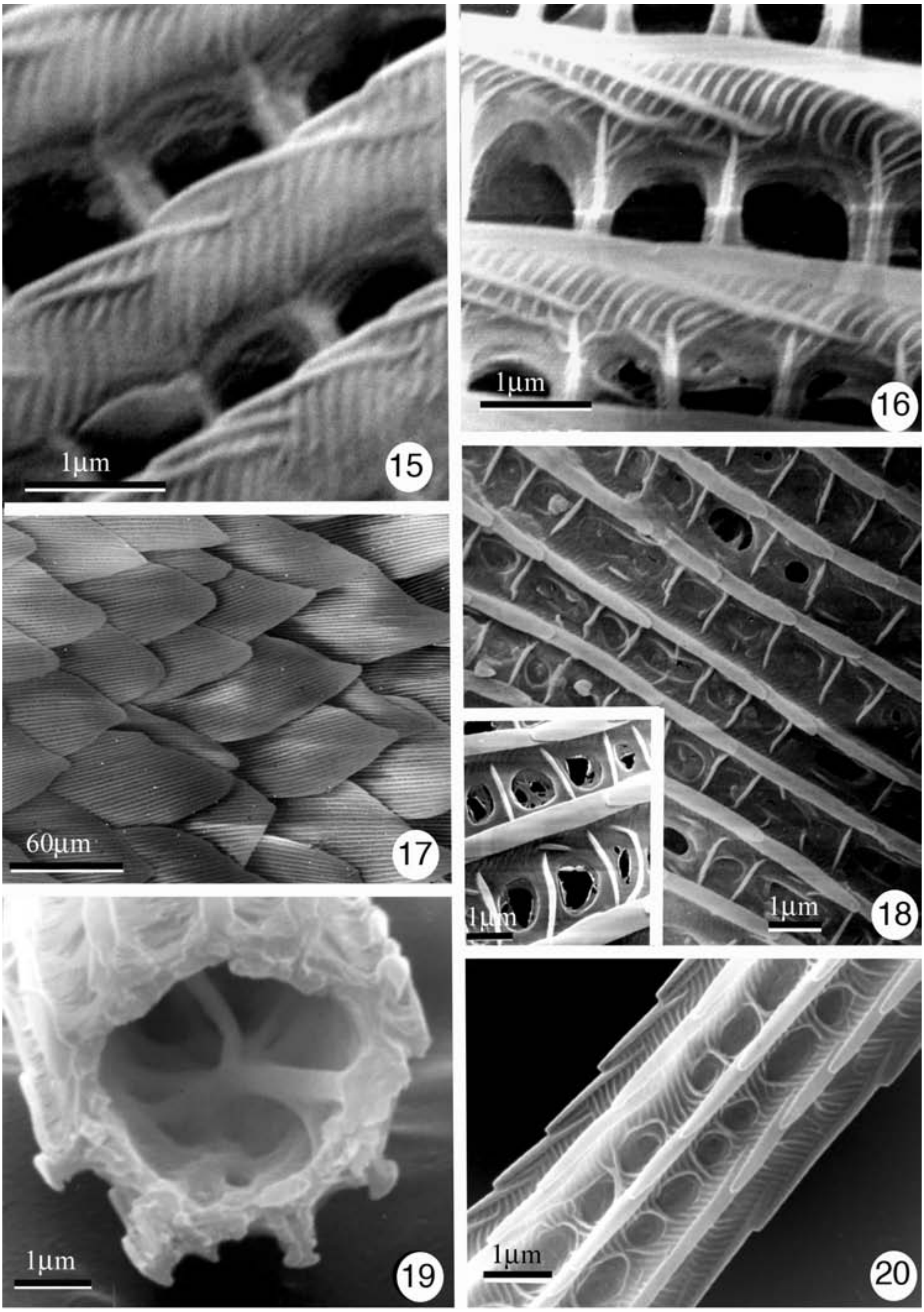
On the "windows", bristles replace scales and appear as thin hair-like structures dispersed though a bare cuticle (Fig. 8). The bristles were hollow; they are only 5 μm wide and had eight spiny ridges, due to the presence of prominent ridge-lamellae (Fig. 19). The micro-ribs were evident around un-opened rounded windows; but the cross-ribs were not evident (Fig. 20). The bristles were almost perpendicular to the cuticle. For this reason, they do not interfere with the incident path of light that inside directly on the cuticle; thus it appears transparent or brilliant as a mirror depending of the incident angle.

