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Egg Plastron of the Bog Copper butterfly *Tharsalea (Epidemia) epixanthe* (Bsd. & Le C. [1835]) (Lycaenidae: Lycaeninae).

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ABSTRACT. The egg of the Bog Copper butterfly, *Tharsalea* (*Epidemia*) *epixanthe*, has a prominent highly-sculptured chorionic surface. Trapped within the chorion is a labyrinth of air spaces which has been proposed as a plastron for gas exchange while the egg is submerged in water. Data derived from scanning electron microscopy (SEM) confirms the plastron should function as predicted. Furthermore, the insulating air spaces should prevent water loss of the diapausing first instar larvae while overwintering.

Key words: Bog Copper, egg, plastron, air spaces, prevention water loss

INTRODUCTION

The Bog Copper *Tharsalea* (*Epidemia*) *epixanthe* (Boisduval & Le Conte [1835]) is a small lycaenid butterfly restricted to acid bogs in eastern North America. The life history and morphology of the immature stages were previously described by the author (Wright, 1983). In all its stages the butterfly is closely associated with its larval host (cranberry). The egg consists of the ovum/embryo, surrounded by a vitelline membrane and an encompassing outer layer (chorion). The highly sculptured chorion (Fig.1) has a honeycomb appearance created by a series of intersecting ridges and depressed pits properly called cells. Beneath the chorion is a labyrinth of air spaces which connect to the environment via small holes (aeropyles). This trapped gas layer beneath the chorion is predicted to serve as a plastron allowing gas exchange to occur when the egg is submerged in water. *Epixanthe* eggs are commonly covered by water droplets following rains (see above photo) and occasionally they are covered for several days after flooding of the bog. Plastronic respiration for this species has not been thoroughly tested and mathematically confirmed as a proof a concept. This paper presents a method to determine feasibility of the idea and to verify the plastron should function as envisioned. Recently, Zhang *et al.* (2020) presented genomic evidence that the egg-diapausing North American coppers can be gathered into a single genus *Tharsalea* Scudder. Diapause in this group occurs as fully-developed first instar larvae within the egg. The chorionic meshwork of their eggs may have a supplemental function beside plastronic respiration.

MATERIALS & METHOD

The water-air interface across the aeropyles is critical for the formation of a plastron. One major factor that determines the efficiency of a plastron is the water-air interface in relation to the weight of the insect. However, calculating the weight of tissue within the egg is not always practical. A more pragmatic method is determining the percentage of the egg's surface area that must be water-air interface to satisfy the conditions of a plastron. Hinton (1969) supplied a useful table of these percentages "for spherical eggs of different diameters and for prolate spheroids of different shapes using a complex equation." In order to compute the total aeropyle surface area of the *epixanthe* egg, scanning electron microscopy (SEM) was employed. Eggs (n=8) were prepared for SEM as in Wright (1983). Eggs were collected ex *Vaccinium macrocarpon* at Forge Pond, Wharton SF, Atlantic County, New Jersey, USA, July 1981 and April 1982.

MEASURING THE WATER-AIR INTERFACE. Aeropyles of the *epixanthe* egg are typically rounded and vary 1-6 µ in diameter. They are heavily concentrated within the walls and base of the cells. By measuring their radius in SEMs, the surface area of each aeropyle may be computed (πr^2). The total aeropyle surface area per standard cell is established by addition. The finalized aeropyle surface area per egg is concluded by multiplying the previous number by the typical number of cells per egg.

