The parasitoid complex associated with the herbivore Hadena bicruris (Lepidoptera: Noctuidae) on Silene latifolia (Caryophyllaceae) in the Netherlands

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Abstract
Larvae of the moth, Hadena bicruris, constitute the most important predispersal seed predator on Silene latifolia (Caryophyllaceae). Parasitoids attacking the larvae of this specialist noctuid can potentially decrease the amount of damage to the plant. This paper describes and quantifies the parasitoid complex associated with H. bicruris in the Netherlands, and documents life history characters of its species. Forty-four percent of larval H. bicruris were parasitized by at least 13 species of parasitoids. The most prevalent of these were the braconids Microplitis tristis (22.9%) and Bracon variator (4.9%) and the ichneumonids Eurylabus tristis (11.7%) and Ophion pteridis (3.4%). Other species occurring sporadically were: the ichneumonids Scambus brevicornis, S. buolianae, Erigorgus cerinops, and Hyposoter sp.; the tachinids Phryxe vulgaris, P. nemea, Blondelia nigripes and Siphona geniculata; and a Mermithidae sp. The ichneumonid hyperparasitoid Mesochorus lanceolatus was found occasionally in larvae of M. tristis. The hyperparasitoid ichneumonids Gelis agilis, G. hortensis and the chalcids Baryscapus endemus, Pteromalus chrysos and P. vibulenus were found in cocoons of both M. tristis and B. variator. The primary parasitoids M. tristis, E. tristis, and to a lesser extent, O. pteridis, are believed to be specialized on Hadena. The effect of the parasitoids on herbivory by their host is probably small because the most common parasitoid species are koinobionts that attack large, late instar hosts. Only B. variator and Scambus spp., which are ectoparasitoids, arrest host development immediately upon parasitism, but like the other parasitoids they kill mainly large L4 or L5 hosts. The gregarious M. tristis and B. variator produce clutches with a female-biased sex ratio, in contrast to the solitary E. tristis where the sex ratio approaches equality. The ectoparasitoid B. variator produces mostly single-sex clutches, probably avoiding deleterious effects of inbreeding. The clutch size distributions suggest that large clutches of M. tristis and B. variator are probably caused by multiple parasitisms.

Keywords: Sex-ratio, caterpillar, clutch size, hyperparasitoids, life history, mutualism
Introduction

Caterpillars of Hadena bicruris Hufnagel (Lepidoptera: Noctuidae) are the most important predispersal seed-feeders on white campion, Silene latifolia Poiret (Caryophyllaceae) in western Europe (Biere & Honders 1996). Wolfe (2002) and Elzinga et al. (2005b) found this herbivore in over 90% of the S. latifolia populations that were sampled in western Europe and the Netherlands, respectively. It often destroys 25–100% of all seed capsules, and may therefore have a major impact on the fitness of the plants (Biere & Honders 1996; Wolfe 2002). Although the adult H. bicruris is also an important pollinator of S. latifolia, damage to seed-capsules by the larvae probably outweighs its positive effects through pollination (Brantjes 1976; Bopp 2003).

Several studies have suggested that parasitoids may cause significant mortality of H. bicruris caterpillars, and may consequently reduce the level of seed predation by this herbivore (Brantjes 1976; Biere et al. 2002; Bopp 2003). However, none of these studies have described and quantified in detail which species of parasitoids were responsible for parasitism. Although taxonomic works on parasitoids (Nixon 1970; Brock 1982; Belshaw 1993) and (pre-electronic) databases (Shenefelt 1980; Yu 1998; Noyes 2001) indicate that several species develop on H. bicruris, these data are, for parasitoids in general, rather scattered, incomplete and may partly be based on erroneous records (Shaw 1994). Furthermore, we have found almost no literature reporting hyperparasitoids in this host–parasitoid complex (except for Schwarz & Shaw 1999). In addition, for the many parasitoid species identified thus far, knowledge of life history parameters such as clutch size, sex ratio, and host stage attacked is very limited, although such data can provide valuable insights into the strategies that different parasitoids employ to exploit suitable hosts in their habitat (Godfray 1994).

The first aim of this paper is to describe and quantify the parasitoid complex, including several hyperparasitoids, associated with the noctuid host, H. bicruris on S. latifolia. We discuss the degree of specialisation of the different parasitoid species on H. bicruris for the different parasitoid species. The second aim is to provide detailed information on the biology of the observed parasitoids. Based on this, we discuss whether the parasitoids in the system may shift the relationship between H. bicruris and S. latifolia from parasitism in the direction of a mutualism. Finally, we discuss patterns in clutch size and sex ratio variation observed for the most prevalent species of parasitoids in the system.

Methods

Host

Hadena bicruris Hufnagel (Lepidoptera: Noctuidae), like other members of this genus (Kephart et al. 2006) (see Hacker 1996 for a revision of the genus), is specialized on the seed capsules of caryophyllaceous plant species, but has a very strong preference for Silene latifolia Poiret (Wirooks & Plassmann 1999; Bopp 2003). Recently, it has been proposed that the species should be split into H. bicruris, common in western Europe and H. capsincola Denis & Schiffermüller that seems to replace the former in eastern and northern Europe (Hacker 1996).

After nectar feeding and possibly pollination of S. latifolia, a female moth may oviposit in the female flower. After extending its ovipositor it deposits an egg, usually on the ovule but occasionally on other parts of the flower (Brantjes 1976). Directly after hatching, the first instar (L1) caterpillar chews a small hole in the ovary, enters the young fruit, and starts to
feed on the ovules and developing seeds. It will stay in this “primary” seed capsule until it has consumed all the seeds, which usually occurs when the fourth or final fifth (hereafter L4 and L5, respectively) instar is reached. At this stage the caterpillar leaves the primary capsule and starts feeding on other “secondary” seed capsules. The caterpillar first chews a large hole in the top of the secondary seed capsule and then progresses to the seeds. When using capsules that have already opened, but have not yet shed seeds, it chews away several sharp capsule teeth before entering the fruit. Late instar caterpillars are usually too large to fit within these seed capsules, and thus their caudal appendages usually protrude from the capsule during feeding bouts (Biere et al. 2002). Large caterpillars will frequently also take refuge in empty seed capsules during the day. At maturity, on average about four weeks after hatching, the caterpillar leaves the food plant and pupates in the soil. Depending on the time of year the moths either emerge after approximately three weeks, or the pupa enters diapause to overwinter (Elzinga et al. 2002).

Adult moths are active from the middle of May until the start of September, usually in two overlapping generations. Although there are two distinct peaks in oviposition, in early June and August, coinciding with the peaks in flowering of S. latifolia (Biere & Honders 1996), eggs are deposited on flowers throughout the season (Bopp 2003).

