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Phylloneta sisyphia (Clerck, 1757) (Araneae: Theridiidae), a Predator of Larvae of *Euphydryas aurinia* (Rottemburg, 1775) (Lepidoptera: Nymphalidae) and its Parasitoid *Erycia furibunda* (Zetterstedt, 1844) (Diptera: Tachinidae)

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Abstract: *Euphydryas aurinia* is one of the most threatened butterflies in Europe due to population decline caused by fragmentation and reduction in the extent of suitable habitats. Several efforts have been addressed to safeguard its species habitats; nevertheless, they have given relatively less emphasis to the role of natural enemies in determining regulatory effects on their host/prey population dynamics. Larval survival is often the key determinant of population size and distribution, so understanding the sources and variation in larval mortality is essential to understanding and predicting butterfly population dynamics. In *E. aurinia*, the larval mortality is mainly given to various abiotic factors, and therefore, to parasitoids and predators. The pre-hibernation caterpillars of *E. aurinia* feed gregariously within a communal silken web and then a larval group might represent an important food source for predators. Various animals have been reported as parasitoids or predators of *E. aurinia* at different stages of its life cycle*;* however, information on predation is still scarce in this butterfly. In this paper, I provide new data on predation events by the spider *Phylloneta sisyphia* (Clerck, 1757) on pre-hibernation larvae of *Euphydryas aurinia* (Rottemburg, 1775), and its parasitoid *Erycia furibunda* (Zetterstedt, 1844) in Central Italy. Finally, I also reported data on visitors (other spiders) of larval webs that could presumably act as predators of caterpillars.

Key words: *Euphydryas aurinia*, marsh fritillary, *Phylloneta sisyphia,* predator*, Erycia furibunda,* larval-pupal parasitoid

Introduction

Efforts for butterfly conservation in Europe have mainly been addressed to safeguard species habitats by means of their quality (host plant abundance, host plant size and vegetation characteristics of host plant cover), environmental variables (soil temperature, air temperature and soil moisture, slope, wind patterns and sun exposure of land), landscape pattern and connectivity (shape and size, presence of corridors) of the sites (Kuhn et al. 2005, New 2013). In line with these topics, *Euphydryas* Scudder, 1872 butterflies, and in particular *Euphydryas aurinia* (Rottemburg, 1775), one of the most threatened butterflies in Europe, have been the subject of much ecological research (Ehrlich & Hanski 2004). In fact, *E. aurinia* is highly selective for both quality and spatial distribution of habitats (Konvička et al. 2003, Tjornlov et al. 2014) and persists in landscapes via metapopulation dynamics, forming spatially restricted colonies interconnected by adult dispersal (Hula et al. 2004); in addition, the species varies geographically in host plant use (see revisions in Singer 2003, 2004, Pinzari et al. 2016). However, relatively less attention has addressed the role of natural enemies in determining regulatory effects on their host/prey population dynamics (Crawley 2009).

Butterfly populations are attacked at all stages of their life cycle by an array of natural enemies, es-

pecially by arthropods and vertebrates. Many works have suggested that invertebrate predators are the main cause of death of butterflies at early stages of their development. A lot of features of caterpillars, such as distastefulness or toxicity, gregariousness, web building, host plant use, body patterns, are thought to have evolved as defences against natural enemies. However, while population biologists agree that predacious animals have shaped the evolution of butterflies, the extent to which the observed abundances and population dynamics, such as fluctuations in butterflies, are influenced by natural enemies is more controversial.