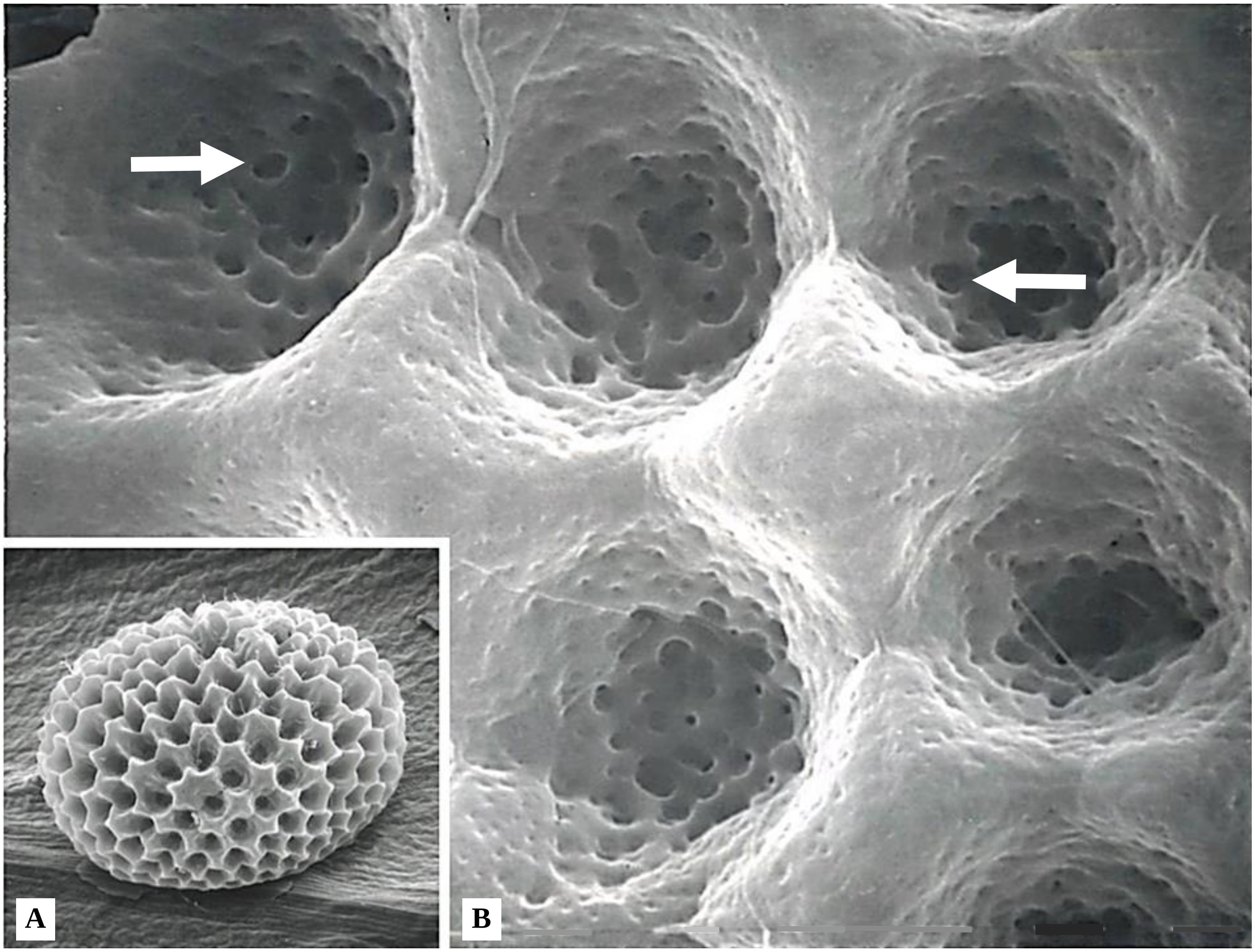

Figure. 1. Scanning electron microscopy (SEM) of *Tharsalea epixanthe* egg. (A.) Whole egg view showing honeycomb pattern. 80x. (B.) Close-up of chorionic cells and aeropyles (arrow). 640x.

CALCULATING EGG SURFACE AREA VIA MODEL. The *epixanthe* egg is not spherical. Thus equations for the surface area of a sphere and hemisphere do not apply. Rather the egg is best described as a spheroid with a flat bottom (Fig. 2). The flat base consists of the transparent vitelline membrane which appears to be non-chorionated. Consequently, two eggs can be stacked back-to-back to build an effective prolate spheroid for which there are standard equations for calculating surface area. Web-based rapid calculators are available for these calculations. The pinched chorionated surface at the equator of the model automatically expands to the red outline for operational calculations.

