Curious Oviposition Behavior in *Phyllium westwoodii* (Phasmatodea: Phylliidae): Preliminary Observations

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**Subject Editor:** Oliver Martin

*J. Insect Sci.* (2015) 15(1): 135; DOI: 10.1093/jisesa/iev111

**ABSTRACT.** We report that in a leaf insect, *Phyllium westwoodii* Wood-Mason (Phasmatodea: Phylliidae), two differing apertures can be used for oviposition, the color of eggs being affected by which aperture is used. Eggs which are forcibly propelled from the internal space within the valvulae of the abdomen are brown, whereas white eggs emerge slowly from the opening between the eighth sternite and the valvulae, and are deposited close to the ventral surface of the female. This unusual oviposition system does not appear to have been previously reported in phasmats or in other insects.

**Key Words:** female efferent system, internal female genitalia, parthenogenesis, Phylliidae, thelytoky

In leaf insects, sexual reproduction produces offspring of both sexes from fertilized eggs. In addition, females are able to reproduce by thelytoky (a type of parthenogenesis) in which only daughters are produced from unfertilized (parthenogenetic) eggs (Brock 1999, Gullan and Cranston 2005). However, the differences between these two methods of reproduction in relation to the female efferent system and oviposition behavior are unknown (Matsuda 1976).

Recently, while rearing *Phyllium westwoodii* Wood-Mason (Phasmatodea: Phylliidae) (Fig. 1), a mid-sized leaf insect widely distributed in Southeast Asia (Hennemann et al. 2009), we noticed that the aperture used for oviposition, the distance that the eggs were propelled and the color of the eggs appeared to differ between fertilized and unfertilized eggs. As far as we are aware, this situation has not been reported previously in phasmats or in other insects. In this article, further details of this interesting phenomenon are described.

**Materials and Methods**

We obtained second-instar larvae of *P. westwoodii* from a dealer and commenced rearing of the insects on host plants (about 50 cm in height) in pots (30 by 16 by 14 cm) placed on a small oval table (36 cm in height) in a room maintained at 25 ± 3°C with a photoperiod of 16:8 (L:D) h. The host plants were mainly *Psidium guajava* with smaller numbers of *Quercus acutisima*, *Myrica rubra*, and *Gaultheria shallon*. After about 1 yr, one male and three females reached the adult stage, and these females began to lay parthenogenetic eggs without mating. We allowed the male to copulate with only one female (Fig. 1), with the other two females being used as a control. Oviposition was monitored and recorded daily using a digital camera (Lumix DMC-FX77; Panasonic Corp., Osaka, Japan) until they died. Egg-ejection distances (from a height of ~80 cm) during oviposition were measured from a position vertically beneath the female settled on its host plant to the point where the eggs landed.

For the examination of internal female genitalia, we used three abdomens, one of which was placed in 5% KOH solution at about 80°C for 15 min, then neutralized in 2% acetic acid. After this treatment, it was washed with distilled water and placed in 80% ethanol for dissection and observation. Chlorazol Black E was used to stain the KOH-treated integument. The two other abdomens were directly dissected in Ringers solution and the female genitalia were removed. The genitalia from both methods were examined under a stereoscopic microscope (model SZ60; Olympus Corp., Tokyo, Japan) with magnification up to ×63. Terminology of the female genitalia follows Matsuda (1976).

**Results**

Unmated female *P. westwoodii* clasped an oviposited egg using the two pairs of valvulae (anterior and posterior) and held it for a short time (Fig. 2A) before swiftly ejecting it away from the body by means of a 360-degree roll of the abdomen. Eggs were propelled to distances of up to 2 m (152.3 ± 78.7 cm, mean ± SD, n = 10 [one female]). The females continued to propel eggs away from the valvulae almost daily. The parthenogenetic eggs were blackish brown and cuboidal with rounded edges (4.0 by 2.2 by 2.5 mm, length by width by height on average, n = 10).

Among the reared females of *P. westwoodii*, 14 d after commencing the laying of brown eggs, we allowed one of the females to copulate with the male. Two days after this, the female produced white eggs from the intersegmental region between the eighth sternite (subgenital plate) and the anterior valvulae (Fig. 2B). These white eggs were similar in size and texture to the brown eggs. However, the white eggs were not flung away using the valvulae but instead emerged slowly from the opening between the eighth sternite and the anterior valvulae. After a short time, they were laid close to and almost underneath the female (3.5 ± 1.6 cm, mean ± SD, n = 10). About 10 min after oviposition, the white eggs darkened to a pale brown with an orange tint and then remained unchanged in color after that point. They were clearly different from the dark-brown eggs laid through the valvulae (Fig. 3). This female laid 18 white eggs over 21 d and simultaneously continued to lay 45 brown eggs from within the valvulae during this period (Fig. 4).

During the 15 d after the laying of white eggs commenced, the male repeatedly copulated with the female, almost daily. The male was then removed and the female ceased laying white eggs 4 d after the separation (Fig. 4). The intersegmental region between the eighth sternite and the anterior valvulae remained closed. However, this female continued to lay brown eggs until a few days before she died. No other unmated females have been observed to lay white eggs from the interspace between the eighth sternite and the anterior valvulae.