In the context of an ongoing survey of the lepidopteran fauna in central Italy (Pinzari et al. 2010, Pinzari & Pinzari 2013, Pinzari et al. 2018a, 2018b, 2018c), we studied a population of *E. aurinia*. We aimed to look at key aspects of its biology, ecology and population dynamics (Pinzari et al. 2016, 2017, 2019a, 2019b) and we focused our attention on the natural enemies of the butterfly *E. aurinia* and, in particular, of its caterpillars. These are gregarious in early stages, live within a communal silken web and represent a good and abundant food source for predators. We argue that they could play a role in a complex multitrophic community that seems to be likely around the larval web. In fact, during our observations, various arthropods, mainly insects and arachnids, have been recorded while frequenting the larval web of *E. aurinia.* They have different dietary reasons, including predators, parasitoids and organisms that gain some benefits from the interaction with butterfly gregarious larvae. In this last case, Pinzari (2016) reported two hemipterans, *Spilostethus saxatilis* (Scopoli, 1763) and *Palomena prasina* (L., 1761), which are typically phytophagous, while feeding on the droppings of the caterpillars of *E. aurinia*. Among parasitoids, the tachinid *Erycia furibunda* (Zetterstedt, 1844) parasitizes the I-III instar larvae of *E. aurinia* (Pinzari et al. 2017). Among predators, the hemipterans *Deraeocoris schach* (Fabricius, 1781) and *Picromerus bidens* (Linnaeus, 1758) prey on the pre-hibernation larvae of *E. aurinia* (Pinzari 2016, Pinzari et al. 2019b) . Regarding predation by spiders, scarce information is available only for other congeneric butterflies. Moore (1987) reported that an unidentified spider consumed the adults of *Euphydryas editha* (Boisduval, 1852) (California) causing male bias in a population. Hence, Dolek et al. (2007) observed the predation on caterpillars of *Euphydryas maturna* (L., 1758) by the crab-spider *Misumena vatia* (Clerck, 1757) (Germany).

Here, I report the observations of predation by the spider *Phylloneta sisyphia* (Clerck, 1757) on pre-hibernation larvae of *Euphydryas aurinia* and its parasitoid *Erycia furibunda* and discuss on the potential role of this spider in predation dynamics.

Materials and Methods

Observations were carried out in 2016 and 2017 (July-August) in an area (2 Ha) nearby the "crossroads Santa Maria del Monte – Fonte Brignola" (ca. 1000 m) in the surroundings of Vallemare (42.4836N, 13.1148E) in the Central Apennines (Rieti, Italy). In the study area, *Euphydryas aurinia* inhabits habitat patches in montane grassland and slopes within a mosaic of wooded areas, hedgerows and fields. Females oviposit on several plants, such as *Gentiana cruciata* L., *Scabiosa columbaria* L. and *Cephalaria leucantha* (L.) Roem. & Schult., and caterpillars feed on these host plants, including also *Lonicera caprifolium* L. (Pinzari et al. 2016).

Initially, the I-V instar caterpillars are gregarious, then the VI-instar larvae are solitary. When caterpillars are gregarious they feed on host plants within a communal silken web (Porter 1981, Warren 1996, Kuussaari et al. 2004, Pinzari et al. 2016). In the study area, in July-August, the larvae of *E. aurinia* are early instars (I-III) and gregariously live inside a silken web.

To gain information on the natural enemies of the caterpillars of *E. aurinia,* during July-August any visitor of the larval webs on gentians was photographed in the field and/or collected on sight. Additionally, notes on the prey-predator interaction were recorded. The attention was given to the larval webs on gentians, rather than the other host plants, because the gentians host large larval webs and are easy to monitor through time.

Insects and spiders collected are kept in Pinzari Collection except for *Phylloneta sisyphia*; this is preserved in the private collection of Dr. Paolo Pantini (Bergamo, Italy) who identified the species. The determination of the other animals was made with the support of Dr. Luigi Lenzini (Rome, Italy) for spiders, and for dipterans by Dr. Pierfilippo Cerretti (Roma, Italy) and Dr. Franco Mason (Verona, Italy). The species identification of the fly belonging to the family Stratiomyidae was based only on photos. All records were reported in details in S1 Appendix.