Study area

To quantify the parasitoid complex associated with H. bicruris and the life history of component species, we performed a study along 100 km of the rivers Rhine and Waal (the main branch after splitting of the Rhine) in the Netherlands from the German border downstream to the town of Gorinchem. In this area we located all S. latifolia patches, 86 in total, ranging in size from 1 to circa 12 000 plants (Elzinga et al. 2005b)

Patches of S. latifolia occur mainly on river dikes and the higher sandy parts of floodplains, but also in disturbed areas near (brick) factory sites. In many places the vegetation is mown twice a year resulting in grasslands with low vegetation height, but other patches are located within nature reserves with grazing cattle, leading to rougher and taller vegetation. In general, S. latifolia seems to prefer habitats that are relatively open through mowing, grazing and other disturbance. In addition to these natural plant patches in the study area some observations were made on a few hundred plants in the experimental garden of our institute in Heteren, the Netherlands, 25 km from the study area.

Sampling and rearing methods

Over the course of three years (2001, 2002 and 2003), we visited the plant patches during the peak flowering periods i.e. June and August and a few weeks later when most seed capsules were ripe. During these visits we collected all visible caterpillars, i.e. caterpillars not present in primary seed capsules, but on the plant or (partly) hiding in secondary seed capsules. In these and previous samplings, live caterpillars located inside primary seed capsules were never found to be parasitized and were thus not considered (Elzinga, pers. obs., n>100). However, occasionally we were also able to collect parasitoid larvae or cocoons from dead caterpillars located inside primary and secondary seed capsules. In addition, caterpillars and immature parasitoids were collected from several hundred S. latifolia plants located in the garden in 2003.

All collected caterpillars and immature parasitoids were taken to the laboratory and placed in separate vials. The instar stage of every caterpillar was determined by examining
the head capsule dimensions (not possible for all of the dead caterpillars) and it was further assessed whether they were alive, dead or paralyzed, and/or parasitized. If caterpillars were alive and not paralyzed, a small block of artificial diet was added to the vial (Elzinga et al. 2002). When caterpillars neared the end of the fifth stadium, the vial was half-filled with vermiculite to allow either pupation or egression of parasitoids.

The number of parasitoid larvae that developed from each host was counted. All dead caterpillars were dissected to assess the presence of immature parasitoid larvae. Secondary clutch size of parasitoids was determined as the total number of larvae it produced on a host. Brood size was calculated as the number of adults emerging from a clutch. For hyperparasitoids, we use the word “brood” to indicate how many adults emerged from a clutch of primary parasitoids since we could observe hyperparasitoids only as adults. Secondary sex ratios (expressed as the proportion of males in a brood) were determined by counting the numbers of males and females that emerged per brood.

All rearings were performed at 25/20°C and 16/8 h light/dark at 70% humidity except for caterpillars collected in late summer. These were reared at 20/15°C and 8/16 h light/dark to initiate diapause (further referred to as autumn conditions). After 6–8 months at 4°C and 8/16 h light/dark and 70% humidity, the diapausing moth pupae and parasitoid cocoons were placed at 25/15°C and 16/8 h light/dark in order to break diapause.

Identification
Specimens of adult parasitoids were identified by several specialists: C. van Achterberg (Braconidae), M. J. Gijswilt (Chalcidoidea), M. Schwarz (Gelis spp.), L. E. N. Sijstermans (Tachinidae) and K. Zwakhals (Ichneumonidae, except Gelis).

Analyses
For quantification of the parasitoid complex we used only data gathered from caterpillars collected in the natural S. latifolia patches (referred to below as “the field”). For rare species, hyperparasitoids and for life history characteristics we used additional data from caterpillars collected in experimental plant patches near the Waal and from the experimental garden. Correlations between sex ratio and secondary clutch size and brood size were analysed with type III generalized linear models with a quasibinomial distribution and a logit link function (McCullagh & Nelder 1989) in the statistics computer program R (Venables et al. 2005). We tested for over- or underdispersion in sex ratio variance with the Meelis test (Hardy & Cook 1995; Krackow et al. 2002).

Results
Primary parasitoids
In total we were able to collect 1773 caterpillars from the field, mainly living L5 larvae (Tables I and II). Of these, 44.2% were parasitized by various species, 44.8% either developed into a moth or died as a pupa with no signs of parasitism, and 11.0% of all caterpillars were dead or died as a caterpillar without indications of parasitism.

Caterpillars were parasitized by at least 13 different species of parasitoids. Although some differences in the level of parasitism per species were observed between years (Table II), the overall pattern was relatively constant (Figure 1). In total we could identify
seven species of hymenopteran primary parasitoids associated with *H. bicruris* along the river Waal: two gregarious braconid species and five solitary ichneumonid species. A very small percentage of caterpillars (less than 1%) were parasitized by at least four tachinid (Diptera) species. All common species appeared to be present during almost the entire flowering season of *S. latifolia*, the food plant of *H. bicruris* (Figure 2). Below we present more detailed information per species.

### Table I. The fate of *H. bicruris* caterpillars collected as different instars in natural *S. latifolia* patches, including those that were dead when collected. Numbers of caterpillars are given. Between brackets parasitoids that developed after *M. tristis* had emerged.

| Instar 3 | Instar 4 | Instar 5 | Unknown |
|----------|----------|----------|----------|
| Nr.      | %        | Nr.      | %        | Nr.      | %        |
| Total Hymenoptera | 28 | 7.1 | 296 | 96.4 | 1392 | 45.0 | 54 | 96.4 |
| *Microplitis tristis* | 38 | 12.8 | 340 | 24.4 | 28 | 49.1 |
| *Eurylabus tristis* | 14 | 7.1 | 193 | 13.9 | 23 | 40.4 |
| *Bracon variator* | 2 | 7.1 | 24(1) | 12.2 | 34(3) | 2.4 | 23 | 40.4 |
| *Bracon brevicornis* | | | | | | |
| *Ophion pteridis* | 4 | 1.4 | 55 | 4.0 | 2(1) | 3.5 |
| Scambus sp. total | 7 | 2.4 | 2 | 0.1 | 1 | 1.8 |
| Scambus brevicornis | 2 | 0.7 | 2(1) | 0.1 | 1 | 1.8 |
| Scambus buolinae | 1 | 0.03 | | | |
| *Eriergus cernops* | | | 2 | 0.1 | |
| *Hyposoter sp.* | | | 1 | 0.1 | |
| Unidentified | 1 | 0.03 | | | |
| Total Tachinidae | 1 | 0.03 | 10 | 0.7 | |
| *Blondelia nigripes* | | | 2 | 0.1 | |
| *Siphona geniculata* | | | 1 | 0.1 | |
| *Phryxe vulgaris* | 1 | 0.03 | 1 | 0.1 | |
| *Phryxe nemea* | | | 1 | 0.1 | |
| Unidentified | 5(1) | 0.4 | | |
| Nematodes sp. | 1(1) | 0.1 | | |
| Pupated | 12 | 42.9 | 145 | 49.0 | 637 | 45.8 | |
| Dead (no sign of parasitism) | 14 | 50.0 | 61 | 20.6 | 118 | 8.5 | 2 | 3.5 |

*Developed once on a host collected in the experimental garden.*

### Hymenoptera

### Braconidae

*Microplitis tristis* Nees

Most parasitism of *H. bicruris* was caused by *Microplitis tristis* (Braconidae: Microgastrinae), a gregarious koinobiont endoparasitoid. On average 22.9% of the collected *H. bicruris* caterpillars were parasitized by *M. tristis*. Parasitoid larvae feed on the haemolymph of the caterpillars and, at maturity, egress from the host and pupate next to it, usually in the soil, but occasionally in a secondary seed capsule. Because the life cycle of the parasitoid is much
shorter than that of its host (Elzinga et al. 2002), we assume that several overlapping generations occur per year. *Microplitis tristis* diapausas as prepupa in the cocoon if reared under autumn conditions.

