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Role of photonic crystals in cabbage white butterfly, *Pieris rapae* and queen butterfly, *Danaus glippus* coloration

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The colors of some Lepidopteron insects were resulted from light scattering and overlapping. If the light contacts the scale insects wings, the nanostructure which existed in the wing scales would reflected the light or absorbed all colors and reflect one color. The obtained results showed that the white butterfly, *Pieris rapae* fore and hind wings have nanostructures called pterin beads. These pterin beads worked as the photonic crystals. These nanostructures are reflecting all light colors so theses wings appear with a white color. All wings scales not straight on the wing basement membrane but it was diagonal and made an angle with the basement membrane of wing. This angle helps the light to reflect and gave the wings colors. In queen butterfly, *Danaus glippus* wings pterin beads were not found. But the longitudinal, parallel ridges, crossribs ridges and lamellae microribs in wing were reflecting, scattering and overlapping the light. The diagonal scales were also in the fore and hind wings of colored butterfly. When the light falls on these scales it reflected and overlapping to make the wings color. These results confirmed that the nanostructures play an important role in both white and colored butterflies. The results also concluded that the nanostructures in the wings are photonic crystals.

Keywords: White butterfly, queen butterfly, wing scales, photonic crystals, pterin beads

INTRODUCTION

Photonic crystals are nanostructures that control of photon motion exactly such as electron in solids. In insects wings coloration it was known that the photonic crystals play an important role in some insects especially Lepidoptera and Coleoptera adults. The role of photonic crystals in insect's wing coloration was summarized in the role of these nanostructures in scattering, reflecting and/or overlapping the colors (Michielsen and Stavenga, 2008). This role also existed in Coleopteran wings such as red palm weevil (Ragaei and Sabry, 2017). Yoshida (2002) recorded that the scales insects wings may play some roles in inhibition of mirror reflection in some lepidopteran wings. Arranged of nanoscale photonic crystals in insects wings can be useful in insects taxonomies (Ragaei and Sabry 2016).

Butterflies are discriminated by the attractive and numerous colors. Some of these colors were due to melanin pigments (Kinoshita and Yoshioka 2005). This called chemical coloration. Other butterflies coloration was due to nanostructures called photonic crystals refection. These types of coloration called physics colorations (Michielsen et al. 2010). A butterfly's rich color can act as camouflage, mate attraction, and warning signal. So, it can be say butterflies get their colors from two different sources. The first one called ordinary color and the second called structural color. On the other hand, Morehouse et al. (2007) found that many animals' colors are due to both structural and chemical mechanisms. The authors found also, Pierids butterflies produce the bright colors (orange and yellow,) by wing scales that contain collections of minute granules. Plskova et al. (2011) observed on the ridges of butterfly wing scales, the wing coloration must be less because of as small width of their ridges as compared with the flat grooves. Stavenga et al. (2014) found that the coloration of the butterfly belong to subfamily Nymphalinae, is due specific patterning of differently colored scales on their wings

This work aims to describe the difference between the wings coloration in white and colored butterflies by using scan electronic microscope (SEM)

MATERIALS AND METHODS

Insects tests

Two insects were tested in this work
1- The adult of white cabbage butterfly, *Pieris rapae*
2- The adult of queen butterfly, *Danaus glippus*.
These insects were obtained from Plant Protection Research Institute, Dokki, Giza, Egypt. The wings of both adults insects were used. This work carried out in the electronic microscope unite, central laboratory, National Research Centre, Cairo.

Both the cabbage white butterfly and queen butterfly adults were killed by chloroform solvent, cleaning manually and freezing. Freezing of the sample very quickly was instead of fixing it. This technique providing the sample stays cold enough, this ‘locks up’ the water and prevents it from evaporating inside the microscope. After that the wings were separated and coated by gold. Coating of samples with gold is required in the field of electron microscopy to enable or improve the imaging of samples. Creating a conductive layer of metal on the sample inhibits charging, reduces thermal damage and improves the secondary electron signal required for topographic examination in the SEM. All images were taken under low vacuum scanning electron microscope (Jeol-JSM-5600 LV in SEM).

RESULTS AND DISCUSSION

Butterflies wings have many and many colors. These colors are used in taxonomy of these butterflies. Some of these colors are produced by melanin pigmentation and other due to interference of lights by nanostructures existed in the wings called photonic crystals. The wings involves of scales in the forewings and hind wings.