DISCUSSION

The majority of butterflies show two scale sizes, which are arranged alternately in each row; the larger ones, called "cover" scales, have a more complex structure; whereas the smaller, "ground" scales, are partially covered by the former (Ghiradella 1994, 1998). That pattern of cover and ground scales was inconspicuous in *R. lebeau*. This moth exhibited at least four different morphological types of scales. Also, their transparent spot, or "window", appeared as a bare area with dispersed bristles that do not interfere with the path of light.

Macroscopically *R. lebeau* varied from chocolate to a dark chocolate-rust and mingles with the dry leaves. Nevertheless, a complex pattern of color and "drawings" are visible through the stereoscope. The complex arrangement is reflected in the existence of at least four morphological types of scales. However, their fine structure was very similar to the description of unspecialized scales, characterized by the presence of ridge-lamella and micro-ribs (Figs. 15 and 16), such as some unspecialized scales of *Morpho* and *Caligo* (Ghiradella 1998) and iridescent scales of some butterflies (Vukusic *et al*. 2001a, b, Vukusic and Sambles 2003). However, the fourth type (single tooth scale) showed a different structure, the micro-ribs are localized only in one side of the ridge and the majority of the windows are not opened. Those that appear open (inset of Fig. 18), might be artifacts caused by ion coating or by electron damage during SEM analysis. To our knowledge, the pattern of short ribs with micro-ribs in only one side of the ridges is a distinctive characteristic of *R. lebeau*, previously not described in other butterflies.

Modified microstructures on the scales account for the brilliant iridescence color of



Figs. 15 to 20. Scanning electron micrographs of scales and bristles of *Rothschildia lebeau*. Figs. 15 and 16: Enlarged area of the ridges that show ridge-lamella as a tilt of cylindrical structures located on the upper side of the ridges ornamented by orthogonally micro-ribs. Fig. 17. Scales with only one tooth and their fine structure is showed in Fig. 18. The micro-ribs are present only on the rib side and not touched by the cross-ribs. Most of these windows are not open. Inset: section with open windows. Figs. 19 and 20. Bristles on the "window": Fig. 19: Cross section of a bristle showing its hollow interior with some support stalks. Externally they have 8 ridges. Fig. 20: Intact bristle, the ridges with ridge-lamellae and surrounding micro-ribs without open windows; cross-ribs are not evident.

several butterflies (Vukusic *et al*. 2001a, b, Vukusic and Sambles 2003). Such specialized modifications evolved as response to environmental changes in the natural history of those insects. Along that line of thinking, why did *R. lebeau* develop the transparent “windows”, which characterize it? Other transparent butterflies developed antireflective structures, such as conglomerates of “nipples” (Ghiradella 1999, Vukusic 2004), possibly to avoid flashes due to reflections on their cuticle, which would make their presence evident to predators. The “windows” of *R. lebeau* apparently evolved in the opposite way, due to the absence of such antireflective structures and the disposition of bristles almost perpendicular to their cuticle that remain bare areas to the cuticle and do not avoid reflections.