**Table II.** The fate of all *H. bicruris* caterpillars collected in natural *S. latifolia* patches over the three years, including those that were dead when collected. Numbers of caterpillars are given. Between brackets parasitoids that developed after *M. tristis* had emerged.

|                | 2001 | 2002 | 2003 | Total |
|----------------|------|------|------|-------|
| Total Hymenoptera | 551  | 529  | 692  | 1772  |
| *Microplitis tristis* | 117  | 168  | 217  | 502   |
| *Eurylabus tristis* | 45   | 81   | 83   | 210   |
| *Bracon variator* | 35(1)| 21   | 27(3)| 83    |
| *Bracon brevicornis* | 1*   |      |      |       |
| *Ophion pteridis* | 14   | 26   | 21(1)| 61    |
| Scambus sp. total | 6    | 2    | 1    | 10    |
| *Scambus brevicornis* | 2    | 1    | 1    | 4     |
| *Scambus buolinae* | 1    | 1    | 1    | 2     |
| *Erigorgus cerinops* | 2    | 0.3  | 2    | 0.1   |
| *Hyposoter sp.* | 1    | 0.1  | 1    | <0.1  |
| Unidentified | 2    | 0.4  |      | 2     |
| Total Tachinidae | 5    | 0.9  | 6    | 11    |
| *Blondelia nigripes* | 2    | 0.3  | 2    | 0.1   |
| *Siphona geniculata* | 1    | 0.1  | 1    | <0.1  |
| *Phryxe vulgaris* | 2    | 0.3  | 2    | 0.1   |
| *Phryxe nemea* | 1    | 0.1  | 1    | <0.1  |
| Unidentified | 5    | 0.9  | (1)  | 5     |
| Nematodes sp. | 1    | 0.2  | (1)  | 1     |
| Pupated | 190  | 34.4 | 290  | 54.9  |
| Dead (no sign of parasitism) | 86 | 15.6 | 40 | 7.6 |

*Developed once on a host collected in the experimental garden.*

![Figure 1](image-url)  
**Figure 1.** The fate of all *H. bicruris* caterpillars collected in natural *S. latifolia* patches. Only the parasitism rate of the most common parasitoid species are shown individually, whereas the others are grouped. Caterpillars that were collected dead or which died without signs of parasitism are assigned to the group Died.
On several occasions \((n=5)\) female parasitoids were observed in the field attacking L5 hosts that were exposed on a secondary seed capsule, either completely or only with the abdomen protruding from the capsule. The wasp was also frequently encountered on primary seed capsules but was never observed parasitizing the caterpillars inside. We assume that it is not able to penetrate through the small hole, that is often blocked by faeces of the \(H. \) bicruris caterpillar, and might be just waiting for the caterpillar to emerge. This corresponds to the observation that the highest percentage of parasitism in the field by \(M. \) tristis was found in L5 instar hosts (Table I), although in captivity the parasitoid is able to attack and develop on all host instars (Elzinga et al. 2002). Furthermore, larger caterpillars are optimal for its development (Elzinga et al. 2003). The secondary clutch size ranged from 1 to 63 with an average of 18.0 \(\pm 0.47\) s.e. (Figure 3A). Although there was a large variation in clutch size, there was no significant difference in clutch size between caterpillars collected as L4 and L5 (\(t\)-test, \(t=-1.287\), d.f. = 365, \(P=0.2\)).

The secondary sex ratio was female biased with an overall value of 0.39 for all sexed adults, significantly deviating from 0.5 \(\left(\chi^2_{0.05,1}=233.9, n=4813, P<0.001\right)\), even though approximately 10% of the broods consisted of only males (Figure 3A). Total eclosion success was 83%. For all-male broods the average eclosion rate was 70%, whereas all-female broods (5%) had an average eclosion rate of only 31%. The largest clutch producing only males was 45 with 36 of these surviving to eclosion. Secondary sex ratio was not significantly influenced by instar (L4 or L5) at which the host was collected (ANOVA, \(F_{1,299}=0.43, P=0.51\)). Clutch size and brood size tended to have a negative effect on the sex ratio (ANOVAs, one outlier removed, \(F_{1,306}=0.064\), Figure 4A, and \(F_{1,309}=0.047\), respectively). Removing all broods with only males yielded only non-significant results. With and without all-male broods, the secondary sex ratio is over-dispersed (Meelis test, \(p<0.001\)), showing that the variance between broods in sex-ratio is larger than binomial.

*Microplitis tristis* has been found in Great Britain in \(H. \) bicruris but also in \(H. \) confusa, \(H. \) rivularis and Hadena indet. (on Dianthus host plants; M. Shaw, pers. comm.). Whereas Nixon (1970) records \(H. \) bicruris, \(H. \) cucubali and two *Plusia* species as hosts for the palearctic *M. tristis*, Shenefelt (1973) lists many host records for the parasitoid, all within the Lepidoptera (including *Hadena* species). Of these only a few, like *Aglais urticae*, occur in our study area besides *H. bicruris*. Our observations suggest that the parasitoid female is strongly attracted to the plant, damaged seed capsules and frass from *H. bicruris* and that newly emerged females in a small vial will easily attack *H. bicruris* caterpillars when offered. Caterpillars of other species (Spodoptera exigua, *Mamestra brassicae*, Chrysodeixis chalcites and *Aglais urticae*), reared in the laboratory, offered in a similar way did not initiate parasitism behaviour \((n=\) approximately five individuals per species) although successful larval development in *C. chalcites* was possible after parasitism was forced (by pressing the abdomen on the host). This was not observed in the other species.
The most important ectoparasitoid present in the system is the gregarious *Bracon variator* (Braconidae: Braconinae). Caterpillars were found either dead or paralyzed and covered with cocoons, larvae or eggs of this parasitoid in 4.9% of the caterpillars collected. Females inject paralyzing venom into the host before oviposition. The larvae then perforate the cuticle with their mandibles and imbibe nutrients from the paralyzed or dead host, before spinning cocoons next to it. Hibernation occurs as prepupa in the cocoons if reared under autumn conditions. *Bracon variator* attacks hosts that are hiding in secondary, but occasionally also primary, seed capsules. In a few cases the paralyzed caterpillar also

