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PREDATION BY NYMPHS OF *PICROMERUS BIDENS*
(HETEROPTERA PENTATOMIDAE ASOPINAE) ON CATERPILLARS
OF *EUPHYDRYAS AURINIA PROVINCIALIS* (LEPIDOPTERA NYMPHALIDAE) IN ITALY

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Pinzari M., Cianferoni F., Fabiani A., Dioli P. – Predation by nymphs of *Picromerus bidens* (Heteroptera Pentatomidae Asopinae) on caterpillars of *Euphydryas aurinia provincialis* (Lepidoptera Nymphalidae) in Italy.

Euphydryas aurinia (Rottemburg 1775) is one of the most seriously threatened European butterflies, as it suffered a severe decline in most countries and it is today listed in the European Community Habitats and Species Directive (92/43/EEC). Although its ecology and biology have been increasingly studied during the 20th Century, the factors affecting its mortality have not been described in detail, and the role of natural enemies on its population dynamics is largely unknown.

In this paper, we present new data on predation events of *P. bidens* (Linnaeus, 1758) on caterpillars of *Euphydryas aurinia* spp. *provincialis* (Boisduval, 1828) in Central Italy, and discuss the stages during which predation could have a stronger impact. Our results show that II to IV instar nymphs of *P. bidens* attack the pre-diapause 3rd instar caterpillars when they live in a communal silken nest, while adults were found on butterfly larval webs, but never preying on caterpillars. The bugs visited a larval group more than once a day and at different times, and showed preference for cooler habitats (i.e., they were not observed on webs of warmer habitats). All together, our observations point out to the potential significant impact that gregarious nymphal instars can have on the population dynamics of *E. aurinia* in cooler habitats, where predator and prey coexist.

KEY WORDS: *Euphydryas aurinia provincialis*, larvae, predator, *Picromerus bidens*.

INTRODUCTION

The ecology and biology of *Euphydryas aurinia aurinia* (Rottemburg, 1775) has been intensively studied in Europe (PORTER, 1981; WARREN, 1993; 1994; 1996; WARREN *et al.*, 1994; KLEMETTI & WAHLBERG, 1997; WAHLBERG, 2001; HULA *et al.*, 2004; SAARINEN *et al.*, 2005; MAZEL, 2006; SVITRA & SIELEZNIEW, 2010; PORTER & ELLIS, 2011; CASACCI *et al.*, 2014) especially owing to its severe decline in most European countries during the 20th century (VAN SWAAY *et al.*, 2010). However, the natural history of its enemies and their role in its population dynamics are scarcely unknown (VAN NAUHUYS & HANSKI, 2004).

Caterpillars of *E. aurinia* sp. *provincialis* show six larval stages; during the first four, they are gregarious and live in a communal silken web (PINZARI *et al.*, 2010, 2016, 2017) on a variety of host plants, likely playing an important role in this complex multitrophic community. In Europe, arthropods and small mammals have been reported as parasitoids or predators of *E. aurinia* (VAN NAUHUYS & HANSKI, 2004). Among insect parasitoids, the impact of braconid wasps on *E. a. aurinia* populations has been studied in detail since the '80s (PORTER, 1981; KANKARE *et al.*, 2005; SHAW *et al.*, 2009), while a more recently study showed the relationship between tachinids and *E. a. provincialis*, as their host (PINZARI *et al.*, 2017). On the contrary, scarce information is available on predation by

heteropterans. In a review by VAN NAUHUYS & HANSKI (2004) only an old report by WILKINSON (1907) is mentioned, on unidentified heteropterans preying *E. a. aurinia* caterpillars. In addition, two recent notes described predation on pre-hibernation caterpillars of *E. a. aurinia* and *E. a. provincialis* by true bugs *Picromerus bidens* (Linnaeus, 1758) *Deraeocoris schach* (Fabricius, 1781) respectively (KONVICKA *et al.*, 2005; PINZARI, 2016b). In these notes, while *D. schach* seems to be an occasional predator of pre-hibernation caterpillars, *P. bidens* looks like a regular and efficient predator, as it was seen at adult stage attacking both butterflies and caterpillars at their fourth larval-instar stage.

