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Observations on the mating behaviour and related copulatory anatomy of *Alaena margaritacea* Eltringham, 1929 (Papilionoidea: Lycaenidae: Poritiinae)

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Abstract: This article records observations made on the mating behaviours and related copulatory anatomy of *Alaena margaritacea* Eltringham, 1929. Several mating sessions were observed, lasting for at least 1 h 38 min. The female seemed to play a predominant role in copulation behaviours. The male abdomen tilted at an unusual angle during copulation. Anatomically, the ostium bursa was found to be covered by a sclerotized cup leading into an orifice. This cup and orifice occurred at a distance from the abdominal tip, corresponding with the unusual tilting of the male abdomen during copulation. Inside the cup, a "waxy" substance was found to cover the orifice. A SEM micrograph of this showed that the substance was folded to increase its volume. This article considers the possibility that this is a sphragis or related structure. However, the current literature emphatically concludes that sphragides do not exist in the family Lycaenidae. The apparently peculiar mating behaviour and anatomy in this species requires further research.

Key words: Poritiinae, endangered species, mating behaviours, genital anatomy, ostium bursa, sphragis.

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INTRODUCTION

Alaena margaritacea Eltringham, 1929 is a critically endangered endemic South African butterfly (Mecenero *et al*., 2020) that is only known from two localities in Limpopo Province (Mecenero *et al*., 2013). The type locality (TL) is < 2 km^2. A second colony, approximately 12 km SE of the TL, was discovered in 2013 by Sylvie Kremer-Köhne (Coetzer, 2015). The health and size of the second colony is poorly known. The TL is situated within a critically endangered vegetation type known as Woodbush Granite Grassland Gm 25 (Mucina & Rutherford, 2006). Less than one percent of this vegetation type remains (Kremer-Köhne, pers. comm.). The present note describes the apparent uniqueness of the mating behaviours and related copulatory anatomy of this threatened species.

MATERIALS AND METHODS

Observations were made by studying the species in situ at the TL, recording some of these on video. A white ribbon with pink pins was placed approximately 40 cm away from the copulation site, which facilitated relocating the pair for recording and determining the duration of the session.

In addition, a captured female specimen was examined under a Scanning Electron Microscope (SEM) at the African Amphibian Conservation Research Group in the School of Biological Sciences of the North-West University, South Africa. The specimens were air dried in a drying oven at 45 ° C overnight. The material was then mounted on aluminium SEM stubs with carbon tape and sputter-coated with gold palladium (120 seconds), using an SPI-Module Sputter Coater (Spi Supplies: Westchester, PA, USA). The SEM image was taken using a Phenom Pro Desktop SEM (Phenom-World B.V., Eindhoven, Netherlands).

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OBSERVATIONS

During seven visits to the TL in December and January 2020–2021, approximately five mating events were observed. Mating occurred strictly in grass tussocks that surround the loose rocks and extensive rock slabs where the larvae feed on crustose Lichinaceae (Coetzer, 2015; Van der Walt *et al*., 2020). Males persistently perched on grass stems at distances of between 5–15 cm down from the tips. Here they rhythmically opened and closed their wings, displaying the bright black and orange patterns on their upper sides.

On each visit, individuals were present at distinct spots in the small breeding area which, currently, spans approximately 650 m^2 (Fig. 1). On arrival at the site, for instance, a first male or two were consistently found in the north-eastern extremity of the area, and females at the south-western extremity—the latter approximately 5 m away from the adjacent montane forest known as Forest Glens.

During one of the observed mating events, the female led the pair up the grass stem for a distance of approximately 10 cm upon disturbance, where they were partly screened

Figure 1 – Google Earth polygon (shaded area) of TL breeding area of *Alaena margaritacea*; an area of about 650 m^2.

Figure 2 – Mating pair, female on top and male below: A – female wafting her wings; B – pair moved to a position where they are screened by an unidentified fern.

by an unidentified maiden-hair fern (Figs 2 A & B). The male followed by working his legs in reverse. The mating session of this pair was measured as lasting for at least 1 hr 38 min. The angle at which the male abdomen connected with that of the female in this pair was found to be tilted, with the point of connection distanced from the female abdomen's posterior end (Figs 3 A & B).