Figure 3. Secondary clutch size (all larvae on a host) distribution of *M. tristis* (A) and *B. variator* (B, data from the field and experimental garden combined) in *H. bicruris*. Colours indicate the sex composition of the resulting broods (including broods of one).
contained larvae of *M. tristis*. Except for one case, the latter could not develop and died inside the host. On one occasion, *B. variator* larvae were found developing on a host from which *M. tristis* larvae had already egressed. Host size ranged from L3 to L5 but the highest percentage parasitism by *B. variator* was found in L4 caterpillars (Table I). On several occasions we observed *B. variator* attacking hosts by entering secondary seed capsules containing L4 and L5 *H. bicruris* caterpillars.

Although the average clutch size increased with the instar stage attacked (2.5 for L3 (*n*=2), 5.09 for L4 (*n*=32) and 6.27 for L5 (*n*=118)), this difference was not statistically significant (ANOVA, \(F_{1,150}=3.065, P=0.082\); *t*-test for instar 4 and 5, \(P=0.085\)). Average clutch size for all collected clutches (including those from the experimental garden) was 5.96 ± 0.31 with most clutches within the range of 1–6, although a maximum of 27 was observed (Figure 3B).

The overall secondary sex ratio was 0.34 and was significantly different from 0.5 (\(\chi^2_{0.05}, F_1=96.3, n=941, P<0.001\), Figure 4B). The majority of the broods were single sex (19% of 140 broods of two or more offspring were only male, 46% were only female) especially if clutch size is smaller than six. Overall sex ratio was 0.22 if all-male broods were discarded. The sex ratio did not change significantly with instar (ANOVA, \(F_{1,140}=2.04, P=0.16\)) nor with clutch size (ANOVA, \(F_{1,164}=1.09, P=0.30\), Figure 4B) or brood size (ANOVA, \(F_{1,166}=1.50, P=0.22\)). With and without all male broods, sex ratio was strongly over-dispersed (Meelis test, \(P<0.001\)) indicating a tendency for broods to contain only one sex (Hardy 1992). Total eclosion rate for broods was 88%. Average eclosion rate for all-male and all-female broods was 86% and 87%, respectively.

Many host species in different insect orders have been described for this palearctic parasitoid, such as Diptera (Tephritidae and Syrphidae), Coleoptera and Lepidoptera (Pickard & Rabaud 1914; Freese 1995; Georgiev & Samuelian 1999; Diaconu & Lozan 2000) including *H. bicruris* (*as Harmodia capsincola*) (Shenefelt 1978). Most species appear to be living in confined spaces such as flowerheads, folded leaves or seed capsules.

*Bracon brevicornis* Wesmael

The gregarious ectoparasitoid *Bracon brevicornis* (Braconidae: Braconinae) was found only once, in the experimental garden (1 July 2003). A paralyzed L5 caterpillar contained a clutch of 14 larvae that developed into nine males and four females within a few weeks. *Bracon brevicornis* is known from many lepidopteran hosts in Europe, Asia, Africa and has been introduced in America. It is an important parasitoid of several pest species, but had not been observed as a parasitoid of *H. bicruris* until now (Shenefelt 1978; Jackson & Butler 1984; Temarak 1984).

Ichneumonidae

*Eurylabus tristis* (Gravenhorst)

*Eurylabus tristis* (Ichneumonidae: Ichneumoninae) is a solitary koinobiont larval-pupal endoparasitoid that was observed in 11.7% of the collected caterpillars. The larva of this species remains in the caterpillar as an L1 and completes development only after the host has pupated. In some dead and dissected caterpillars, including a few that had been parasitized by *M. tristis*, larvae of *E. tristis* were observed, in one instance apparently
attacking a second conspecific larva with its mandibles. Diapause occurs in the larval stage in the host pupa when reared under autumn conditions. Occasionally, *H. bicruris* collected as caterpillars early in the season and reared under summer conditions went into diapause after pupation. From some of these pupae *E. tristis* emerged after diapause was broken. Adults were observed attacking L5 hosts that were hiding in secondary seed capsules by

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**Figure 4.** Average sex ratios (proportion males in brood) for different secondary clutch sizes (all larvae on a host) of *M. tristis* (A, averages calculated over two consecutive clutch sizes) and *B. variator* (B) in *H. bicruris*. The dashed line is the correlation between sex ratio and clutch size of *M. tristis* as derived from a generalized linear model on the underlying raw data. The horizontal lines indicate the overall average sex ratios. Numbers on top of the graphs give the number of observations on which the averages are based.
inserting their abdomen through the entrance hole. Most parasitism was found in L5 instar hosts, but some L4 were parasitized as well (Table I). The sex ratio was not significantly different from 0.5 ($\chi^2_{0.05,1}=0.33$, $n=194$, n.s.).

Only four noctuid hosts are mentioned in Taxapad (Yu 1998) for this palaearctic species: *H. bicruris*, *H. albimacula*, *H. perplexa* and an unlikely record on *Panolis flammea*, a pine feeding noctuid. In Great Britain it has been found on *H. bicruris* as well (M. Shaw, pers. comm.).

**Ophion pteridis** Kriechbaumer

*Ophion pteridis* (Ichneumonidae: Ophioninae) is a solitary koinobiont endoparasitoid, which was recovered from 3.4% of the caterpillars. It feeds on host haemolymph but eventually consumes the entire soft tissue of the host before egress. Occasionally it egresses from the host just after pupation, but mostly from L5 hosts just before they pupate. It pupates next to the host in the soil in a brown cocoon with a distinct light brown band. Most parasitism by *O. pteridis* was found in L5 hosts (Table I). Facultative diapause occurs as prepupae in the cocoons. The sex ratio observed from 19 individuals was 0.26 and this was significantly different from 0.5 ($\chi^2_{0.05,1}=6.37$, $n=19$, $P=0.04$).

Brock (1982) describes *Lacanobia (=Mamestra) pisi* and *L. oleracea* as hosts and observes that the parasitoid regularly parasitises night-feeding noctuid caterpillars in seed pods of *Campanula*. However, we assume that this must be a misidentification for *Silene*, as he refers to a few species of *Hadena*, including several observations on *H. bicruris*, all of which do not occur on *Campanula*. Taxapad (Yu 1998) also mentions *Gallopistria juventina* as a possible host. *Ophion pteridis* is a palaearctic nocturnal parasitoid that can be trapped with light traps (Nabli et al. 1999), and is widely distributed and fairly common in the Netherlands (Oosterbroek 1978).

