2012, *Entomologist's Gazette* **63**: 251–257

Larval parasitoids of *Rivula sericealis* (Scopoli) (Lepidoptera: Noctuidae) in Britain, including notes on the biology of *Cotesia subordinaria* (Tobias) (Hymenoptera: Braconidae, Microgastrinae), a solitary-cum-gregarious parasitoid

MARK R. SHAW[1]

National Museums of Scotland, Chambers Street, Edinburgh, EH1 1JF, U.K.
markshaw@xenarcha.com

Synopsis

Sampling larvae of *Rivula sericealis* revealed few parasitoids. It is shown experimentally that *Cotesia subordinaria* generally develops as a solitary parasitoid in host larvae attacked in their first three instars, but typically forms broods of two to three individuals when ovipositing into fourth and fifth (= final) instar host larvae.

Key words: Hymenoptera, Braconidae, Microgastrinae, *Cotesia subordinaria*, parasitoid, biology.

Introduction

The small partly plurivoltine noctuid moth *Rivula sericealis* (Scopoli) is somewhat atypical in appearance in the British fauna, regarding both the adult (e.g. Townsend, Waring & Lewington, 2007) and the larva (e.g. Porter, 1997), and indeed its history of subfamilial classification has been very unsettled.

Potential hosts that occupy relatively isolated positions, whether ecologically, behaviourally, morphologically or phylogenetically, are promising candidates to have specialised and/or phylogenetically interesting parasitoids – the more so if the host is, like *R. sericealis* in Britain, common enough potentially to sustain them. With this in mind an effort was made to collect and rear larvae of *R. sericealis*, concentrating on its first (overwintered) generation, at various sites in Britain over the past few years (Table 1). Short-term (summer) cultures of the moth were also maintained for observation and in order to provide experimental opportunities with parasitoids.

The moth is generally common over much of Britain, occurring in a wide variety of grassy sites, perhaps most commonly in somewhat sheltered situations. The larva feeds on various grasses, in my experience *Brachypodium sylvaticum*, *Dactylis glomerata* and *Molinea caerulea* but doubtless many others – though the smaller and softer-bladed grasses appear to be markedly less favoured – and it is also recorded from sedges. There are five larval instars and the winter is spent as a diapausing third instar larva, which contracts and assumes a particularly intense green coloration for the purpose. The actively feeding post-diapause larvae can be collected from late March to May, with some growth differentiation that results in a long emergence period of the first generation adults. The adults are long-lived and there is then a succession of at least partial additional generations, such that adults are on the wing more or less continuously from late May well into September, with summer generation larvae in various stages of growth from June until third instar larvae start to enter diapause in the autumn. All larval instars are more or less sparsely setose.

[1] Honorary Research Associate.

Materials and Methods

For some time it was frustratingly difficult to collect samples of larvae, although observations in culture suggested that they rest well up on grass blades from which they dislodge easily. Sweeping, at least in still weather when it seems likely the larvae were dropping ahead of the net, largely failed; though under slightly breezy conditions in the late afternoon it was sometimes successful. Vacuum-sampling tussock-forming grasses was much more effective, and most larvae were collected by this means. Sampling was restricted to the overwintered generation (April–May), and larvae of the third, fourth and fifth instars were all collected, with nothing to suggest that they were not equally amenable to sampling. The three sites at which large samples were collected were each chosen in the knowledge that the rogadine braconid parasitoid *Heterogamus dispar* (Haliday) had a strong local population, in pursuit of the (evidently incorrect) hunch that *R. sericealis* might prove to be its unknown host.