Results

Various arachnids used to frequent the larval webs of *E. aurinia* on gentians. Spiders (Araneae) and harvestmen are likely recorded on the stems of *G. cruciata* neighbouring the larval web. In particu-

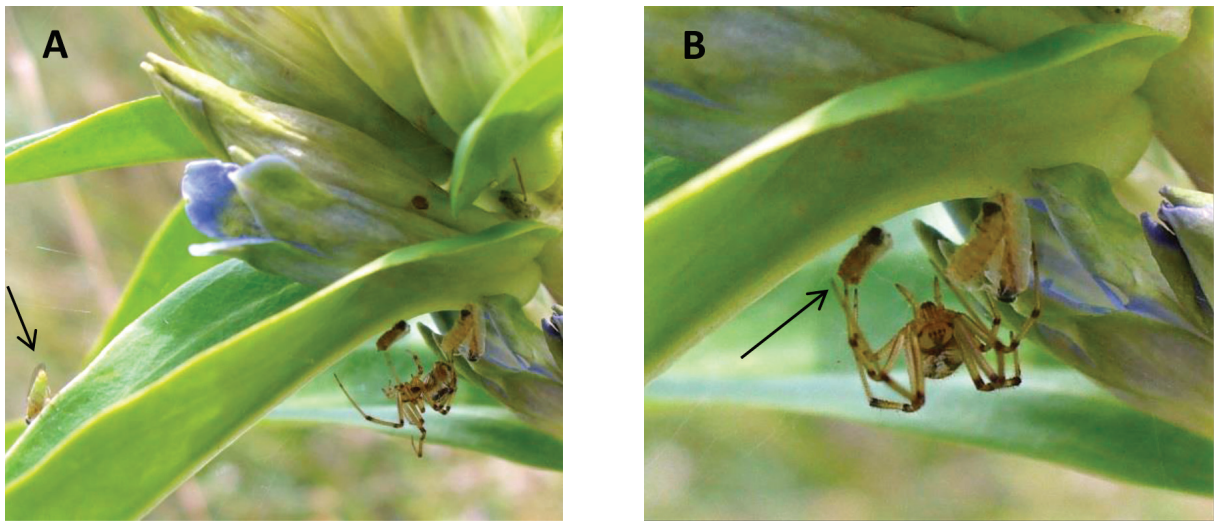

Fig. 1. *Phylloneta sisyphia* on silken threads on *Gentiana cruciata* (24 July 2016): A) a fly of *Odontomyia* sp. rested on a leaf; B) while feeding on the other three flies inside cocoons.

lar, I recorded seven arachnid species: *Phylloneta sisyphia* (Walckenaer, 1802) (Theridiidae), *Oxyopes lineatus* Latreille, 1806 (Oxyopidae), *Xysticus erraticus* (Blackwall, 1834) (Thomisidae), *Pisaura mirabilis* (Clerck, 1757) (Pisauridae), *Philaeus chrysops* (Poda, 1761) (Salticidae), *Pellenes* sp. Simon, 1876 (Salticidae), *Araneus diadematus* Clerck, 1757 (Araneidae) and also the harvestmen *Phalangium opilio* Linnaeus, 1761 (Phalangiidae) (S1 Appendix).

I only observed an individual of *P. sisyphia* while preying on a caterpillar of *E. aurinia* on the butterfly larval web and, after that, 2-3 larvae of the butterfly in cocoons that were proximate to the spider. *Phylloneta sisyphia* used to frequent the larval webs and also prey other visitors (Figs. 1 and 2). In the study area, *P. sisyphia* weaves its silken web around the inflorescences of *G. cruciata* and its spider web was often nearby the butterfly larval web and sometimes merged together with it.