**Erigorgus cerinops** (Gravenhorst)

Two females of the solitary larval-pupal endoparasitoid *Erigorgus cerinops* (Ichneumonidae: Anomaloninae) were found in caterpillars collected as L5 (on 20 June 2003 and 29 August 2003 respectively). The first eclosed from the host pupa within the same summer, the other the following year after diapause. Several noctuid hosts are mentioned in the literature for this palaearctic parasitoid (Schnee 1993; Yu 1998); this is the first published record from a *Hadena* species.

**Scambus** sp.

On nine occasions a *Scambus* species (Ichneumonidae: Pimplinae) was observed as a parasitoid of *H. bicruris*, mostly on L4 but occasionally on L5 hosts (Table I). Two species could be identified, and a few others did not develop into adults. These *Scambus* species are both solitary ectoparasitoids that paralyze their host before oviposition. They spin cocoons next to the carcass, after feeding primarily on imbibed host haemolymph. The two *Scambus* species are both palaearctic but *S. buolianae* has been released as a biocontrol agent in Canada for the European pine shoot moth *Rhyacionia buoliana* (Lepidoptera: Tortricidae) (Leius 1961; Fitton et al. 1988).

*Scambus brevicornis* (Gravenhorst) was collected five times (28 August 2001, 29 August 2001, 26 June 2002, 12 June 2003 (2x)) on paralyzed or dead L4 and L5 hosts. One was found on a caterpillar from which *M. tristis* had already eclosed. All identified specimens were females.
This species has been found in large numbers on caterpillars of the leafroller *Adoxophyes orona* in apple orchards in the Netherlands (Evenhuis & Vlug 1983) but occurs on insect hosts from many different orders such as Coleoptera, Diptera (Tephritidae), Hymenoptera, and many Lepidoptera (Solbreck & Sillén-Tullberg 1986; Fitton et al. 1988; Molau et al. 1989) including *Hadena rivularis* (Yu 1998). It appears that most of these hosts occupy flower heads, folded leaves, seed capsules or other confined spaces.

*Scambus buolianae* (Hartig) was found three times (on 11 September 2001, 10 September 2002 and 3 July 2003) as larvae and all were females. Like *S. brevicornis* this species has a wide range of mostly confined living hosts (Leius 1961; Yu 1998). *Hadena bicurris* has previously been described as a host for *S. buolianae* (Fitton et al. 1988).

**Unidentified species**

We were able to identify a single *Hyposoter* (Ichneumonidae: Campopleginae) female to genus level only. This individual developed within the same season, from a caterpillar collected on 20-6-2003 as L5.

**Diptera**

**Tachinidae**

Several tachinids did not emerge from their pupae for unknown reasons and could therefore not be identified to the species. In total, tachinids contributed to only 0.6% of the observed parasitism (Table II). Most tachinid larvae egressed from L5 hosts (Table I), whereas on three occasions the larvae emerged after pupation of the caterpillar, and on a further two occasions after diapause of the host pupa. Four gregarious koinobiont endoparasitoid tachinid species were identified.

*Blondelia nigripes* (Fallén) (Exoristinae: Blondeliini) was obtained from three hosts. One clutch contained three females, one contained a solitary male, and the third (found in an experimental plant patch near the river Waal) consisted of three females and one male. The parasitized caterpillars were collected as L5 (on 12 June 2003, 3 July 2003 and 7 July 2003 resp.) and the flies emerged several weeks later in the laboratory. *Blondelia* species are equipped with a piercing ovipositor which is used to inject an incubated egg directly into the body of a host (Dowden 1933). *B. nigriceps* is a very polyphagous species attacking numerous Lepidoptera from different groups, mostly glabrous caterpillars, and also occasionally Symphyta larvae (a.o. Tschorsnig & Herting 1994; Ford et al. 2000; Avci & Kara 2002).

Another species collected was *Siphona geniculata* (De Geer) sensu Andersen (formerly *S. cristata* Fabricius) (Tachininae: Siphonini). Three individuals eclosed from one caterpillar, collected as L5 (at 12-6-2003) from which two females emerged a few weeks later. This gregarious species has been associated mainly with large Lepidoptera, particularly Noctuidae (Ford & Shaw 1991; Belshaw 1993; Tschorsnig & Herting 1994; Ford et al. 2000).

*Phryxe vulgaris* (Fallén) (Exoristinae: Eryciini) was recorded twice in *H. bicurris* larvae, one collected as L5 (on 12 June 2003), the other as L4 (on 29 August 2003) from which, respectively, three females and one male, and one female and one male developed; the first clutch egressed from the caterpillar, the second from the host pupa after diapause. It has
been found previously on *H. bicruris* (in Belshaw 1993) and is extremely polyphagous on different families of Lepidoptera that contain relatively large caterpillars (Tschorsnig & Herting 1994). It is also known from the nearctic region (O’Hara 1999).

*Phryxe nemea* (Meigen) (Exoristinae: Eryciini) was recovered from one caterpillar, collected as L5 (at 23 June 2003), from which five females developed after emerging from the host larva. This species is highly polyphagous on exposed caterpillars of many families of Lepidoptera, including *H. bicruris* (Belshaw 1993), although extremely hairy caterpillars seem to be avoided (Tschorsnig & Herting 1994; Ford et al. 2000). *Phryxe* species deposit their eggs on the outside of the host. After hatching shortly after, the larvae then perforate the larval cuticle and burrow into the host.

**Nematodes**

We observed parasitism by nematodes in two caterpillars, of which one had already been parasitized by *M. tristis*. We presume that these large (up to 18 cm long) nematodes were of the genus *Hexamerminis* (Nematoda: Mermithidae) (Wouts 1984), but they could not be identified further.

**Hyperparasitoids**

We found six species of hymenopteran hyperparasitoids associated with the primary parasitoids *M. tristis* and *B. variator*. Nine out of the 33 clutches of *M. tristis* that we collected in the field and in the experimental garden as recently eclosed larvae or as cocoons contained a hyperparasitoid and 21 out of 73 cocoon clutches of *B. variator* (plus two larval clutches). Furthermore, a total of six clutches of *M. tristis*, collected before eclosion from the host, contained a hyperparasitoid.

**Ichneumonidae**

*Mesochorus lanceolatus* Schwenke

*Mesochorus lanceolatus* Schwenke (Ichneumonidae: Mesochorinae), the only true hyperparasitoid in our study, hyperparasitises *M. tristis* larvae when they are still in the host. In total we found six clutches of *M. tristis* parasitized by *M. lanceolatus*. Only three were recorded from the field, whereas the others were recovered from experimental plant plots along the river Waal and in the experimental garden. In all cases the clutches of *M. tristis*, ranging from 14 to 30 individuals, were only partly parasitized so that in addition to broods of 3–28 hyperparasitoids, adult *M. tristis* also eclosed. One of the broods of *M. lanceolatus* contained only males which might indicate oviposition by an unmated female. The other broods contained both females and males. All broods were collected in July or September 2000, 2001 or 2002. Half of the broods diapaused within the cocoons of *M. tristis*.