The anatomy of the female genitalia was further examined (Fig. 5). The oviput opened just above the middle of the eighth sternite, behind which the white eggs were laid. The large bursa copulatrix, which functions as a reservoir for the spermaphore, was located above the...
oviduct, and opened above the aperture of the oviduct. A pair of the spermathecae was attached above the bursa near its opening. A pair of slender accessory glands also arose from beneath the bursa near its opening. However, no duct was present in the internal space within the valvulae where brown eggs emerged, although a wide membranous invagination existed there.

Discussion

Because this distinctive oviposition behavior was observed 2 d after copulation and ceased 4 d after the removal of the male (Fig. 4), we infer that the differences in egg coloration, in the position of the opening used for oviposition, and in the egg-ejection distances reflect the difference between unfertilized and fertilized eggs: brown eggs from parthenogenesis and white eggs from gamogenesis (Figs. 2 and 3). Before mating, this isolated female laid only brown eggs from the internal space within the valvulae. As she had not mated prior to this, we believe the brown eggs were unfertilized, although we did not test them. In contrast to the isolated female, it appears that a mated female is able to lay eggs from two locations; the internal space within the valvulae and from the intersegmental region just posterior to the eighth sternite. Some of white and brown eggs have recently hatched, but we are unable to ascertain whether the eggs produced from the different locations were fertilized or not because most of them are still eggs.

Our anatomical investigation did not reveal any structural differences in internal female genitalia between this leaf insect and other known Phasmatodea (Matsuda 1976); the species possessed a single oviduct (Fig. 5). This implies that both brown and white eggs are laid from the same oviduct.

Presumably, the brown egg to be emitted from the ostium makes a wide invagination within the valvulae as they are opening. It is then clasped by the valvulae and slung away (Fig. 2A). The wide invagination has been termed the “fertilization pocket” (Matsuda 1976), but it does not seem to have the appropriate morphology for fertilization. In contrast, the white egg produced from the ostium is directly released from the interspace between the eighth sternite and the anterior valvulae when the latter are closing (Fig. 2B). When a female adult successfully

![Fig. 1. The female P. westwoodii used in this study.](image)

![Fig. 2. Posterior portion of the female abdomen (lateral view). (A) Brown egg being laid from the valvulae before mating. (B) White egg appearing in the opening between the eighth sternite and the anterior valvulae, after mating (the same individual shown in A). The arrows indicate the intersegmental region between the eighth sternite and the anterior valvulae.](image)
mates with a male, a spermatophore with a sperm sac is attached to the female genitalia (Großer 2011). There can be little doubt that this action and function become an important trigger for the selection of egg-laying procedure.

Although we are unable to offer a reason for the difference in egg color, we speculate unfertilized eggs are slung away to scatter the next generation near potential food sources, thereby preventing food supply exhaustion. The reason why fertilized eggs are deposited close to the female may be that this increases the mating probability of the next generation.

As another hypothesis, there is a possibility that the two egg types could be due to pressure inflicted by the copulating male somehow forcing the eggs out of the female’s usual pass, resulting in the peculiar deposition. If so, however, the female would be expected to deposit white eggs very soon after copulation due to the increased pressure. As color changes were recorded both 2 d postcopulation and 4 d postseparation, we speculate that pressure is unlikely to be a factor due to the length of time passed. Nevertheless, because of the lack of additional data at this time, we are unable to rule out this possibility.

It is unknown whether these behavioral and physiological features are universal in *P. westwoodii* and in other phylliid species. In this experiment, however, only one female was studied, meaning that reexamination of the features is required to clarify the generality. Regrettably, we have no living adults at present, so we are currently unable to continue observation. To date, 6 white eggs and 17 brown eggs have hatched, but we cannot determine the sex from the shape of the abdomen as these nymphs have not yet reached the third instar or more. We hypothesize that only females will emerge from brown eggs, whereas both males and females will originate from white eggs. We now propose to test this theory by surveying the sex ratio of the next generation, via examination of the chromosome numbers of individuals emerging from both types of eggs, and by further physiological and morphological investigations into the female genitalia.

![Fig. 3. The dark-brown egg (right) is an unfertilized (parthenogenetic) egg laid before mating. The white (center) and pale-brown (left) eggs are probably fertilized eggs laid after mating (the center egg was photographed shortly after oviposition).](image)

![Fig. 4. The numbers of brown and white eggs laid by the female.](image)

![Fig. 5. Genitalia and ovipositor of the female (lateral view). (a) anterior valvula; (b) posterior valvula; (c) eighth sternite (subgenital plate); (d) opening of oviduct; (e) oviduct; (f) bursa copulatrix; (g) spermatheca; (h) accessory gland; (i) wide invagination (fertilization pocket sensu Kalusche [1972] shown in Matsuda [1976]); (j), gut (proctodaeum).](image)
Acknowledgments

We would like to express our great thanks to Paul D. Brock (The Natural History Museum, London), Oskar V. Conle (Zoologische Staatssammlung München, Munich), Kenji Nishida (Universidad de Costa Rica, San José), Toyohei Saigusa (Kyushu University, Fukuoka), Kei-ichi Ishikawa (Leaf Stick, Tokyo), Neil Moffat (Tokyo), and Yuki Arai (Tokyo) for their kind support, valuable advice, and identification of phylliid species.

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Received 22 May 2015; accepted 28 August 2015.