Pre-hibernation caterpillars of *E. a. provincialis* feed gregariously and move from plant to plant (PINZARI *et al.*, 2016), representing an important food source for *P. bidens*, especially when they are also in a gregarious phase. In fact, gregarious behaviour is typical of their I-II instar nymphs (LARIVIÈRE & LAROCHELLE, 1989; DE CLERCQ, 2000) and, according to KONVICKA *et al.* (2005), this species could represent a substantial mortality factor in populations of butterflies.

Within this framework, in the context of an ongoing survey of the Lepidoptera fauna in central Italy (PINZARI *et al.*, 2010; PINZARI & SBORDONI, 2013; PINZARI, 2016a; PINZARI *et al.*, 2018a, 2018b, 2019a, PINZARI & PINZARI, 2019a, 2019b), in 2010 we started a study on several aspects of the biology of *E. aurinia* sp. *provincialis* in

Central Italy; we described its larval host plants preference (PINZARI *et al.*, 2016), adult population dynamics (MANUELA PINZARI, unpublished data), mating behaviour (PINZARI *et al.*, 2019), and, finally, we identified its main predators (PINZARI, 2016; PINZARI, 2019) and parasitoids (PINZARI *et al.*, 2017). Here, we report our observations on bug nymphs of *P. bidens* preying the caterpillars of *E. a. provincialis*.

MATERIALS AND METHODS

STUDY SPECIES

Euphydryas aurinia provincialis (Lepidoptera: Nymphalidae) are widespread over the Central Apennines and, in particular, on Monti Reatini (data from Osservatorio per la Biodiversità del Lazio). In the study area (see below), they occupy habitat patches in montane grassland and slopes, within a mosaic of different habitats including wooded and rocky areas, hedgerows, fields and less steep areas which are occasionally ploughed and cultivated.

They have one generation per year: they lay their eggs in large clumps under plant leaves in May-June, and develop into six larval instars. The first three instars are gregarious: they spin a substantial nest between the leaves of the food plants and live together from May to August within the communal silken web; the IV instar-larvae enter diapause in August-September and overwinter in a larval web (hibernaculum) close to the ground. By February, caterpillars are again active, they complete their life-cycle and pupate by the end of April. Adults start to emerge in May (PINZARI *et al.*, 2016).

Picromerus bidens (Hemiptera: Heteroptera: Pentatomidae, Asopinae) is distributed in continental Italy and in Sicily (FARACI *et al.*, 1995). It is associated with a wide range of habitats, mainly on shrubs and forests of open forest edges, meadows and gardens, preferring fresh, shaded and humid areas (LARIVIÈRE & LAROCHELLE, 1989). The proximity of fresh vegetation is essential to the successful development of nymphs and adults (MAYNÉ & BRENY, 1948) and for reproductive activity (MAHDIAN *et al.*, 2008). Females deposit egg batches (typically 35 to 45 eggs), as many as five and varying in size (SCHUMAKER, 1911; MAYNÉ & BRENY, 1948b; JAVAHERY, 1986; LARIVIÈRE & LAROCHELLE, 1989), and the nymphs hatch in spring (MAYNÉ & BRENY, 1948a; 1948b). There are five nymphal instars which, depending on ecological conditions, take from 25 to 60 days to complete development (STRAWINSKI, 1927; SOUTHWOOD & LESTON, 1959; JAVAHERY, 1986; DE CLERCQ, 2000; MUSOLIN & SAULICH, 2000; SAULICH & MUSOLIN, 2014). During the I instar, the nymphs live close together in aggregations that vary in size in relationship to the number of eggs for individual batches. The II instar nymphs also tend to be gregarious, living in groups of 3-56 individuals, although they start to disperse intermittently to find prey. Activity and mobility increases from the III to the V nymphal instar (JAVAHERY, 1986).