Host range assessment for most *Mesochorus* species is in its infancy, but all species described for Europe appear to parasitise hosts in the Braconidae, Ichneumonidae and Tachinidae (Schwenke 1999). Often, they seem to be specialized on a primary host species and not on the secondary host, although some hyperparasitize many lepidopteran species. *M. lanceolatus* was described from a single specimen, originating in Sweden, and no previous host records exist (Schwenke 1999).
Gelis spp.

We found two species of hyperparasitoids from the idiobiont genus Gelis (Ichneumonidae: Cryptinae) developing solitarily in cocoons of M. tristis and B. variator.

Gelis agilis (Fabricius) was found in cocoon clutches of both M. tristis and B. variator (on 8 July 2002 and 29 August 2001 resp.). We found three small broods in the natural plant populations; three were obtained from a clutch of 16 and four from a clutch of four M. tristis cocoons and two from a B. variator clutch of four. Also Schwarz and Shaw (1999) observed that G. agilis was found in small broods, usually parasitizing only a small fraction of the clutch of the primary host. At our institute (NIOO-CTE), we have reared this species on Cotesia glomerata cocoons in the laboratory for several years. Unmated females produced small numbers of female offspring, confirming that this species is thelytokous (Schwarz & Shaw 1999). We did not observe any hibernation. They can be extremely long-lived, >2 months at 25°C under laboratory conditions (J.A. Harvey, pers. obs.), and probably overwinter as adults (Schwarz & Shaw 1999). Gelis agilis has been found on many hosts, mainly small Lepidoptera, such as Coleophoridae (presumably as a primary parasitoid) and many Hymenoptera (Lei et al. 1997), including a Bracon sp. on H. bicruris (Schwarz & Shaw 1999).

Gelis hortensis (Christ) was found twice in M. tristis and once in B. variator cocoons from clutches that had been partly parasitized. One parasitized M. tristis clutch (15 Gelis from a M. tristis clutch of 30) was found in a natural plant population (on 26-6-2002) and the other (six from nine), in addition to one on B. variator (one from one), in the experimental garden (September 2002 and August 2003, respectively). Broods consisted of both apterous males and females. Two females (the rest of the brood had emerged before collection) and one male from the brood in B. variator hibernated, which is in contrast to the findings for this species of Schwarz and Shaw (1999), who did not observe any pre-adult hibernation. We were able to rear the species on Cotesia glomerata cocoons in the laboratory for one generation. Parasitism rates for C. glomerata cocoons were very small and only males emerged, indicating that the females had probably not mated successfully. The species has been found parasitizing Lepidoptera, mostly species of Coleophoridae (presumably as a primary parasitoid) and many Hymenoptera, especially Braconidae (as a pseudohyperparasitoid) (Schwarz & Shaw 1999).

Chalcidoidea

All other hyperparasitoids that were found developing on M. tristis and B. variator cocoons belong to the superfamily Chalcidoidea. The three identified Chalcidoidea are palaearctic species (Noyes 2001) and were mentioned in a recent species list for the Netherlands (Gijswijt 2003).

Baryscapus endemus (Walker) (Eulophidae: Tetrastichinae) was the most abundant pseudohyperparasitoid. In the field it was found twice, once on a clutch of M. tristis (12 September 2001) and once on B. variator (14 September 2001). In the garden we observed it twice on M. tristis and 12 times on B. variator (collected in July, August or September 2002 and 2003). Although most hosts were collected as cocoons, hosts were twice collected as larvae (B. variator and recently emerged M. tristis), suggesting that it is an endoparasitoid. Broods ranged from one to five individuals, often parasitizing host clutches only partially. All specimens (n=35) appeared to be females, suggesting thelytokous reproduction. Almost all broods hibernated (one only partially). This species has been recorded from various hosts in different insect orders (Coleoptera, Hemiptera,
Hymenoptera and small Lepidoptera) and as a hyperparasitoid on hymenopteran parasitoid species, including several braconids (Noyes 2001).

*Pteromalus vibulenus* (Walker) (Pteromalidae: Pteromalinae) was found once in the field on cocoons of a clutch of *M. tristis* (28 August 2001) and four times in the garden on cocoons of *B. variator* (August and September 2001 and 2003). Once, two individuals emerged from one host cocoon, showing facultative gregarious behaviour. Broods ranging from one to four either consisted of males or were of mixed sex. Hibernation occurs when the insects are maintained under autumn conditions. *Pteromalus vibulenus* has been found on parasitoid species and on many species of primary hosts (Coleoptera, small Lepidoptera, Hymenoptera) and is cited several times as a hyperparasitoid of *Apanteles (= Cotesia) glomeratus* on *Pieris brassicae* (Graham 1969; Noyes 2001).

*Pteromalus chrysos* Walker (Pteromalidae: Pteromalinae) was observed once in the field developing in cocoons of *B. variator* (1 August 2002). The clutch of five hosts was partly parasitized (brood of four) and adults emerged without diapausing. This species has been identified previously from tachinid and hymenopteran parasitoids including several *Apanteles* (*sensu lato*) species and other Braconids and from many primary hosts (Coleoptera, Diptera, Hymenoptera and small Lepidoptera) (Graham 1969; Noyes 2001).

**Discussion**

*The parasitoid complex associated with* *H. bicruris*

Figure 5 provides an overview of the parasitoid complex associated with *H. bicruris* as observed in our study. We found that 44% of all collected *H. bicruris* caterpillars were parasitized, but because collecting the caterpillars may have reduced the time that they were vulnerable to parasitism, the natural parasitism rate may be higher. Four species, *M. tristis, E. tristis, B. variator* and *O. pteridis*, are by far the most prevalent parasitoids of *H. bicruris*. The other species of parasitoids were observed only once or a few times, and were absent from collections some years.

Several sources list additional hymenopteran parasitoid species associated with *H. bicruris* and other *Hadena* species (Shenefelt 1980; Yu 1998), but the reliability of many of these records is doubtful (see next section). Other tachinids that have been mentioned as parasitoids are *Epicampocera succincta* (Exoristiniae: Eryciini) (Ford & Shaw 1991) and

![Figure 5](image-url)

Figure 5. The parasitoid complex as observed on *H. bicruris* in the Netherlands along the river Waal. Solid lines show the most important parasitoid species. Bold names indicate possible specialist species.
Compsilura concinnata (Exoristinae: Blondeliini) (Hammond & Smith, 1953 in Belshaw 1993). None of these records come from Dutch source populations and might suggest regional differences in the composition of parasitoid complexes. As H. bicruris is widespread in Europe it would be necessary to study the parasitoid complexes in other countries to provide further information about possible regional differences. Because many other Hadena species have similar lifestyles (i.e. feeding on seed-capsules of Caryophyllaceae) it would be interesting to compare their parasitoid complexes with that of H. bicruris.