Short-term (summer) cultures of *Rivula sericealis* were established from combinations of reared moths and those collected at light. Both mating and oviposition occurred easily in closed plastic boxes (14 × 8 × 6 cm) bottom-lined with tissue paper in which 3 : 1 water : honey solution on cotton wool was continuously present. Small sections of suitable grass blades resulted in egg aggregation, but were not necessary for provoking oviposition which occurred readily at the edges of the tissue and also more sparsely over the entire surface of the container. Rearing of wild larvae was conducted under standard protocols in a shaded and well-ventilated outdoor shed (cf. Shaw, 1997). Experimentally parasitised hosts were reared under natural summer daylight (July; Edinburgh, U.K.) indoors at 18–22° C. All larvae, whether wild-collected or in culture, were fed on *Dactylis glomerata* in closed tissue-lined plastic boxes: in the case of wild-collected larvae and those that had been parasitised under experimental conditions, cohorts were counted and all larvae accounted for with each food change (at least weekly; daily inspection during times of cocoon formation in culture). Parasitoid cocoons together with host larval remains were removed and stored individually in 7.5 × 2.5 cm corked glass tubes to await adult emergence. Experimental exposures of cultured (parasitoid-free) *R. sericealis* larvae to virgin females of *Cotesia subordinaria* (Tobias) were done singly, under continuous observation, in 7.5 × 2.5 cm corked glass tubes. Host larvae of known age and instar, typically at rest beside recent feeding damage, were offered on short cut sections of foodplant, though identical oviposition behaviour resulted when hosts were offered naked and some runs were done this way. To promote longevity, female parasitoids were kept individually in 7.5 × 2.5 cm corked glass tubes at 8–11° C in a domestic refrigerator when not being offered hosts: a smear of very dilute honey : water solution was maintained in the tube and each female was allowed an hour or so of activity at room temperature (18–22° C) every day, during which she could feed. Experiments were performed during this activity period, and females were limited to ten hosts per day. Two females were involved: one lived for 41 days and the other for 20 days. The first death was the result of overheating on a long, hot car journey and the second followed an ill-judged attempt to pair the female with her sons, which left her exhausted. All experimental ovipositions occurred when the females were apparently in their prime.

Table 1. Sampling sites and parasitoids reared from *Rivula sericealis*.

Site (date)	Food plant	No. collected	Unparasitised	*Cotesia subordinaria*	*Hyposter* sp(p).
Gait Barrows NNR, Lancs.(15/16.v.2008)	Mixed grasses and sedges	50	47	0	3
Monks Wood NNR, Hunts. (16.v.2009)	*Brachypodium sylvaticum*	31	26	0	5
Eastleach, Glos. (various April–May 2007–2009)	Mainly *Dactylis glomerata*	13	10	3	0
Miscellaneous aggregated (April–May 2008–2009)	Various grasses and sedges	16	15	0	1

Moderate numbers of wild larvae were collected at three sites, in each case from at least predominantly different grasses (Table 1): other collections (several sites on Anglesey; Flanders Moss, Stirlingshire; Castor Hanglands NNR, Northamptonshire) were too small to be accounted for individually and are lumped as 'Miscellaneous'. Larvae harbouring parasitoids were in all cases substantially retarded and it is confidently believed that no mortality from larval parasitoids had occurred before sampling took place. However, it is possible that for late-collected samples some, though probably few, advanced and unparasitised hosts had already left the sampling arena to pupate, resulting in the level of parasitism to have been slightly over-estimated if so. The parasitoids reared were all solitary with respect to the host.

Results

Overall the rearing results from wild larvae (Table 1) were disappointing, as nothing really unusual was found and parasitisation rates were low, although the *Cotesia* species reared from Eastleach was previously unknown in Britain (see Shaw, 2012). The single or possibly two (K. Horstmann, pers. comm.) *Hyposoter* (Ichneumonidae: Campopleginae) species reared have not yet been determined: in all cases whitish cocoons were formed within the final instar host's larval skin. An unidentified *Mesochorus* (Ichneumonidae: Mesochorinae) species was reared as a true hyperparasitoid from two of the *Hyposoter* cocoons from Monks Wood larvae.