Concerning feeding behaviour, the I instar-nymphs do not feed but only take up water (DE CLERCQ, 2000) or suck liquids from a wide diversity of plants (MAYNÉ & BRENY, 1948b; SOUTHWOOD & LESTON, 1959). Animal food is required from the II or III nymphal instars (MAYNÉ & BRENY, 1948b). *Picromerus bidens* is a highly polyphagous bug that preys on larvae, pupae and adults of more than 250 species from several insect orders (LARIVIÈRE & LAROCHELLE, 1989). It is often found on plants that are attacked by leaf-eating larvae, particularly of Lepidoptera and Coleoptera (STRAWINSKY, 1927; MAYNÉ & BRENY, 1948a,

1948b). It is a little vagile and disperses mainly by walking (MAYNÉ & BRENY, 1948b; JAVAHERY, 1986; LARIVIÈRE & LAROCHELLE, 1989).

STUDY AREA AND LARVAL WEBS

Our observations were carried out in 2015-2016 in an area (2 ha) near the “crossroads Santa Maria del Monte - Fonte Brignola” (1300 m). Here, we monitored 115 larval webs of *E. a. provincialis* (62 in 2015 and 53 in 2016).

To daily (and more easily) observe the interaction between predators and prey, we also collected an egg batch of *E. a. provincialis* and established it on a host plant of *Scabiosa columbaria* L. (Dipsacaceae) in a clearing between trees and shrubs (*Fraxinus ornus* L., *Fagus sylvestris* Gaertn., *Quercus* sp., *Salix caprea* L., *Malus* sp., *Acer* sp., *Juniperus* sp., *Cytisus* sp., *Prunus* sp., *Lonicera caprifolium* L., *Clematis vitalba* L., *Rubus* sp., *Abies alba* Mill.). The area was close to our private laboratory in Colle Marcone (1121 m a.s.l.) (PINZARI *et al.*, 2010). In this “artificial” larval web, eggs hatched successfully and larvae developed naturally. We visited this larval web during day light, as (or even more) often than the natural larval webs (i.e., every 2-3 hours per day), and also at night time, to ascertain possible nocturnal activities of the predators.

Over the two years, a total of 50 days were spent monitoring all egg batches and larval webs of *E. a. provincialis* focusing on: (i) the eggs (from May to the end of June), (ii) the pre-diapause gregarious phase (I-III instar on larval webs, from May to September), (iii) the diapause phase (IV instar in larval webs, from September to mid-February), and (iv) the post-diapause gregarious phase (IV instar, from mid-February to mid-March). We counted and collected heteropterans on butterfly larval webs. Sampling was carried out every 7-15 days throughout the year, except during winter (November to February), in all localities, visiting the larval webs along the same transect path. The path for monitoring the “natural” webs was marked by numbered flags, located at each of the host plants.

For each bug observed, we recorded the date and time of the observation, the exposure of the predator to sunlight (shade or sun), the position of the predator (plant or larval web), the behaviour of the predator (predation or not), and established the prey attacked by the predator. To identify the species of predators, some individuals were collected as vouchers and preserved dried (private collection, Pinzari - Rome, Italy), while others were only photographed.

RESULTS

OBSERVATIONS ON *PICROMERUS BIDENS*

During our observations on the 115 larval webs, we did not record the presence of *P. bidens*, which was instead observed on the “artificial” web in Colle Marcone, while it was preying on caterpillars. In particular, we sampled six nymphs and three adults in 2015, and observed 13 individuals without collecting them. Only a single nymph was collected in 2016 as voucher.

Twice in June 2015 and once in July 2016 (Fig. I), we observed II to IV instar nymphs of *P. bidens* on a larval web of *E. a. provincialis*, while preying on the pre-diapause III instar caterpillars (Fig. II, 1-4). No V instar nymphs were observed. Nymphs and adults of *P. bidens* were not seen in August; on the contrary, adults were found on larval webs or in their vicinity, on three occasions. In particular, we found: (i) a female at a distance of ca. 1 m from the larval web, on 26th September

2015; (ii) a male attacking a larva of an unidentified sawfly (Hymenoptera, Symphyta) (Fig. II, 5) near a larval web, on 29th September 2015; (iii) an adult (sex not identified) on the larval web, but not attacking the caterpillars, on 4th October 2015 (Fig. II, 5-6).