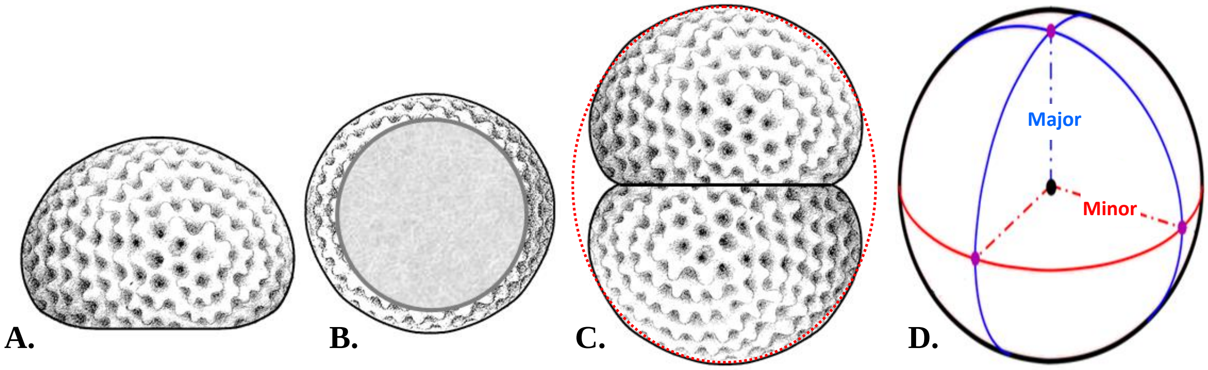

Figure. 2. Transition from single egg to two stacked eggs to form a prolate spheroid. A. Lateral view. B. Ventral view of non-chorionated base. C. Stacked eggs. D. Diagram of prolate spheroid showing major semi-axis (blue) and minor semi-axis (red).

SUMMARY OF ORDER OF METHOD CALCULATIONS

A. Aeropyle surface area per egg (direct measurements).

1. Determine the surface area (μ^2) of each individual aeropyle within a cell using πr2.
2. Determine the total aeropyle surface area (μ^2) per cell using addition.
3. Determine the number of cells (n) per egg using addition.
4. Determine the aeropyle surface area per egg (μ^2) using multiplication. Convert to mm^2.

B. Surface area of two-egg prolate (model).

1. Using two stacked eggs as an operational prolate spheroid, determine the larger semi-axis (polar radius) and the smaller semi-axis (equitorial radius) in mm.
2. Open a web-based calculator for determining the surface area of the prolate spheroid. Recommend: https://www.easycalculation.com/shapes/surface-area-of-prolate-spheroid.php
3. Record the surface area in mm^2.

C. Percentage of egg surface that is water-air interface (aeropyles).

1. Double aeropyle surface area of a single egg to balance the two-egg prolate spheroid model.
2. Divide aeropyle surface area by the total prolate spheroid surface area. Record percent (%).

D. Consult Hinton's Table. Percentage of Surface Area That Must Be Water-Air Interface

1. In vertical left-hand column of the table, determine "b/a" using the quotient of minor semi-axis ÷ major semi-axis.
2. In horizontal column at the top of the table, use the diameter (mm) of the prolate spheroid.
3. Where these columns intersect, record the percentage in parentheses.

RESULTS

SUMMARY OF CALCULATED VALUES in brackets.

A. Aeropyle surface area per egg. [0.148 mm^2]
(592.7 μ^2 x 250 = 148,175 μ^2)
B. Surface area of two-egg (model). [2.105 mm^2]
(Web-based calculator) Larger semi-axis = 0.480 mm. Smaller semi-axis = 0.375 mm.
C. Percentage of egg that is water-air interface. [14.1%]
(0.296 mm^2 ÷ 2.105 mm^2 = 0.141) Single egg numbers from model generate the same %.
B. Hinton's Table. Surface Area that Must be Water-Air Interface [14.2%]
b/a = 0.375 mm ÷ 0.480 **=** 0.781 (0.80 is closest value in left-hand vertical column.)
Egg diameter = 0.75 mm. (0.8 is closest value in horizontal column.)

The surface area of the *epixanthe* egg which is water-air interface (aeropyles) (14.1%) surprisingly aligns with the minimum percentage required for plastronic respiration (14.2%) in Hinton's table.

AIR SPACES. The *epixanthe* egg maintains a large collection of air spaces within its chorion. Shown below on the left (Fig. 3A) is a cross-section of a chorionic cell wall and ridge that was fractured during SEM preparation providing a unique view of these air spaces. This arrangement discloses two distinct chambers aligned one on top of the other. The top chamber (orange) contains a fine lattice of small spaces surrounding a central space; the bottom chamber (yellow) is nearly 3x larger and contains numerous air spaces of varying sizes supported by struts contacting the lower chorion (Fig. 3B). All air spaces within both networks interconnect, and, because they encounter penetrating aeropyles on the chorionic surface, they presumptively participate in plastronic respiration. The unique two-chamber arrangement suggests that each chamber formed at a different stage during egg formation (choriogenesis) in the female ovariole.