The presence of *P. sisyphia* was recorded on (or nearby) the butterfly larval web in the following circumstances: (i) a female while resting on silken threads anchored to the leaves and the flowers of the plant; then, it was photographed while preying a fly belonging to *Odontomyia* sp. (Diptera: Stratiomyidae) (Fig. 1); (ii) an individual while moving on the surface of a butterfly silken web; this was merged with the spider web on the flowers of *G. cruciata*; (iii) another individual while preying on a caterpillar of *E. aurinia*; in Fig. 2, the same spider already previously captured and immobilised in cocoons two or three larvae of the butterfly and also a female of the tachinid, *E. furibunda* (Diptera, Tachinidae); and finally, (iv) an individual while moving on silken bridge between two flowers of *G. cruciata*.

Fig. 2. *Phylloneta sisyphia* while preying on caterpillars of *Euphydryas aurinia* (black arrow) and *Erycia furibunda* (12 August 2016) wrapped in the spider web (white arrow).

Discussion

In *Euphydryas* spp., both caterpillars and butterflies use the foliar compounds (iridoids) for their own defence against natural enemies (e.g. Bowers 1980, 1981, Bowers & Puttick 1986, Stermitz et al. 1986, 1994, Franke et al. 1987, Gardner & Stermitz 1988). *Euphydryas aurinia* share with the conspecific butterflies both the sequestration of iridoids (Penuelas et al. 2006) and the aposematic vivacious colour wing pattern (Long et al. 2014).

These findings suggest that both caterpillars and adults of *E. aurinia* should be distasteful or toxic to predators. On the contrary, various organisms live at the expense of *E. aurinia* (van Nouhuys & Hanski 2004) and, during my observations, I recorded various insects and arachnids inhabiting the nests of *E. aurinia* when the caterpillars were at the I-III instar before their winter diapause (unpublished data).

Spiders are usually generalist predators of a wide range of insects, including moths and butterflies. My observations showed that among arachnids different species of Araneae are visitors to the larval webs of *E. aurinia*. For such a generalist, the pre-hibernation caterpillars of *E. aurinia* might represent an important food source for the observed spiders and, even more, *Phylloneta sisyphia*. The habit of this species to weave its spider web on the flowers of gentians is advantageous to easily prey both caterpillars of *E. aurinia* and other insects that visit these plants for different dietary reasons. Two examples of this were the predation events by *P. sisyphia* on the stratiomyid flies of genus *Odontomyia* and the tachinids fly *Erycia furibunda*. The flies of the genus *Odontomyia* presumably feed on flowers of *G. cruciata* (personal communication, Dr. Constantino, L.M. Department of Entomology, Colombia), while *E. furibunda* parasitizes the pre-hibernation larvae of *E. aurinia* (Pinzari et al. 2016).

The single observation of predatory behaviour in *P. sisyphia* does not allow affirming that this species is a habitual predator of *E. aurinia* larvae, however it might attack them quite efficiently and visit a single larval group repeatedly through time (Fig. 2), acting as a potential and substantial mortality factor for my endangered study species. At present, the larval mortality of *E. aurinia* is attributed to environmental conditions, climate and nourishment, and therefore, to some parasitoids such as tachinids (Pinzari et al. 2017, Tschorning 2017) and braconids (Porter 1983, Kankare et al. 2005), as well as predators such as hemipterans (Konvička et al. 2005, Pinzari 2016, Pinzari et al. 2019b). The predatory effect on larvae of *E. aurinia* by *P. sisyphia* is difficult to estimate but it is likely twofold: it affects both the population size of the butterfly by preying caterpillars and the population of the parasitoids by preying the gravid females of *E. furibunda* (Fig. 2). Indeed, female parasitoids are used to rest on larval nest of *E. aurinia* to find the host, *E. aurinia* caterpillars (Pinzari et al. 2017), and lay hundreds of eggs limiting the host population size.