As far as sunlight exposure is concerned, the larval web in Colle Marcone was located within woodland and shrubs. It was in shade until 11 AM; during the following hours it was in sunlight until 3 PM and then in moderate-shade until sunset. We found *P. bidens* on host larval webs or nearby throughout the day (Fig. III). Our data show that different stages of this bug occurred at the larval webs at different times of a day. In particular, the II instar nymph visited the larval webs of *E. a. provincialis* mainly during the morning, while the III instar nymph stayed on the nest longer into the

Fig. III – *Picromerus bidens*: clock time of the observations of nymphs and adults in 2015-2016 at Loc. Colle Marcone.

Fig. I – Occurrence of nymphs and adults of *Picromerus bidens* on the larval web of *E. a. provincialis* (Loc. Colle Marcone, 1121 m a.s.l.) in 2015-2016. Some records could involve individuals that have been observed repeatedly.

afternoon (Fig. III). During nocturnal observations, we did not find any predators either on larval web or in their vicinity.

NOTES ON PREDATION BEHAVIOUR

We observed seven nymphs of *P. bidens* feeding on the caterpillars of *E. a. provincialis*.

During predation, the nymphs remained at the external edge of larval web and explored only the web surface of the older webbing of the nest. When they found a potential prey (i.e., the nearest caterpillar), they attacked the larva by piercing it with its stylet: they raised it with their beak, and moved away; then, they hid between leaves at a distance of ca. 20-30 cm from the nest and consumed the prey (Fig. II, 1-3).

On the 29th of June 2015, a II instar nymph approached the larval mass only when the nest was in sunlight and larvae were still inside the silk web (Fig. II, 1).

Fig. II – Predation by *P. bidens* on gregarious larvae of *E. a. provincialis* in a larval web on *L. caprifolium*: 1) a II instar nymph (29.VI.2015); 2) a II instar nymph (27.VI.2015); 3) a III instar nymph (29.VI.2015); 4) a IV instar nymph (29.VI.2015); 5) an adult while preying on a larva of unidentified sawfly (Symphyta) close to a larval web (27.IX.2015); 6) an adult on a larval web (4.X.2015).

We observed adults of *P. bidens* on a larval web of *E. a. provincialis* but we never recorded their predation events on caterpillars. On the larval web surface, we photographed only one adult preying, but it fed on a larva of unidentified sawfly (Hymenoptera, Symphyta) (Fig. II, 5).

DISCUSSION

Picromerus bidens is a generalist predator of a wide range of insects, including moths and butterflies (LARIVIÈRE & LAROCHELLE, 1989). For such a generalist, the pre-hibernation caterpillars of *E. a. provincialis* might represent an important food source for adult bugs and, even more, for their gregarious nymphs.

Gregarious behaviour is typical of the I-II instar nymphs of *P. bidens* (LARIVIÈRE & LAROCHELLE, 1989; DE CLERCQ, 2000), whenever the nymphs discover a large source of food (i.e., a butterfly larval web) they remain around it, even if they are no longer gregarious (III and IV nymphal instars). As they grow into the V instar stage, they may need larger preys and, therefore, abandon the butterfly larval webs (JAVAHERY, 1986).

During our observations, nymphs of *P. bidens* attacked caterpillars of *E. a. provincialis* quite efficiently and visited a single larval group repeatedly through time (from II to IV nymphal instars, Fig. III), acting as a potential and substantial mortality factor for our endangered study species.

Similar prey behaviour was, in fact, described for the V nymphal instar of *P. bidens* on the endangered *E. maturna* in Germany (DOLEK *et al.*, 2007), and authors raised their worries on its potential impact on this species.

The absence of I instar nymphs *P. bidens* on larval webs of *E. a. provincialis* collates with the fact that they are not predators at this age (SOUTHWOOD & LESTON, 1959). Adults can instead attend larval webs and then, we surmise, feed on butterfly larvae, as already observed for *E. a. aurinia* by KONVICKA *et al.* (2005) in western Bohemia, Czech Republic. Unfortunately, we never recorded predation events on caterpillars by adult bugs.