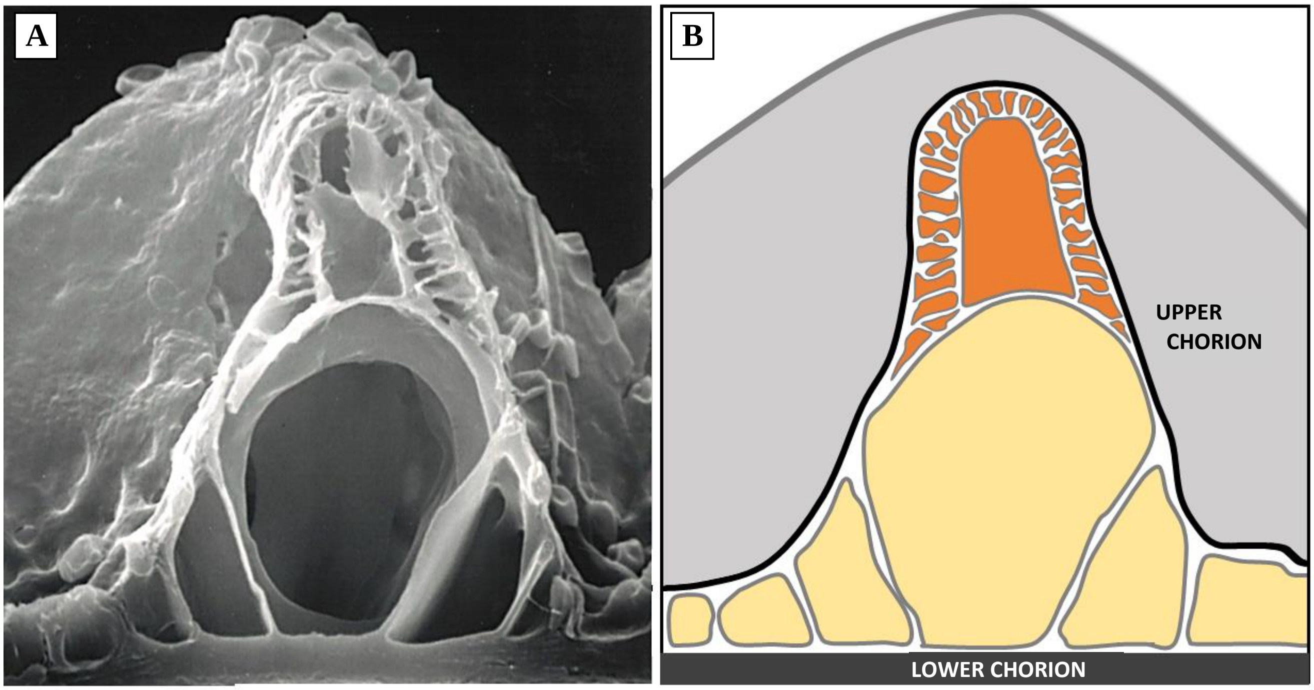

Figure. 3. Air spaces within chorion of *Tharsalea epixanthe* egg. (A.) SEM cross-section of a chorionic cell wall and ridge. 320x. (B.) Diagram highlighting two distinct chambers of air spaces in the upper chorion by separate colors.

DISCUSSION

The complex structure of Nearctic lycaenine eggs has been documented in several studies using scanning electron microscopy (Ferris, 1977; Miller & Brown, 1979; Downey & Allyn, 1981; Wright, 1983; Wright, 2008). In most cases, morphology of the intricate surface suggests the presence of a plastron within the chorion. A plastron by definition consists of a gas film of constant volume and an extensive water-air interface (Hinton, 1969). For an egg plastron to function efficiently when submerged in water, the total water-air interface (= aeropyles) must satisfy the oxygen demands of tissue (embryo, first instar) within the egg. Calculating the required water-air interface is complex process. In most insects with plastrons, the water-air interface requirement is 10^5 to 10^6 u^2 per mg of tissue. Hinton (1969) converted this requisite to a percentage of total egg surface area. This is particularly useful for eggs with flat bottoms, an area which does not participate in respiration. The prolate spheroid model eliminates flat bottoms once eggs are placed back-to-back. The introduction of scanning electron microscopy has made it possible to obtain accurate measurements of aeropyles. In the current study, the surface area of the *epixanthe* egg that is water-air interface (14.1%) equals the minimum percentage required for plastronic respiration (14.2%) in Hinton's Table 1. By this method, the SEM data confirms the *epixanthe* egg plastron will function.