The cabbage white butterfly, *Pieris rapae* wings colorations

The cabbage white butterfly, *P. rapae* adults have white scales on the upper of both forewings

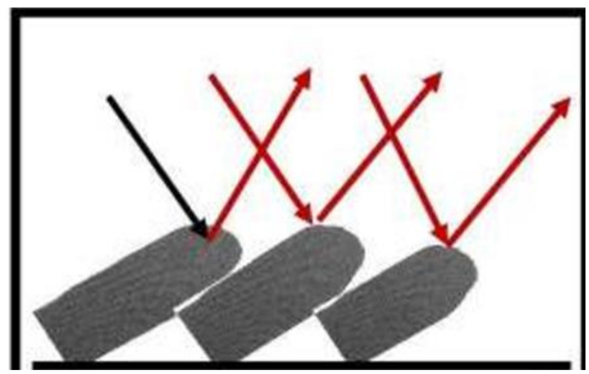

Fig. 1. The diagonal scales of butterfly

and hindwings. By using the scan electronic microscope (SEM), the scales are not straighted but diagonal and made an angle with the basement membrane of wing (Fig. 1). This diagonal shape is allowing of these scales to reflect, scatter and overlap the light which contacts with these scales. Johnsen and Widder (1999) showed that the pterins beads size was existed for light scattering and the two types of wing scales (‘cover’ and ‘ground’ scales) together produce wide-angle scattered light.

The nanostructures which are on these scales (pternis beads) also, reflect all visible spectra of light. So, these scale are appears with white colors. Some areas in both the fore- and hindwings have some black spots (Fig. 2a). In these areas the nanostructure are not existed (2d), so these wings have some black light. So, it can be said that the diagonal shape of the white butterfly scales play an important role in coloration. The same shape was observed in the hindwings. So, the hindwings are appearing with the white color and have some black spots (Fig. 2e).

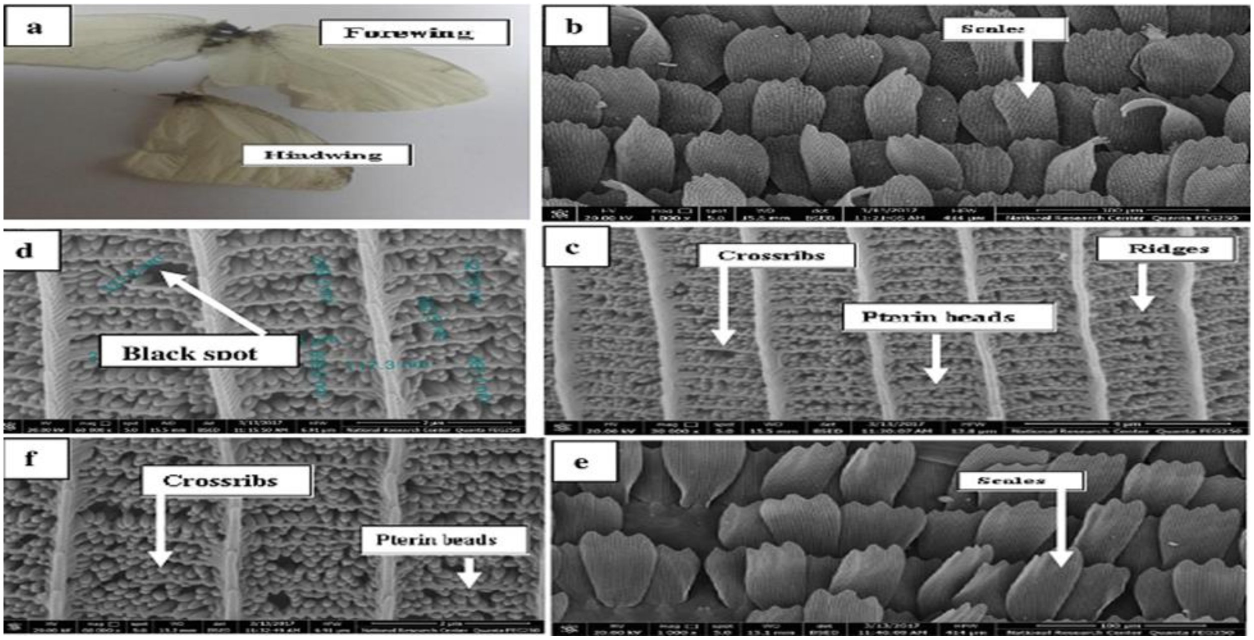

Fig. 2. SEM for the forewing and hind wing of the cabbage white butterfly (a) showing the scales of forewing (b), the ridges, crossribs, pternis beads of forewing (c), the size of ridges, pternis of forewing (d), the scales of hindwing (e) and (f) the ridges, crossribs, pternis beads of the hindwing

