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Peculiar behaviour of a female *Alaena margaritacea* (Eltringham, 1929) (Lepidoptera: Lycaenidae: Poritiinae)

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Etienne Terblanche
3 Kerk Street, Haenertsburg, South Africa. Email: jetpapermoon@gmail.com

Abstract: A female *Alaena margaritacea* was observed using her legs to groom grass blades on a slope, the type locality, near Haenertsburg, Limpopo Province, South Africa. The behaviour was video-recorded. The article describes the behaviour's peculiarities and stages such as reiterative grooming at blade tips. Using extant literature, it posits two hypotheses for the behaviour's function: that it involves territorial, sexual scent-marking or that it aims to repel competing feeders such as ants by the putative use of semiochemicals.

Key words: *Alaena margaritacea*, ethology, grass-grooming, scent-marking, sexual attraction, feeding competition, pheromones, semiochemicals.

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INTRODUCTION

Alaena margaritacea is critically endangered (Coetzer, 2015) and its type locality, approximately three kilometres south-west of Haenertsburg in the Limpopo Province of South Africa, is situated within critically endangered vegetation known as Woodbush Granite Grassland Gm 25 (see Mucina & Rutherford, 2006). The site is located on a grassy slope at the fringe of Forest Glens (as it is named on the 1:50 000 map). About halfway up the slope one finds a cluster of lichen-covered rocks where *A. margaritacea* larvae feed on the lichens (Williams, 2006; Coetzer, 2015). Slope and forest meet sharply here in the manner of an ecotone that characterises much of the environment around Haenertsburg.

A second site was discovered in 2013 by Sylvie Kremer-Köhne when surveying veld flowers (pers. comm.) on the farm Boshoff, twelve kilometres south of Haenertsburg, within least-concern vegetation categorised as Wolkberg Dolomite Grassland Gm 26 (Mucina & Rutherford, 2006; Coetzer, 2015). This major discovery did not alter the species' conservation status (Coetzer, 2015), but multiplied the research potential around its ethology, ecology, distribution and conservation, while boosting hope that it will continue to exist.

This article describes peculiar behaviour displayed by a female *A. margaritacea*, observed and video-recorded at the type locality.

OBSERVATIONS

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During the end of December 2019 and beginning of January 2020, the author paid four visits to the type locality where the butterfly had been emerging year after year around Christmas day. The observed behaviour took place on 1 January 2020 at 2:45 p.m. in a pocket of tussock-grass blades. It lasted four minutes. The insect walked methodically along the blade surfaces horizontally, diagonally and perpendicularly while wriggling its forelegs and rubbing or stroking the blade surfaces with its mid- and hind legs. Its wings were rhythmically and slowly fanned at regular intervals to their open position and back (Fig. 1). Heightened leg activity was seen at the blade tips.

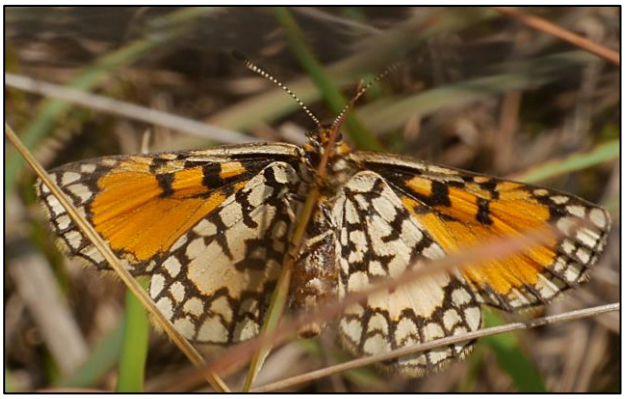

Figure 1 – Fanning: as the female *A. margaritacea* groomed the grass blades, she consistently fanned her wings relatively slowly at intervals to their open position and back.

An umbrella term suggests itself for naming the behaviour, as found in Elmquist *et al.* (2018), who refer to this type of legwork on substrata as "grooming." At first glance, any observer who encounters it will be struck by the aptness of the term. In addition to grooming grass, the observed *A. margaritacea* on occasion poked her

abdomen quickly and in quick succession into the air around the blades lower down in the grass pocket, mostly while hanging upside down. Concomitant with poking, walking along blades continued.

The overall behaviour commenced lower down in the grass pocket. The female worked her way upwards but dropped downwards again on one or two occasions. As indicated, she engaged in increased grooming at the thin, sharp blade tips, notably at individual blades slanting upwards. Unlike other blade parts, the tips were groomed reiteratively.

This activity can be usefully divided into five stages. First, the insect slowly climbed to the blade tip from lower down. Legwork increased once she had reached the end near the tip, including the quick waving of forelegs, occasionally stretching these out nearly to their full open extent as part of the waving process. Hind legs tapped or rubbed the blade surface.