For several species of Lepidoptera it has been shown that egg parasitism is an important mortality factor (Hirose 1994). Although we collected several hundreds of H. bicruris eggs on different occasions and reared caterpillars from them, we could find no evidence for egg parasitism. The eggs of H. bicruris are usually found on the ovary deep in the calyx of the flower, offering a possible spatial protection from egg parasitoids. However, for the related H. compta on the plant Dianthus sylvestris, it has been observed in Switzerland that the parasitoid Exetastes rufipes (Ichneumonidae: Banchinae) uses its prolonged ovipositor to reach the egg or newly hatched caterpillar (Erhardt 1989). As we never collected pupae of H. bicuris from the soil, we do not know whether and to what extent pupal parasitism occurs.

Level of specialisation of the primary parasitoids on H. bicruris

Determining the level of specialization of a parasitoid on a particular host is extremely difficult. It is impossible to test whether all species occurring in a region are potential hosts for a parasitoid. Therefore one often has to rely on literature describing observations of a parasitoid developing on a certain host species. However, there are many objections and problems to abstracting host ranges from other literature (Shaw 1994). Among the main objections are the numerous misidentifications and the uncertain status of names of both host and parasitoid species, the biased sampling efforts towards pest and easy to sample host species, and the equal weight given to regular and “freak” associations (Shaw 1994). For example, a closer study on Cotesia (=Apanteles) glomerata, a braconid related to M. tristis, revealed that 49 out of 57 reports of host species appeared to be incorrect (Shaw 1982).

Some general observations can, however, be made. Koinobiont endoparasitoids (with the exception of tachinids, see Belshaw 1994), are generally seen as relatively specialist parasitoids (Shaw 1994). Due to their intimate contact with the host tissue, which requires in most cases adaptation to the host immune defence system and regulation of host growth and behaviour, it is generally believed that strong coevolution between hosts and parasitoids has been very important for these species (Whitfield 1994). However, physiological constraints are not only believed to limit current host range, but also host searching behaviour is often highly adapted to a specific host species or group (Askew 1994).

Recent studies have investigated the genetic structure of a microgastrine (Braconidae) parasitoid on different host species. It appeared that Cotesia species on Melitaeini actually consist of genetically different clades specialized on different host species, suggesting that host specialisation might be even more common than originally thought, especially for koinobiont endoparasitoids (Kankare & Shaw 2004).

Many parasitoids (but especially idiobiont ectoparasitoids) that are considered as generalists and thus capable of developing on many different insect species, or even orders, are actually specialized on a certain habitat characteristic or an aspect of host ecology, such as hosts living concealed in a plant structure (Shaw 1994). Many of the parasitoid species
observed on *H. bicruris* in this study might be considered as generalists. This is especially true for those taxa that were encountered only rarely (e.g. *Scambus* sp.), the tachinids and others that are highly polyphagous on different insect taxa. The *Scambus* species, and also the much more abundant *B. variator*, seem to be specialized on hosts that live concealed in plant tissues (Shaw 1994), which can also be inferred from the relatively long ovipositor that these species possess. *Ophion pteridis* has been recovered from several noctuid species but is regularly found on *H. bicruris* (Brock 1982) and might be considered to be an oligophage, specialised on a limited number of noctuids. Based on its behaviour and its numerous host records on *Hadena* sp., we think that *M. tristis* is a specialist. Also the records for *E. tristis* indicate it is specialized on *Hadena* species.

Summarizing, we think that *H. bicruris* is the only host in the region for *Microplitis tristis* and *Eurylabus tristis*, and it is an important host for *Ophion pteridis* and *Bracon variator*. All of the other parasitoids probably attack alternative hosts in the region that are much more important in maintaining their numbers.

**Hyperparasitoids**

In some multi-trophic systems hyperparasitism has a major impact on the population dynamics of the primary parasitoid (Lei et al. 1997; van Nouhuys & Hanski 2000). In our system around a quarter of the recovered clutches of *M. tristis* and *B. variator* appeared to be hyperparasitized. We did not observe one particular species responsible for most hyperparasitism of *M. tristis* but the most abundant hyperparasitoid in the garden on *B. variator* was *B. endemus*. Because *B. variator* always pupates in seed capsules, it is probably much more vulnerable than *M. tristis* which usually pupates in the soil. How important hyperparasitism is for the population dynamics of these parasitoids remains unclear.

All collected hyperparasitoids are highly generalistic, with the exception of *M. lanceolatus*. Although host ranges of *Mesochorus* species are not well documented it has been suggested that most of them depend quite heavily on the secondary host, i.e. in host searching/acceptance they home in on the Lepidopteran host, rather than on their tachinid or hymenopteran primary hosts (Schwenke 1999). The host ranges of the pseudo-hyperparasitoids, *Gelis* spp. and Chalcidoidea, are very wide and they can potentially attack most parasitoid species that produce small cocoons or pupae. *Gelis* species may also act as primary parasitoids or as hyperparasitoids of hyperparasitoids (Schwarz & Shaw 1999).

**Effects of the parasitoids on the plant–host interaction**

The moth *H. bicruris* is generally considered to be a parasite of its host plant *S. latifolia* as the effects of pollination by the adult moth do not outweigh the detrimental effects through seed feeding (Brantjes 1976; Bopp 2003). Several studies mention the possibility that due to the high rate of parasitism the number of seeds destroyed by the herbivore hosts might decrease (Biere et al. 2002; Bopp 2003; Elzinga et al. 2003) and even to such an extent that the positive effect of pollination by the adult *H. bicruris* might counteract the negative effects of herbivory by the moth’s offspring. This would lead to mutualism or commensalism, in which the plant and the moth both benefit from each other (Kephart et al. 2006). However, our data on parasitism do not support this idea.

First, only a small percentage of parasitism is caused by idiobiont parasitoids (*B. variator, Scambus* sp.) which arrest host growth. The other parasitoids are koinobionts and allow the
host to continue feeding and growing for variable periods after parasitism. Although for *M. tristis* it has been experimentally observed that caterpillars feed less when parasitized, this has only been shown for clutches much smaller than the natural clutch sizes (Elzinga et al. 2003). Even if parasitoids reduce food consumption by *H. bicruris*, most parasitism occurs in the latest stages of the caterpillar when one or more seed capsules have already been destroyed. *Eurylabus tristis* may have little or no impact on food plant consumption by its host as the larva develops only after pupation of the caterpillar. Also *O. pteridis* probably does not cause a substantial decrease in the number of destroyed seeds as it emerges just prior to pupation (or even just after).