When the light contacts with the wings the nanostructure called pterins bead (Fig. 2c and 2f) existed on both the fore- and hindwings are reflecting all visible spectra of light. The size of these nanostructures is ranged between 312.9 to 249.3 nm (Fig. 2d). These nanostructures reflect all spectra light and produce the white colors. In the black areas these pternis beads absorb all the spectra light and produce the black colors. Stavenga et al. (2014) reported that the white color in butterflies was produced by the reflectance of lights on nano-beads located on the wing scales. Stavenga et al. (2010) found that in the absence of absorbing pigments (nanostructures beads only) the scattering of incident light is wavelength independent, resulting in a white scale color. Luke et al. (2009) removing these pterins beads and observed that overall reflectance was decreased by a third when the pternis beads were absent. Stavenga and Arikawa (2006) found that the reflectance is high only above 450 nm, but it is minor below 400 nm, because the scales of *P. rapae* contain a substantial amount of UV-absorbing pteridins. Stavenga et al. (2004) found that the pternis beads which existed on the wing scales of white butterfly may be cause the distinct matt–white color and function to increase the reflectance amplitude. The authors also found the black spots in the white wings are completely free from pternis beads. On the other hand, Blakley (1969) found that pternis beads of the white butterfly wing scales were not exhibit any appreciable absorption at either wavelength (543 nm and 633 nm).

The queen butterfly wings coloration

The queen butterfly, *Danaus glippus* has many colors; orange, red, yellow and black. These colors are due to pigments or by color interferences (Fig. 3a). The forewing scales also, are not straighten to allow the spectra light reflect (Fig. 3b). These scales with diagonal shape allow spectra light to scatter, reflect and overlap to produce the wing colors. The wing scales are containing also the ridges and crossribs. This structure is allowing to the light scattering and overlapping. So, it can be said that the ridges and crossribs act as light reflectors. The distance between these ridges are ranged from 2.015 to 2.025 µm, but the distance between all crossribs ranged from 432.2 to 492.9 nm (Fig. 3d).