Experimental rearings

The results of exposing first, second and third instar hosts to the virgin females of *C. subordinaria* (Table 2) were essentially similar, though development times to the cocoon stage varied a little, partly because the host was not killed until its fourth instar in each case, and first instar hosts suffered high mortality, probably immediately as a result of oviposition trauma. The females were attracted to host feeding sites and faeces (even in the absence of hosts), and acceptance of hosts, including those in proecdysis, was immediate on contact. Oviposition was rapid (insertions of under a second) and apparently at random sites into the host's haemocoel, with little host reaction (in a few cases the head was raised briefly). The female parasitoid usually left the host immediately afterwards in a way that

Table 2. Outcomes of experimental ovipositions by two females of *Cotesia subordinaria* into *Rivula sericealis* larvae of different instars.

Female	Host instar	Host age (days)	Number parasitised	Number surviving 2 days	Min (mean) days to parasitoid cocoons	1 parasitoid	2 parasitoids	3 parasitoids	Host pupae
1	1st	1	10	5	17 (17.6)*	5	0	0	0
2	1st	1	10	6	14 (14.6)	5	0	0	1
1	1/2 proecdysis	3	18	15	12 (14.1)	15	0	0	0
2	1/2 proecdysis	3	16	15	12 (13.4)	14	0	0	1
1	2nd	5	10	9	13 (13.6)	6	2	0	1
2	2nd	5	10	8	13 (13.4)	8	0	0	0
1	3rd	8	21	20	13 (13.9)	17	3	0	0
1	4th	12–15	11	11	14 (15.2)	5	4	0	2
1	5th	14–18	5	5	13 (13.0)	0	2**	2	1

* Retardation of this batch was probably the result of partial desiccation of food, but this did not lead to additional mortality.
** In both cases the host died and the fully-grown parasitoid larvae failed to erupt.

in the wild would clearly prevent immediate rediscovery (often by flight). In about a quarter of cases, however, rediscovery and superparasitism did occur (with comparable ovipositor insertion times) before the host could be removed. Unfortunately no unusual outcome of this was anticipated, and no segregation of superparasitised hosts was attempted. However, while most cases of multiple oviposition certainly resulted in only one parasitoid developing (clear, because of the numbers involved), in a few others two parasitoids successfully developed (Table 2).

When hosts were offered in their fourth or fifth (final) instars, however, ovipositor insertion was generally for a longer period (more than one and sometimes over two seconds) and when the female parasitoid was ovipositing her hind legs were usually raised in the air (this did not occur with smaller hosts, although the host's setae were an evident impediment from the third instar onwards). Because host removal was fully efficient in the case of fourth and fifth instar hosts (completely preventing rediscovery), when broods of more than a single parasitoid resulted it was clear that more than one egg had been laid with a single insertion of the ovipositor (Table 2). It was only possible to offer one female (♀ 1, Table 2) fourth and fifth instar hosts: although numbers were small, the proportion of apparently parasitised fourth and fifth instar hosts to suppress all parasitoids and go on to form viable pupae was significantly higher than when hosts were attacked by this female in their first three instars (Table 2) (Fisher's exact test, $p = 0.0432$). Hosts parasitised in the fourth instar sometimes (3 of 9) remained in that instar until parasitoid eruption, but more often (6 of 9) progressed to the fifth instar before this occurred, irrespective of whether parasitoid development was solitary or gregarious. Overall parasitoid development was solitary in only 5 of the 13 hosts attacked in their fourth or fifth instars in which parasitoids became established, the difference in this respect between these instars and the first three instars being significant if only ♀ 1 is considered (Fisher's exact test, $p = 0.0285$), or highly so on the basis of progeny of both females (Fisher's exact test, $p = 0.0054$). In two cases hosts attacked as fifth instar larvae died without parasitoid eruption, and dissection revealed two fully grown but moribund parasitoid larvae within; as development had progressed so far it seems unlikely that the host had suppressed the parasitoids in any active sense and the deaths are unexplained. Three parasitoid individuals successfully developed in two other hosts attacked in this instar.

Cocoons were generally formed on and aligned with a grass stem; if gregarious they were often positioned side by side. The host usually lived for a day or two after parasitoid eruption. All progeny of the virgin females was male, and the adults emerged from cocoons after about eight days at 18–22° C.