Second, she dropped approximately five centimetres down the blade and, third, climbed back to the tip. Legwork continued as described above, but less intensely. Fourth, she reverse-climbed down the blade for about one centimetre. Fifth, she climbed back to the tip yet again, where her forelegs touched the hind legs. She also hung from the blade with the ventral aspect of her thorax close to its surface and the feet of the mid-legs interlocked away from the opposite surface.

Antennae remained mostly in their lifted position. Weak antennation occurred once or twice only: the antennae were not pushed down to a large degree or regularly, in contrast with other lycaenids nectaring or grooming. As soon as the grooming episode was complete, the female came to rest at the blade tip (Fig. 2). Resting lasted longer than grooming.

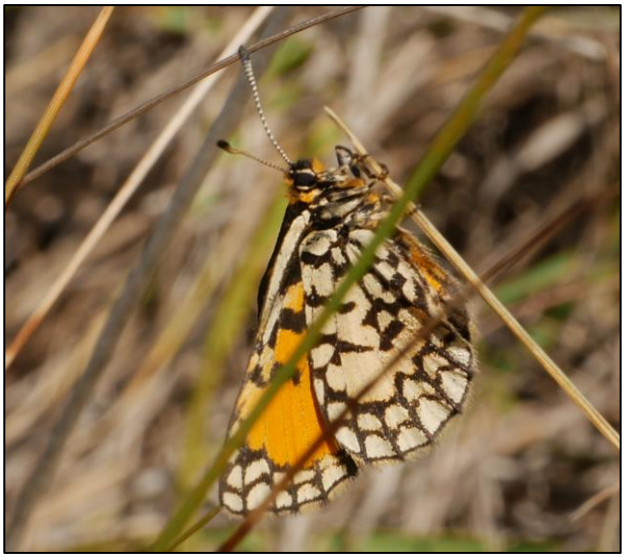

Figure 2 – Resting/perching once the grooming session had been performed.

Some movements described above are too quick to capture on video, hence photographs could not be pulled out of the footage to illustrate these here. Video footage of these behaviours can therefore be watched on Vimeo, URL: https://vimeo.com/416060510. If possible, the material should be watched at a resolution quality of 1080 or above. Table 1 below has been drawn up to assist in following the aspects of the behaviour as described above.

Table 1 – Time frames on the video where the specific behaviours of the female *A. margaritacea* can be viewed.

Behaviour	**Time frame on video**
Grooming (legwork on grass surfaces)	0 min 00 sec – 4 min 25 sec (throughout)
Fanning (rhythmic wafting of wings)	0 min 30 sec – 0 min 50 sec
Poking (abdomen quickly into the air)	0 min 51 sec – 1 min 25 sec 2 min 03 sec – 2 min 13 sec
Reiterative grooming at blade tips:	
Stage 1 (climbing blade from below)	3 min 00 sec – 3 min 43 sec
Stage 2 (dropping down the blade) Stage 3 (climbing back to the tip)	3 min 45 sec – 4 min 00 sec
Stage 4 (reverse-climbing down the blade) Stage 5 (climbing back to the tip again)	4 min 02 sec – 4 min 22 sec
Resting (on a different blade tip)	4 min 29 sec – 5 min 12 sec (on video, but lasted longer of course)

DISCUSSION

The methodical and insistent nature of the behaviour as it arose in the field was striking. It did not appear incidental but purposeful. Sideways movement along grass blades especially indicated that the behaviour was extraordinary. It carried the risk that the insect was more visible, potentially increasing the probability of being attacked by predators.

In this species oviposition takes place on rocks covered by lichens. The nearest rocks were about one metre from the spot where the observation was made and the insect stayed strictly within the grass pocket. It is unlikely that the behaviour was related to oviposition in any direct manner. But what would the behaviour's function then be? As will be explained briefly, extant knowledge points to the distribution of attractant-/ scent-marking-/ territorial pheromones or repellent semiochemicals.

It has long been known that insects, including the Lepidoptera, distribute pheromones to mark territory and attract mates. Among insects, females predominate in this behaviour (Landolt, 1997). In butterflies (not moths), one mainly associates territoriality with lycaenid males engaged in hilltopping. Given that the observed behaviour of this female lycaenid was unrelated to oviposition, one nonetheless posits that she chemically marked the blade pocket, perhaps to attract males. Grooming could have been related to this for reasons set out below.

Among insects (including Lepidoptera), legwork relates to known pheromonal behaviours. Some of these may be relevant to *A. margaritacea*'s grass-grooming activity. For instance, butterflies employ forelegs for smelling or "tasting" substrates (Arikawa, 2001), wasps groom surfaces to scent-mark them (Elmquist *et al*., 2018), some butterflies carry ductless pheromonal glands not only

abdominally but also in their legs (Pinzari *et al*., 2018), and the female of *Phingeris teleius* was found to perform "a kind of specific dance, walking around a flower head, drumming with forelegs and often opening its wings" to distribute anti-oviposition pheromones (Sielezniew & Stankiewicz-Fiedurek, 2013).