From this study it is clear that most parasitism occurs in L4 or L5 hosts and that only a very limited number of hosts are attacked as L3 or earlier. Only *B. variator* appears to access its hosts occasionally in a primary seed capsule, as we observed L3 caterpillars parasitized by this species twice. Ovipositions by *B. variator* and *E. tristis* were observed on L4 and L5 caterpillars that were seeking refuge in empty secondary seed capsules. These capsules have a large entrance opening at the top through which *E. tristis* can insert its abdomen and *B. variator* can enter completely. When feeding from secondary seed capsules the abdomen of *H. bicruris* is often exposed while the head is concealed in the seed capsule. During this period the caterpillar is probably most vulnerable to attack from tachinids that need to be in close contact with the host to oviposit and for parasitoids with short ovipositors, like *M. tristis*.

We conclude that, even in the presence of a high parasitism rate, the relationship between *S. latifolia* and *H. bicruris* probably does not shift from parasitism to commensalism or mutualism (Kephart et al. 2006) because the parasitoids do not substantially decrease the food consumption of *H. bicruris*. Whether the parasitoids have a positive impact on the plants through a decrease in the number of adult moths in the next generation will depend on whether parasitism results in a decrease in the number of deposited moth eggs per plant.

**Clutch size and sex ratio of the main parasitoids of *H. bicruris***

In *M. tristis* and *B. variator*, secondary clutch sizes vary considerably and are largely independent of host size. *Microplitis tristis* produces an average clutch size of approximately 18 offspring. This is very close to the optimum clutch size for *M. tristis* in large *H. bicruris* hosts under laboratory conditions, as has been shown in a previous experiment, where individual offspring weight and survival were compared for different clutch sizes (Elzinga et al. 2003). However, there is considerable variation in clutch size which is poorly understood. Experiments have shown that host size did not influence clutch size (Elzinga et al. 2003) and that parasitoid age could only cause a reduction in clutch size if the parasitoid lives abnormally long (Elzinga et al. 2005a).

One of the reasons for smaller than optimal clutch sizes might be that a parasitoid is unable to complete oviposition because the host, in defending itself, disturbs the female during oviposition (Ikawa & Suzuki 1982). On the other hand, larger clutch sizes might be caused by multiple parasitisms either by the same female or by a conspecific (Gu et al. 2003). In experimental rearings under favourable conditions, we have never observed clutches larger than 28 (Elzinga, pers. obs.), suggesting that the large clutches (up to 63) found in the field are the result of superparasitism.

*Bracon variator* produces an average clutch size of six, but again, a large variation was observed. However, competition for food greatly affects offspring size of *B. variator*. When clutch size is large, small parasitoids emerge whereas small clutches can produce individuals
that are much bigger (Elzinga, pers. obs.). The consequences for individual fitness of size differences are not known, but many studies of other parasitoid species show that small individual females have shorter lifespans and produce fewer eggs than larger conspecifics (e.g. Visser 1994). As in M. tristis, the large clutches might be the result of occasional superparasitism.

Parasitoids are expected to produce different offspring sex-ratios depending on their mating strategy. In general, species in which mating frequently occurs between siblings (Local Mate Competition) are expected to show a female biased sex-ratio (Hamilton 1967; Godfray 1994). In contrast, species that mate randomly are more frequently expected to produce an equal sex-ratio (Fisher 1930; Hardy 1994). In general, gregarious species are thought to have a higher degree of sibling mating than solitary species due to a high chance of mating within a brood. Indeed, female-biased sex ratios are very common in gregarious hymenopterans or solitary species attacking gregariously living hosts (Godfray 1994). Our data do not show otherwise; E. tristis, a solitary parasitoid, shows an almost exactly equal number of male and female offspring, in contrast to the gregarious B. variator and M. tristis that clearly show female-biased sex ratios.

However, there was a clear difference in the distribution of sex ratios across individual broods between these two gregarious parasitoids. Micoplitis tristis mostly produced mixed broods of males and females. The larger number of single sex male broods may be explained by a small percentage of unmated females that produce unfertilised haploid eggs that develop into males. Bracon variator on the other hand more frequently produces single-sex broods, including many all-female broods. Not only are small clutches of up to six individuals almost all single sex, but also most of the larger clutches are. Laying single-sex clutches may incur different types of benefit to a female parasitoid (Hardy 1992). First, it is possible that survival and development of siblings is better in single-sex clutches than in mixed clutches. However, our data on clutch survival do not show lower survival of mixed broods. Therefore a second explanation might be more likely. In two close relatives of B. variator, B. hebetor and B. serinopae, but also in other Hymenopteran species, it is known that sex is determined by the segregation of several alleles at a single sex-determination locus (Godfray 1994; Antolin et al. 2003). In species where sex determination is linked to heterozygosity at sex loci (so called complementary sex determination), outbreeding may reduce the probability of producing unfertile diploid males (Antolin et al. 2003). Although we do not know whether B. variator possesses a similar sex determination system, the many single-sex broods strongly suggest that this species avoids inbreeding. The fact that several large broods consist of both males and females may further suggest that these clutches are a product of superparasitism.

If inbreeding is indeed avoided by B. variator, then the overall female biased sex ratio is unexplained (Heimpel 1997). For B. hebetor it has been suggested that female-biased offspring may compensate for the production of male offspring by un-mated females (Ode et al. 1997), but this would not explain why the overall sex ratio is lower than 0.5. Other explanations suggest that higher intra-brood competition for males or a higher mating competition for males due to lower male dispersal may have favoured evolution toward a female biased sex ratio (Ode et al. 1996, 1998).

Conclusions

At least 13 species of both hymenopteran and tachinid species of primary parasitoids parasitize almost half of the caterpillars of H. bicurris, although 95% of the parasitism is
caused by only four hymenopteran species. Although it is difficult to show specialization of a parasitoid species on a particular host, the two most abundant species of parasitoids, *M. tristis* and *E. tristis* are likely to be, at least locally, specialists. The other species will probably have alternative hosts in the region.

We found six species of mainly generalist hyperparasitoids on *M. tristis* and *B. variator*. Only *Mesochorus lanceolatus* might be a specialist on *M. tristis*. The other five species are all well known to be generalistic.

Three of the most abundant parasitoids are koinobionts that kill the caterpillars at a late stage and will have only limited direct effect on seed herbivory. It is therefore unlikely that parasitoids will shift the parasitic relationship of the pollinating *H. bicruris* with *S. latifolia* to a more mutualistic one.

Both *B. variator* and *M. tristis* exhibit a large variance in clutch sizes and offspring sex allocation. Only for the former species can clutch size be partly explained by variation in size of *H. bicruris* hosts, but the other variance remains unexplained. Secondary sex ratio in the gregarious species *M. tristis* and *B. variator* is female biased, in contrast to the solitary *E. tristis* which has a sex ratio of 0.5. *B. variator* produces many single sex broods, suggesting that inbreeding is rare in this species.

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