Discussion

Despite their relative 'unusualness' in the British fauna, the larvae of *Rivula sericealis* proved to be only lightly parasitised, and not by any unusual parasitoid genera. One (or perhaps more: K. Horstmann, pers. comm.) unidentified species of *Hyposoter* (Ichneumonidae: Campopleginae), pupating in a cocoon formed within the skin of the final instar larva, was reared from a total of three sites (Table 1), but always in fairly low numbers. Cocoon formation within the host skin is not unusual for *Hyposoter* species, especially those specialising in

parasitizing more or less spiny or setose larvae. Three examples of *Cotesia subordinaria* resulted from sampling at a single site in successive years: a further reared male of this species from a different site had been given to me some years ago from a host that was unidentified at the time, swept from *Brachypodium sylvaticum* at Shoreham, Sussex, coll. and em. 1995 (*I. D. Ferguson*). Fortunately the host remains had been retained and it is now clearly seen that they belong to *R. sericealis*, and the adult parasitoid could be identified once the cultured males were available for comparison. Otherwise I am unaware of parasitoids previously reared from *R. sericealis*.

Cotesia subordinaria is, however, an interesting species of *Cotesia*, both because it is rather distinctive in the British fauna and more particularly because of its variable development as a solitary or gregarious parasitoid. This plasticity has a clear relationship with host size, and among ichneumonoid idiobiont ectoparasitoids (e.g. some genera of Pimplinae and Xoridinae (Ichneumonidae) and Braconinae and Doryctinae (Braconidae)) such a relationship would not be unusual. However, among ichneumonoid koinobiont endoparasitoids it is much more noteworthy. Somewhat similar situations arise in some *Cotesia* species that have large broods in late instars of their hosts, but develop small broods (at the extreme, with sizes down to one) in young larvae of the same species. This arises particularly in colonial hosts such as Melitaeini butterflies whose larvae diapause at a small size, carrying small broods of the parasitoid through the winter with them, which become adult in early spring and parasitise healthy individuals of the same host generation (Shaw, Stefanescu & Nouhuys, 2009). Similar successive usage of a single host generation is seen in certain *Cotesia* parasitoids of other hosts such as Zygaenidae, Arctiidae and Lasiocampidae (M. R. Shaw, unpublished data), though in most of these cases the parasitoid is basically highly gregarious, with large brood sizes in the well-grown host stages. One well-known species, *Cotesia melanoscula* (Ratzeburg) (= *solitaria* (Ratzeburg)) parasitises various Lymantriidae and is able to use the host generation successively at a wide range of sizes – but in this case the parasitoid always maintains strictly solitary development (Parker, 1935, as *Apanteles solitarius*).

The plasticity seen in *C. subordinaria* seems not to reflect the opportunism of having successive generations with ever-larger broods, developing on an ageing host cohort, not least because its host is generally available in a range of sizes during the parasitoid's flight period. Rather it may reflect an adaptive strategy of superparasitism of older hosts in order to survive the host's physiological defences, as in these larger hosts this degree of gregariousness seemed not only fairly usual, but also parasitoid progeny seemed at greater risk of being suppressed. (Note that even if only one parasitoid developed to the cocoon stage, as in some parasitised in their fourth instar, its survival may have depended on suppressed siblings.) Among the many adaptive and non-adaptive underlying causes of self-superparasitism (Godfray, 1994; Dorn & Beckage, 2007), avoidance of suppression by the host has sometimes been demonstrated (e.g. Sagarra *et al.*, 2000; Buchori, Sahari & Spiratna, 2009). Strong adaptive evidence has been advanced for the solitary microgastrine braconid *Microplitis rufiventris* Kokujev in which the siblicidal behaviour seen in superparasitised early instar hosts was relaxed in the less suitable later instar hosts in which superparasitism improved parasitoid survival (Khafagi & Hegazi, 2008). More work would be needed to substantiate this in the case of *C. subordinaria*, but the indications that

this may be happening are evident. Although not on a scale to suggest that it is an adaptive strategy, gregarious development in another essentially solitary *Cotesia* species, *C. marginiventris* (Cresson), has been observed as a rare outcome of superparasitism (Riddick, 2002; 2008) in early instar hosts. I am unaware of any experiments with this parasitoid involving older hosts, but it would be interesting to know if gregarious development under those circumstances becomes more frequent and hence suggestive of an adaptive strategy.