In this framework, although the impact of natural enemies, such as predators, on population size requires further research, *P. sisyphia* could likely play a role in this complex multitrophic community around the larval webs and represent an important regulatory factor influencing the population of *E. aurinia.* Finally, since *E. aurinia* suffers a severe decline in most European countries, a major knowledge of edibility/inedibility of this species by documenting predation events could be interesting in terms of butterfly conservation. It would be fascinating to study whether spiders and other predators that visit the larval webs may act as a mortality factor on population of *E. aurinia*, as suggested for some *Euphydryas* butterflies (Vrabec & Jindra 1998; Konvička et al. 2005).

Supporting Information

Appendix: Records and Material Examined.

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Appendix

Records and Material Examined

Araneae

Araneus diadematus Clerck, 1757 (Araneae, Araneidae). Material examined: Italy, Vallemare (RI), Crossroads Santa Maria del Monte – Fonte Brignola, 1100 m, 23 August 2016, M. Pinzari legit (Coll. M. Pinzari, Rome, Italy).

Oxyopes lineatus Latreille, 1806 (Araneae, Oxyopidae). Material examined: Italy, Vallemare (RI), Crossroads Santa Maria del Monte – Fonte Brignola, 1100 m, 14 July 2017, M. Pinzari legit (Coll. M. Pinzari, Rome, Italy).

Xysticus erraticus (Blackwall, 1834)(Araneae, Thomisidae). Material examined: Italy, Vallemare (RI), Crossroads Santa Maria del Monte – Fonte Brignola, 1100 m, 14 July 2017, 1 ex, M. Pinzari legit; idem, 11 August 2016, 1 ex, M. Pinzari legit (Coll. M. Pinzari, Rome, Italy)

Pisaura mirabilis (Clerck, 1757)(Araneae, Pisauridae). Material examined: Italy, Vallemare (RI), Crossroads Santa Maria del Monte – Fonte Brignola, 1100 m, 7 Aug 2017, M. Pinzari legit (Coll. M. Pinzari, Rome, Italy).

Philaeus chrysops (Poda, 1761)(Araneae, Salticidae). Material examined: Italy, Vallemare (RI), Crossroads Santa Maria del Monte – Fonte Brignola, 1100 m, 29 August 2016, 1 ex, M. Pinzari legit (Coll. M. Pinzari, Rome, Italy).

Pellenes sp. Simon, 1876 (Araneae, Salticidae). Italy, Vallemare (RI), Crossroads Santa Maria del Monte – Fonte Brignola, 1100 m, 19 Aug 2017, 1 ex, M. Pinzari legit (Coll. M. Pinzari, Rome, Italy).

Phylloneta sisyphia (Walckenaer, 1802) (Araneae, Theridiidae). Material examined: Italy, Vallemare (RI), Crossroads Santa Maria del Monte – Fonte Brignola, 1100 m, 24 July 2016, M. Pinzari legit, 1#, (Coll. P. Pantini, Bergamo, Italy), photo by Mario Pinzari; idem, 12 August 2016, M. Pinzari legit, 1 ex (Coll. M. Pinzari, Roma); idem, 22 August 2016, M. Pinzari legit, 1 ex, photo by Mario Pinzari.

Opiliones

Phalangium opilio Linnaeus, 1761 (Phalangiidae). Material examined: Italy, Vallemare (RI), Crossroads Santa Maria del Monte – Fonte Brignola, 1100 m, 28 August 2017, 1 ex, M. Pinzari legit (Coll. M. Pinzari, Rome, Italy).

Diptera

Erycia furibunda (Zetterstedt 1844) (Diptera, Tachinidae). Material examined: Italy, Vallemare (RI), Crossroads Santa Maria del Monte – Fonte Brignola, 1100 m, 1 #, 12 Aug 2016 M. Pinzari legit (Coll. M. Pinzari, Rome, Italy).

Odontomyia sp. (Diptera, Stratiomyidae). Material examined: Italy, Vallemare (RI), Crossroads Santa Maria del Monte – Fonte Brignola, 1100 m, 1 #, 24 Jul 201, 1 adult, 2-3 larvae (photo by Mario Pinzari) (Fig. 2).