To evaluate the predatory impact on *E. a. provincialis*, two major factors need to be taken into account: the habitat of the interacting organisms and the population size of predators and prey.

In the study area, *E. a. provincialis* can be found in both “cool and humid” and “warm and arid” habitat patches in montane grassland. On the contrary, according to JAVAHERY (1986), *P. bidens* in our study area prefers “cool and humid” patches and does not tolerate high temperatures. During our observations, when the larval web was in sunlight, both adult and nymph bugs used to hide in the vegetation in shaded areas, similarly to its prey; the caterpillars of *E. a. provincialis* also remained in the shaded parts of their host plant and retreated into the larval webs, when the temperature was getting higher. We therefore recorded the coexistence of *P. bidens* and *E. a. provincialis* only in Colle Marcone, which is characterized by cool and damp microclimate, while we never observed them together in warmer areas of our study area (such as “crossroads Santa Maria del Monte - Fonte Brignola”). As we did not observe *P. bidens* on any of the 115 webs in the field, we can state with some certainty that *P. bidens* was (and it is) absent in those warmer areas; nevertheless, we were not able to quantify the exact population size of bugs in the cool site where it occurred together with our butterfly. For this reason, we believe that a dedicated study carried out in the cooler

habitats of *E. a. provincialis* would be needed, to ascertain the occurrence of *P. bidens*, to estimate the abundance of bugs and butterflies and, therefore, the impact of this predator on butterfly mortality.

It is also important to underline that consumption and developmental duration of the predatory stages of *P. bidens* is affected by temperature (MAHDIAN *et al.*, 2006a, 2006b, 2008), which means that the species could adopt different developmental and feeding strategies in different habitats and the predatory effect on the butterfly population size could vary seasonally.

The predatory effect on larvae of *E. a. provincialis* by *P. bidens* was difficult to gauge, as we could not use permanent marking on the nymphs (because of their moulting), and individuals on larval webs could have been observed and counted repeatedly. However, we could estimate the minimum and maximum numbers of nymphs, considering that we captured six individual nymphs and observed 13 predator events (see Results). If the individuals observed were different, the total number of nymphs on our plant would be 19 (i.e., six captured plus 13 observed); if instead we observed only one individual, our total number would be 7 (i.e., six captured plus 1 observed). This range of 7-13 nymphs is consistent with that found by JAVAHERY (1986), who observed aggregations of 3-56 individuals.

The number of caterpillars in larval webs greatly varies depending on the host plant: from the few dozen larvae on *Scabiosa columbaria* to hundreds on *Gentiana cruciata* L. (Gentianaceae) (PINZARI *et al.*, 2017). If we consider that nymphs return several times on the same plant to eat, and gravitate around the larval web at the III-IV instar stages, it is clear that they can represent an important threat for the caterpillar survival. Moreover, due to the gregarious behaviour of predators and preys, *P. bidens* nymphs of only a single egg batch could easily annihilate an entire larval web of caterpillars, with serious consequences on the survival of butterflies in that target area.

CONCLUDING REMARKS

In *Euphydryas* butterflies, larval mortality caused by generalist predators as *P. bidens* does occur and is occasionally severe (EHRlich & HANSKI, 2004). *Picromerus bidens* could represent an important regulatory factor influencing the population of *E. a. provincialis*, as suggested for the rare butterfly *E. maturna* (VRABEC & JINDRA, 1998; VOINOT & ESSAYAN, 2015). The gregarious predatory behaviour displayed by early nymphal stages of *P. bidens* could strongly affect butterfly population size, especially in the case of small larval webs. However, the predatory impact of *P. bidens* on *E. a. provincialis* should be further evaluated, within a larger study including the effect of also other predators – among which, the true bug *Deraeocoris schach* (PINZARI, 2016), the theridiid spider *Phylloneta sisypbia* (Clerck, 1757) (PINZARI, 2019), the specialized parasitoids, tachinid fly *Erycia furibunda* (PINZARI *et al.*, 2016) and likely many other unidentified species.

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