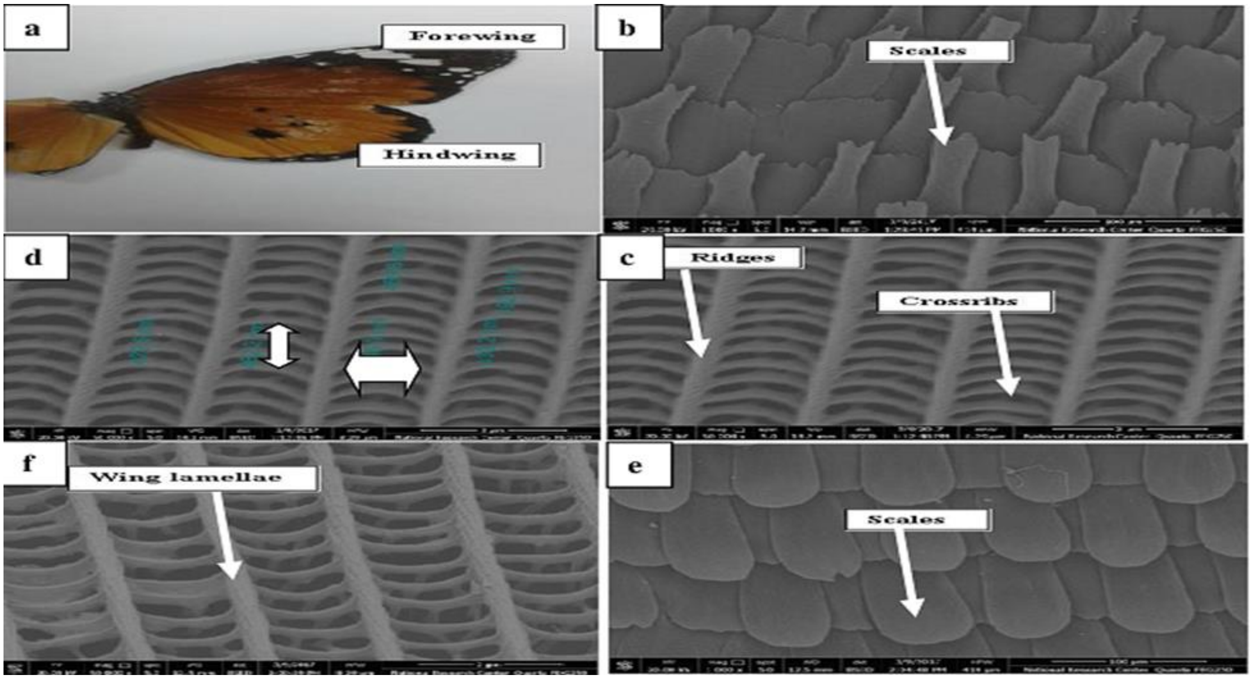

Figure. 3. SEM for the forewing and hind wing of the queen butterfly (a) showing the scales of forewing (b), the ridges, crossribs, pternis beads of forewing (c), the size of ridges, pternis of forewing (d), the scales of hindwing (e) and (f) the wing lamellae, crossribs, pternis beads of the hindwing

The same shapes are also observed in the hindwing (Fig. 3e, f).

As clear in Figure (3e) the wing scales consists of two types; cover scales and ground scales. This shape is making of the cover scales largely overlap the ground scales. Figure (3d) showed that the cuticle on the scales of queen butterfly wings is containing of nano- and microstructures. These structures may be producing the wings coloration. The crossribs that existed from the sides of ridges on the wing scale (Fig. 3c) separate incoming light waves, causing the waves to spread as they travel through spaces between the structures. These spreading light waves allow the spectra light to interfere with each other to produce the wings colors. The ridges and crossribs are playing an important role in insect coloration. The light reflected by the ridges and more or less diffusely scattered by the crossribs. It is known that the sunlight appears as a white light. This because it is a combination of different colors blended together, which all corresponds to different wavelengths. When the white light hits the slits, most of the wavelengths of light approach in phase, but was put out of phase when they bounce off the slits. The scales ridges in wings are playing the role of the slits. As show in Figure (3b and 3e) the scales make an angel on the basement membrane of wing. This angle causes the dull color, and because the valleys between the ridges are rounded, it is easier to view the ridges from any angle, without a change in color or brightness. Figure 3f show that the photonic crystals between the crossribs are responsible to light reflection. Due to the photonic crystals under the ridges and the diffractions caused by ridges and crossribs, the lights reflecting and overlapping formed the wings colors. The same observation was found by Saranathan et al. (2010). The authors found that the photonic crystals which existed in the body of the wing scales as either disjoint crystallites. Photonic crystals in butterfly wing scales are the ideal sources of modern technology in the future. Kinoshita et al. (2008) found that the multilayered ridges in *Morpho* species cause the bright blue of the wings. Rutowski et al. (2007) considered that the ridges in the scale wings act as iridescent reflectors, which shift the light wavelengths to shorter wavelengths when the angle of light incidence increases.

CONCLUSION

Finally, it was concluded that the white butterfly has a pternis beads (as a nanostructure) which responsible to this insects appears with the white colors. These beads reflect all light colors so, it appears with white color. In case of queen butterfly (colored butterfly) some nanostructure such as crossribs and ridges are responsible to light scattering, reflecting and overlapping to produce the wings colors. Both pternis beads and crossribs and ridges act as the photonic crystals in wings colors. The results confirmed that the colors of white butterfly and queen butterfly not due to melanin pigmentation but color interference

CONFLICT OF INTEREST

The present study was performed in absence of any conflict of interest.

ACKNOWLEGEMENT

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AUTHOR CONTRIBUTIONS

Mohamed Ragaei; put the idea and collect the data, Sabry KH; designed the figures and write the manuscript, Farag NA; reviewed the manuscript and collect the data, Huda AH, carried out the SEM for all tested insects and Abdel-Rahman; collect the tested insects and review.

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