Acknowledgements

Rob Petley-Jones and Chris Gardiner (English Nature, now Natural England) granted permission to do fieldwork at respectively Gait Barrows NNR and Monk's Wood NNR; my sister Nanina Shaw-Reade did much in support of fieldwork on her land at Eastleach; Keith Bland helped to collect larvae at several sites; Gavin Broad provided access to the BMNH collection; and Ian Ferguson donated a reared male of *C. subordinaria* to NMS, providing a second U.K. distributional record. I am most grateful to them all.

References

Buchori, D., Sahari, B. & Spiratna, E. 2009. Encapsulation and hemocyte numbers in *Crocidolomia pavonana* and *Spodoptera litura* Fabricius (Lepidoptera) attacked by parasitoid *Eriborus argentopilosus* Cameron (Hymenoptera). *HAYATI Journal of Biosciences* **16**: 135–141.

Dorn, S. & Beckage, N. E. 2007. Superparasitism in gregarious hymenopteran parasitoids: ecological, behavioural and physiological perspectives. *Physiological Entomology* **32**: 199–211.

Godfray, H. C. J. 1994. *Parasitoids*[:] *Behavioural and evolutionary ecology.* ix, 473 pp. Princeton.

Khafagi, W. E. & Hegazi, E. M. 2008. Does superparasitism improve host suitability for parasitoid development? A case study in the *Microplitis rufiventris*-*Spodoptera littoralis* system. *Biocontrol* **53**: 427–438.

Parker, D. L. 1935. *Apanteles solitarius* (Ratzburg), an introduced braconid parasite of the satin moth. *V. S. Department of Agriculture Technical Bulletin* no. 477: 17 pp.

Porter, J. 1997. *The colour Identification Guide to Caterpillars of the British Isles* xii, 275 pp. Harmonsworth.

Riddick, E. W. 2002. Superparasitism occasionally predisposes *Cotesia marginiventis* (Cresson) (Hymenoptera: Braconidae) to develop gregariously in *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae). *Journal of Entomological Science* **37**:1–9.

——— 2008. Sting frequency and progeny production of lab-cultured *Cotesia marginiventris*. *Biocontrol* **53**: 295–302.

Sagarra, L. A., Peterkin, D. D., Vincent, C. & Stewart, R. K. 2000. Immune response of the hibiscus mealybug, *Maconellicoccus hirsutus* Green (Homoptera: Pseudococcidae), to oviposition of the parasitoid *Anagyrus kamali* Moursi (Hymenoptera: Encyrtidae). *Journal of Insect Physiology* **46**: 647–653.

Shaw, M. R. 1997. *Rearing parasitic Hymenoptera* [*The Amateur Entomologist* **25**] 46 pp. Orpington.

——— 2012. Notes on some European Microgastrinae (Hymenoptera: Braconidae) in the National Museums of Scotland, with twenty species new to Britain, new host data, taxonomic changes and remarks, and descriptions of two new species of *Microgaster* Latreille. *Entomologist's Gazette* **63**: 173–201.

Shaw, M. R., Stefanescu, C. & Nouhuys, S. van 2009. Parasitoids of European butterflies, pp. 130–156. *In* Settele, J., Shreeve, T. G., Konvička, M. & Van Dyck, H. (Eds), *Ecology of Butterflies in Europe*. Cambridge.

Townsend, M., Waring, P. & Lewington, R. 2007. *Concise Guide to the Moths of Great Britain and Ireland* 160 pp. Gillingham (Dorset).