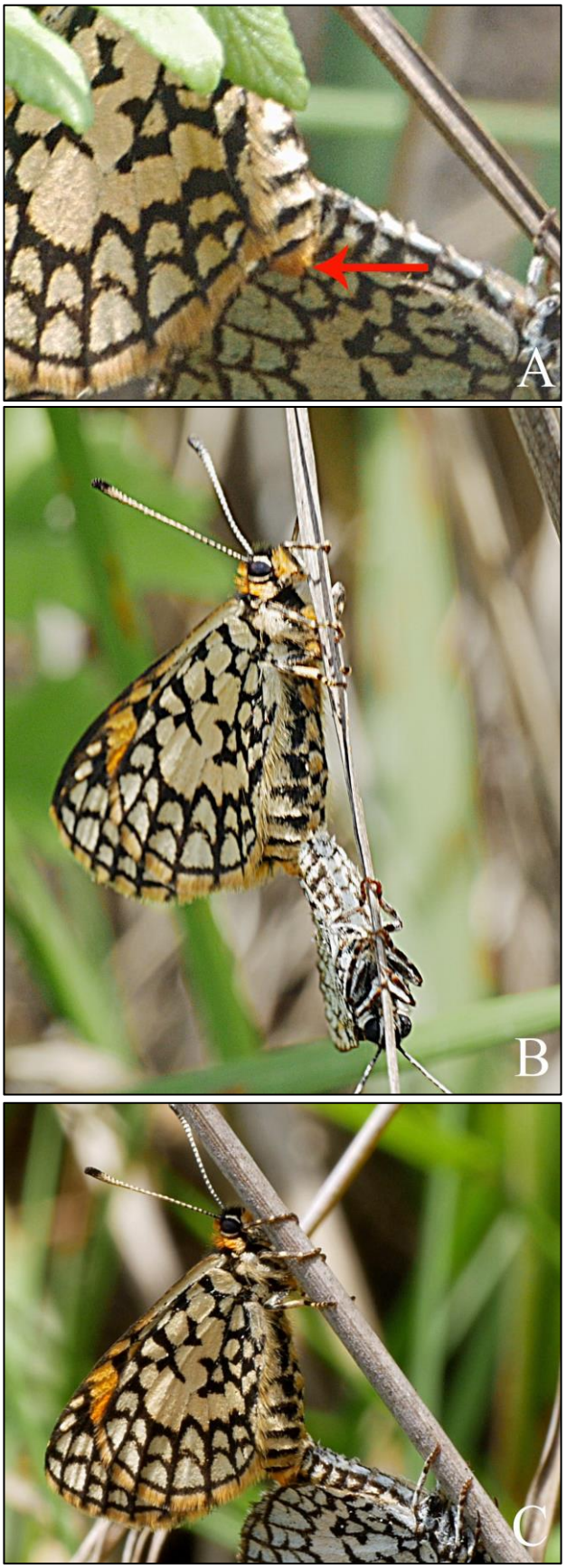

Figure 3 – Mating pair, female on top and male below: A – red arrow indicates tip of female abdomen; B – images showing the titled angle of copulation: C – close-up of Fig. 3B.

Closer examination of a different, captured female specimen showed that the ostium bursa, that is, the vaginal orifice or opening, was partially covered by a shiny, sclerotized, button-like structure (Fig. 4). This structure is situated at a distance away from the posterior abdominal tip, which corresponds with the field observation of the tilted angle of copulation, as described above. This button-like structure was also observed in other living females at the TL (Fig. 5).

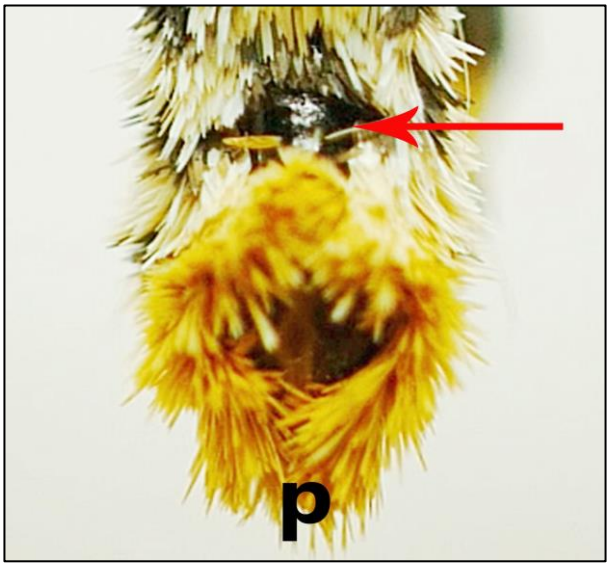

Figure 4 – Abdomen of a female specimen. The red arrow points to the shiny black button-like structure situated at a relative distance away from the posterior tip (marked "p"). This button or sclerotized cup is part of the complex ostium bursa, which partially covers the vaginal orifice.

Figure 5 – The red arrow points to the shiny black button ventrally at a distance away from the posterior tip of a living female abdomen.

A SEM micrograph of this structure brought additional aspects into focus (Fig. 6). It is a cup that partially covers the vaginal orifice and opens into it. Inside this cup, external to or on top of the orifice, a folded substance is visible. Under a light microscope, where it was viewed prior to SEM, this substance appeared "waxy". While preparing the specimen for SEM, Willie Landman made the same observation. The SEM micrograph shows that the substance is folded to create gaps that increase its volume.