Some years prior to witnessing *A. margaritacea*'s grooming, the author saw puzzling behaviour by a male lycaenid, *Aphnaeus hutchinsoni* (Trimen, 1887) (Lycaenidae: Aphnaeinae), which appeared to rub a prominent dry twig on a hilltop with his mid- and hind legs while walking slowly to one end of the twig and halfway back, as can be seen on a YouTube video (URL https://youtu.be/D2auxbuY0Lc) where this behaviour and that of the female *A. margaritacea* are compared.

Against the background of these known behaviours – legwork employed to smell and/ or distribute attractant- or territorial pheromones – a first hypothesis can be posited towards explaining the behaviour's function.

First hypothesis: pheromonal attraction – sexual behaviour

One conjectures that the *A. margaritacea* female smelled or "tasted" the blade surfaces with her forelegs to achieve precise pheromonal distribution, especially at the blade tips. Perhaps she smelled for pheromones of another female to counter them. Mid- and hind legs might have been involved in scent-marking the blades to attract males on aggregate or individually, while marking out a territory. If in aggregate, the groomed blade pocket would form a lek where competition among males could be increased. In the case of the observed behaviour, the lek would have been roughly the size of a standard ruler (30 cm long) cubed, 0.027 m^3. The noticeably extensive grooming at blade tips could mean that the female marked territorial "hot spots" in the same vein as male lycaenids' territorial behaviour on hilltops. The more remote possibility is that this female *A. margaritacea* was searching for male pheromones. Since this would, however, involve a mixture of male and female pheromones, since she poked the air to distribute her own chemicals, it appears unlikely.

Poking (hence contracting) the abdomen might point to the existence of pheromonal glands within the abdomen, as is frequently the case among Lepidoptera, especially between segments eight and nine (Ma & Roelofs, 2002). The *A. margaritacea* female under discussion appeared to rub the ventrolateral side of her abdomen and/ or its tip on grass blade surfaces, though this is a tentative observation that needs observational confirmation. The possibility that such pheromonal glands do exist in her abdomen will be examined under the guidance of Jonathan Taylor, a protist and microscope specialist in the School of Biological Studies at the North-West University of Potchefstroom.

Wafting of wings, a known behaviour referred to as "fanning," serves to distribute pheromones among satyrine butterflies (Pinzari *et al.*, 2018). However, the satyrines fanned only when males and females were in close proximity to each other so as to facilitate detailed species recognition. Though the *A. margaritacea* female studied here may have used fanning as an additional way of spreading pheromones, she may equally have used it to advertise her bold upper side wing patterns for the purposes of identification by mates, aposematic-/ pseudo-aposematic protection or both. Fanning brings a second hypothesis into focus.

Second hypothesis: distribution of repellent semiochemicals – feeding behaviour

Mark Williams (pers. comm.) pointed out that there was another recorded behaviour that could shed light on the function of *A. margaritacea*'s grass-grooming. Farquharson (1921) described his observation of behaviour displayed by another species within the poritiinid complex, *Teratoneura isabellae* (Dudgeon, 1909) and his report sheds potential light on the behaviour under examination here. The relevant passages are cited immediately below.

Farquharson had been trying for years to witness a lycaenid ovipositing. His factual, lively letter indicates this anticipation when he describes how an individual *T. isabellae* flitted on to a branch where coccids (Homoptera) were residing. "I suppose my anxiety to see [lycaenid oviposition]," he writes, "prevented me from 'tumbling' to what really was doing:"

> The butterfly lit just at the tip of the branch, the Coccids being about an inch [2.5 centimetres] behind that. It proceeded to walk backwards rather slowly and deliberately, the abdomen inclined upwards at a fairly steep angle to the thorax, and the wings opening and closing fairly rapidly – though not by any means nervously or excitedly – and gently beating the twig. The ants retreated backwards, making hardly any resistance at all, though some dodged to the underside of the twig and ran forwards. The butterfly having gone back about three inches then suddenly dropped the abdomen so that it rested on the twig and ran rapidly forward, the tip of the abdomen brushing the twig as it did so. The backward manoeuvre was repeated, this time on the underside of the twig, the wings then hanging downwards, the abdomen flexed as before.