The *epixanthe* egg faces a dual challenge while safeguarding the first instar. It must be structured to evade drowning in water, plus prevent desiccation in dry environments. This is especially important during first instar diapause within the egg. In New Jersey, this period extends from July to next April (9 months). Reduced metabolic activity might reduce oxygen needs during diapause, but supplementary air spaces are critical for the prevention of water loss from insect tissue. Interconnecting air spaces help trap humid air reducing the concentration gradient of water vapor which reduces water loss.

Layers of the lepidopteran egg are deposited in a well-ordered sequence during oogenesis within the female ovariole (Fehrenbach, 2003; Telfer, 2009; Carter *et al.* 2013). Their acquisition progresses under the influence of follicular epithelium. The complex system of air spaces of the chorion (trabecular layer) is generated by microprojections of follicular cells which act as spacers between developing cavities. The trabecular layer evolved multiple times within the higher Ditrysia (Hinton (1981). As evident from the wide diversity of egg sculpture within Ditrysia, oogenesis is a prime target of natural selection.

Evolution of the elaborate egg chorion of the *Tharsalea* needs systematic inquiry. In some cases, as in subgenus *Epidemia*, the structure of the egg surface offers reliable criteria to distinguish eggs at the level of species (Wright, 2008). In a broad sense, one may postulate that ecological constraints and life history strategies of the *Tharsalea* impacted their egg size and sculpture during phylogeny. The switch to first instar diapause in the egg demanded an increased volume of air spaces. Consequentially, in SEMs of their eggs, we find more chorionic cells and expanded ridges compared to eggs of *Lycaena* which diapause as larvae outside the egg. Chorionic ridges serve as wall-like sides of each individual cell. Where they intersect, they often generate pronounced peaks extending well above the cup-shaped cells. (See red-outlined peak of *epixanthe* egg in Fig. 4.) Beneath this peak lays the upper chamber of air spaces in the *epixanthe* chorion, which is the last to be laid down during choriogenesis. This feature may be a useful marker for phylogenetic comparison.

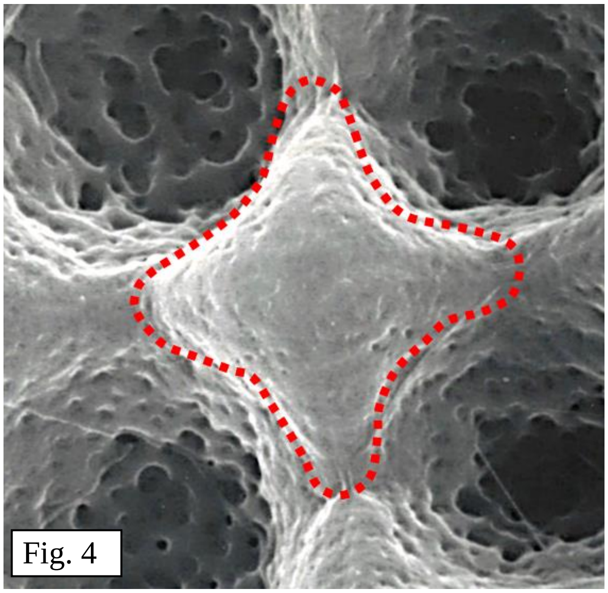
Fig. 4

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SUPPLEMENTAL DATA

Hinton (1969) recognized the egg shell (outer chorion) has a greater surface area than the metabolically active part of the egg (ovum, first instar) by a factor of the square of linear dimensions. The percentage of the egg shell that must be water-air interface is therefore smaller by this factor. To correct this, he supplied the formula $(r/R)^2$ to be applied to the percentages in his table.

r = radius of the egg without the shell
R = radius of the egg with the shell

The *epixanthe* correction factor is 70%. In the final analysis, the minimum percentage of surface area that must be water-air interface in the *epixanthe* egg is lowered to 10%. This threshold strengthens the conclusion the *epixanthe* egg plastron will comfortably function as predicted with a little extra to spare.

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