In butterflies with structural color, some tonalities emerge according the angle of illumination; moreover, an example of grazing illumination was described by Laurence *et al.* (2002) to explain a specialized iridescence mechanism. Our observations suggest that in *R. lebeau* such type of illumination could be associated with a mirror-like reflection of light from the “windows” (Fig. 6). Why did this moth develop structures that could make it more evident instead of hiding it? Janzen (1984) interpreted the “windows” of *R. lebeau* as imitation of holes in a rotten leaf. Refereing to other species, Lawrence *et al*. (2002) and Sweeney *et al*. (2003) suggested that the exhibition of brilliant colors may serve intraspecific communication, both among competitors and potential mates. In that sense, we suggest a different hypothesis: the “windows” of *R. lebeau* could be mirrors that send brilliant signals in the moonlight during the night when this moth flies. Those possible light signals could be a form of intra-species communication, since there is evidence that some moths have really good night vision (Ghiradella 2004, personal communication). However, the use of “moon light” in intra-specific and inter-specific interactions by this species has not been recorded. Experimental field studies to ascertain the real significance of the “windows” of *Rothschildia lebeau* are suggested to future researchers.

ACKNOWLEDGMENTS

We thank Hellen Ghiradella for her helpful and valuable suggestions on the manuscript, Daniel H. Janzen for his comments on an earlier draft (albeit he did not agree with the communication hypothesis that we present here), Felipe Chavarría (Área de Conservación de Guanacaste) for providing specimens, Illeana Holtz (Faculty of Microbiology) for helping with spectrophotometer measurements, and Julían Monge-Nágera for helpful suggestions and grammar corrections. This research was financed by Grant 801-99-515 from the Research Vice-presidency of the University of Costa Rica and facilitated by National Science Foundation Grants MCB 0084223 and 0084222.

RESUMEN

El nombre común de la mariposa nocturna *Rothschildia lebeau* (Saturniidae) es “cuatro ventanas”, porque exhibe una zona transparente en cada ala. Las escamas de las áreas coloreadas y las cerdas de las “ventanas” fueron analizadas al estereoscopio y al microscopio electrónico de rastreo. Al menos se identificaron cuatro tipos morfológicos de escamas similares a las escamas no especializadas de otras mariposas. En la “ventana” las escamas han sido sustituidas por cerdas que no interfieren el paso de la luz, confiriéndoles la transparencia que las caracteriza. No obstante, si el ala es iluminada en ángulo rasante refleja la luz como un espejo. Dos hipótesis para explicar la evolución de estas “ventanas” son el mimetismo y la comunicación.

Palabras clave: Microestructura, microscopía electrónica de rastreo, escamas, cerdas, mariposa nocturna, comunicación interespecífica, reflectión de luz, *Rothschildia lebeau*.

REFERENCES

Ghiradella, H. 1985. Structure and development of iridescent lepidopteran scales: The Papionidae as a showcase family. Ann. Entomol. Soc. Am. 78: 252-264.

Ghiradella, H. 1994. Structure of butterfly scales: Patterning in an insect cuticle. Microscop. Res. 27: 429-438.

Ghiradella, H. 1998. Hairs, bristles, and scales. *In* Microscopic anatomy of invertebrates. Vol 11A: Insecta. p. 257-287.

Ghiradella, H. 1999. Shining armor: Structural colors in inscects. OPN 10: 47-48.

Janzen, D.H. 1984. Weather-related color polymorphism of *Rothschildia lebeau* (Saturniidae). Bull. E. S. A. 16-20.

Laurence, C., P. Vukusic & R. Samples. 2002. Grazing-incidence iridescence from a butterfly wing. Appl. Optics 41: 437- 441.

Sweeney, A., Jiggins C. & Johnsen S. 2003. Polarized light as a butterfly mating signal. Nature 423: 31-32.

Vukusic, P., J.R. Samples & H. Ghiradella. 2000. Optical classification of microstructure in butterfly wing-scales. Photonic Sci. N. 6: 61-66.

Vukusic, P., J.R. Samples, C. Laurence & G. Wakey. 2001a. Sculpture-multilayer optical effects in two species of *Papilio* butterfly. Appl. Optics 40: 1116-1125.

Vukusic, P., J.R. Samples, C. R. Laurence & R. J. Wootton. 2001b. Limited-view iriescence in the butterfly *Ancyluris meliboeus*. Proc. R. Soc. Lond. 269: 7-14.

Vukusic, P. 2003. Sambles. Photonic structures in biology. Nature 424: 852-855.

Vukusic, P. 2004. Natural Photonics. Physics World. (February): 35-39.