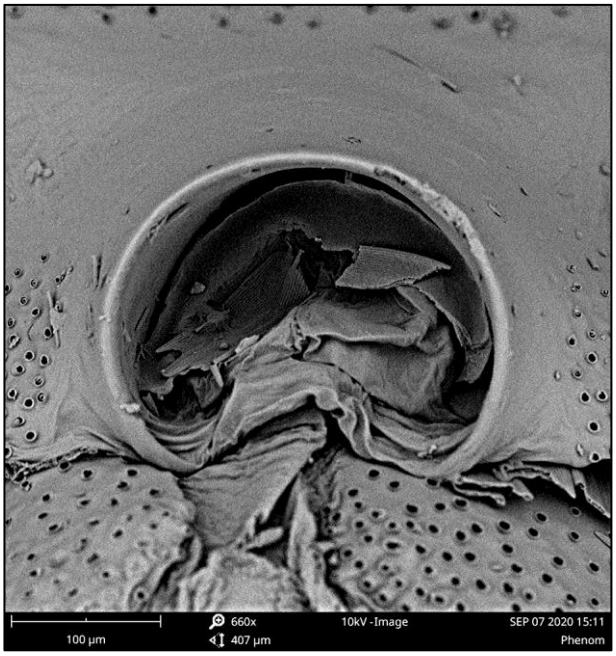

Figure 6 – SEM micrograph of ostium bursa of *A. margaritacea* female. It is situated ventrally towards the posterior end of the abdomen. Note the hardened cup and the substance covering the orifice (image: W. Landman).

DISCUSSION

The most intriguing observation among those described above is the substance covering the ostium bursa. Putatively, it could be a tiny sphragis. In their comprehensive review of sphragides in Lepidoptera, Carvelho *et al*. (2017) reported that the occurrence of sphragides is restricted to one percent of the Order; they pertinently assert that no lycaenids have ever been found to carry these structures.

They give a range of interrelated definitions and descriptive characteristics, differentiating a sphragis, from other structures. These are to be considered if one wants to demonstrate its occurrence in *A. margaritacea*. The first and most basic is that a sphragis is a structure that plugs the vaginal orifice, deposited by a mating male to prevent secondary mating (Carvalho *et al*., 2017). They further state that a sphragis must be *external.* Internal plugs, poorly researched, fall outside the ambit of the definition. They note that the substance of sphragides indeed appears "waxy," while in some cases it is folded to increase its volume so as to debar ardent attempts at secondary mating even more effectively (Carvalho *et al*., 2017).

A sphragis must furthermore cover large areas of the female abdomen. Another part of their article however states that small sphragides do occur. If the substance covering the ostium bursa of the female insect under examination here is a sphragis, it should be considered that it is smaller than usual. If it were a sphragis, it would of course belong to a much smaller insect (wingspan = 29 mm), while sphragides are normally associated with much larger insects of the families Papilionidae and Nymphalidae (Carvalho *et al*. 2017).

Further specimens of *A. margaritacea* (or a common congener such as *A. amazoula*) should be examined to confirm that the occurrence of the substance is not facultative; that it is in fact a male plug. Some features point in the direction of a sphragis, quasi-sphragis, or a unique new phenomenon. The most visible among these, probably also the most important, is the external positioning of the substance on top of the orifice. Its "waxy" appearance and folded quality further intimate the possibility of a sphragis.

Carvalho *et al*. (2017) have found further that sphragides correspond with complex genital anatomies. *A. margaritacea*'s female genital anatomy appears to fit this requirement. The complexity is at least indicated by the existence of the cup itself, the apparent plug inside it, and the distance of the ostium bursa from the abdominal tip.

The long duration of copulation, though not completely unusual among butterflies (see Hughes *et al*., 2000), may be related to the deposition of a plug. Again, further research is required to affirm or deny the hypothesis that the substance is an external plug, if not a unique sphragis.

Consider, however, that observations on *A. margaritacea* have been severely restricted by low numbers of individuals over the past three seasons. During the 2020–2021 season, an average of three to four individuals were seen each day at the TL, with a maximum of seven on one of the days. The ratio of individuals was furthermore heavily skewed in favour of males, as is often the case among Lepidoptera; unfortunately, females were rarely seen.

The observations described and those reported by Terblanche (2020) seem to indicate overall that females play a salient, peculiar role in the reproduction of this species. The fact that the female "leads" an escape from disturbance during mating, as observed, seems to stress this recognition.

Finally, these observations emphasise the sheer uniqueness of *A. margaritacea* among Lycaenidae and lepidopterans at large. The need to conserve the insect should first and foremost enjoy preference with a view to the intrinsic value of each species on the earth but, in this case, that value is heightened by the fact that this species may shed unique light on the behaviours and evolution of lepidopterans. As such, the species can serve as a distinctive foil against which aspects of other species can be examined and contrasted. For instance, the insect's restriction of mating behaviours to grass, while behaviours in the other life stages are strictly limited to rocks carrying Lichinaceae, offers a particularly well-defined opportunity for conservation studies, given that both of these extensively different substrata are essential to *A. margaritacea*'s survival.

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