There are striking similarities here with *A. margaritacea's* behaviour at the level of somatic activities, to which the present article returns; Farquharson continued to hope for oviposition, confirming the principle in the world of natural historical observation that one can stumble on an important discovery when looking for something else:

> I was still waiting for the egg-laying marvel. I thought I was to see it to some purpose, when "she," if that really was the sex, let the abdomen rest on the twig. But [W. A.] Lamborn used to swear that females that really wanted to oviposit and knew that one wanted to know the food-plant, really did that to annoy the onlooker [!]: so I kept on hoping, till "she" suddenly stopped over the Coccids, unrolled a very slender proboscis and proceeded to absorb the [coccid] secretion so much prized by the ants. Occasionally an ant would venture along, but retreated without attacking. In a short time the butterfly flew away, circled round for a bit and came back to another twig, where the same performance was repeated. By this time I had formed the conclusion that she was deliberately chasing the ants off what they doubtless regard as their own particular prey. What exactly is the "force majeure" to which the ants yield I do not know; the flapping of the wings isn't a very formidable thing, but it seemed to act and the ants did keep their distance. [The movements described and the position of the abdomen suggest strongly

that the butterfly produces and fans towards the ants some odour disliked by them.—ed. note, E.B. Poulton]

Some resemblances between these behaviours and those observed in *A. margaritacea* are noticeable: fanning, the inclined abdomen, grooming various surfaces of the substratum, grooming reiteratively, moving backwards down the substrate, hanging upside down and the unrelatedness of the behaviour to oviposition. These parallels and the fact that *A. margaritacea* poked the air with her abdomen only lower down in the blade pocket may tally with the hypothesis that she kept competing feeders such as ants away. If this was the case, reiterative grooming of blade tips might have been aimed at this kind of deterrence, though close examination of the video material does not reveal the presence of coccids in these areas.

But do imagos of *A. margaritacea* actually feed? No feeding has been observed over four visits, certainly not on the available veld flowers. Williams (pers. comm.) said he is not aware that any species of Poritiinae has been observed nectaring from flowers. However, there are numerous reports of honeydew imbibition from Homoptera and extra-floral nectaries.

Jonathan Taylor and the author did confirm under a light microscope that the insect possesses a small and infolded, hidden, orange-and-pink proboscis. The imagos therefore feed, but what they feed on remains an open question: the behaviour studied here might point to coccid secretions in competition with ants, viz. the second hypothesis to be tested.

Interestingly, this second hypothesis suggests that Farquharson's "force majeure" – clearly a puzzle to him since *T. isabellae*'s fanning was too gentle to chase the ants away – could be none other than semiochemicals inducing an ant retreat. To his credit, Poulton, as can be seen in his editorial comment cited in square brackets above, was already aware that a repellent "odour" was involved. But only in 1959 did the existence of semiochemicals become a more exact scientific fact, 28 years after the publication of Farquharson's letter and based on 20 years' worth of chemical work, when Butenandt *et al.* (1959) published the organic chemical structures of what they termed (in German) *Sexual-Lockstoff* (sexual attractant-materials). Thus semiochemicals entered the world of insect science. Farquharson's and Poulton's intuitions were confirmed: the *force majeure*, the semiochemicals, did in fact turn out to be a considerable agent at play across the insect kingdom.

One is therefore in a position to conjecture in tandem with this second hypothesis that *A. margaritacea* might mimic semiochemicals present among ants, which are known for reacting swiftly to a number of semiochemicals in a variety of manners within and across their colonies (Hölldobler & Wilson, 1990). *Alaena margaritacea* might have distributed these in the lower parts of the pocket of blades to deter ants from reaching food materials for which they compete.

Overall, the function of the observed behaviour could therefore be to attract mates, mark territory, repel competing females or repel feeding competitors such as ants. That this female *A. margaritacea* came to rest on a blade tip providing good visibility over the surroundings once the grooming session had been performed hints that she was looking out for potential mates and/ or predators; or that she was due to feed on organisms or materials on the grass blades at a later stage.

These prospects require further research, including observation of sexual behaviours between and among females and males, establishing the feeding habits of imagos, careful examination of grass blades and areas in the immediate vicinity for ant or coccid activity, studying the abdomen and legs microscopically to identify structures, organelles, organs, and tissues (hence functions) and, if possible, chemical sampling and analysis.

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I wish to acknowledge here also the resilience of our "forefather" lepidopterists such as Charles Farquharson who carried out hard and excellent work without modern medical technology, mobile freezers, tarred roads, four by four vehicles, GPS equipment, Google Earth Pro, and the like, while patiently achieving astute results that continue to shed light on our puzzles, in this case very nearly a hundred years later. Torben Larsen's tome entitled *Butterflies of West Africa* celebrates his amazement at Farquharson's field work. Referring to his beloved *Isabellas*, Larsen (2005) notes that Farquharson figured out the early stages of *T. isabellae* (published in the 1921 writings mentioned above): "given the rarity of the species," Larsen continues, "it was amazing that this was one of the first Lipteninae ever to be bred. But then, Farquharson was something else again."

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