SHORT COMMUNICATION

Siblicidal behaviour by larvae of the gregarious parasitoid *Cotesia vanessae*

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Abstract

Contrasting life histories distinguish solitary from gregarious parasitoids. Females of solitary species typically lay one egg in a host; when more than one parasitoid is present in the host, larvae will kill their rivals so that only one parasitoid completes development. Females of gregarious species typically lay multiple eggs in the same host with the resultant larvae co-existing to complete development. Here we provide an unusual report of siblicide by larvae of a gregarious parasitoid; i.e., *Cotesia vanessae* (Reinhard) (Hymenoptera: Braconidae) developing in noctuid caterpillars (Lepidoptera). Siblicidal behaviour has not previously been reported with larvae of gregarious Braconidae. We speculate that this behaviour reflects a trade-off between the finite amount of resources within the host available for larval development, and selection to optimize use of these resources. 'Flooding' the host with eggs allows the female to use the finite resources of the host to their fullest extent, regardless of host size. This strategy also may allow the female to overwhelm the host's immune system to enhance survival of her progeny in otherwise marginal host species. It further may enhance the ability of the female's progeny to competitively exclude the larvae of conspecific females or larvae of other parasitoid species co-occurring in the host. Siblicide allows for self-regulation of brood size when host resources are insufficient to support egg-to-adult development of all eggs initially laid in the host.

Keywords

Trichoplusia ni, competitive exclusion, siblicide, host-parasitoid interaction, Braconidae, Noctuidae

A finite amount of resources are available within a host to support parasitoid development. Gregarious parasitoids typically lay multiple eggs per host, whereas solitary parasitoids typically lay one egg per host. Resource competition arises when a parasitoid lays multiple eggs in the host during one ovipositional bout, when conspecific gregarious or solitary parasitoids parasitize the same individual host multiple times (superparasitism), or when multiple species oviposit in the same individual host (multiparasitism).

Competition can take various forms along a continuum defined by extremes of scramble competition versus contest competition (Nicholson 1954). In the former, the resource is more or less equally partitioned among all contestants. They all may survive when there is a surfeit of resources, but all may die when resources are limiting. In the latter, resource distribution is unequal with 'winners' and 'losers'. Solitary parasitoids display an extreme form of contest competition. In cases of superparasitism, they will kill even siblings (siblicide) with only one parasitoid surviving to complete development (Mayhew and van Alphen 1999; Pexton and Mayhew 2002). In contrast, gregarious parasitoids tend towards scramble competition. They are physically tolerant of each other with multiple individuals surviving to emerge from the host (Dorn and Beckage 2007) and siblicide is rare (but see Bartlett and Ball (1964), Bartlett and Medved (1966), and Grbić et al. (1992)).

Here we report high rates of siblicide among larvae of the gregarious parasitoid *Cotesia vanessae* (Reinhard) (Hymenoptera: Braconidae). Comprised of both sexual and parthenogenetic populations (Stefanescu et al. 2012), this species parasitizes caterpillars of certain Nymphalini (Nymphalidae) and Noctuidae (Lepidoptera) (Hervet 2017). Females lay multiple eggs in a single oviposition bout that usually lasts only a few seconds. The developing larvae feed on their host's haemolymph and fat tissues before egressing en masse from the host's sides prior to pupating (Hervet et al. 2014). We have observed anywhere from one to 250 parasitoids emerging from one individual host, and averages of between 32 and 148 adult parasitoids produced per host, depending upon the host species (Hervet 2017).

In previous experiments using a parthenogenetic population, we assessed a range of lepidopteran species as potential hosts for *C. vanessae* by parasitizing early 4th instar caterpillars. Female *C. vanessae* were placed in contact with the caterpillar until a single bout of oviposition was observed. The parasitized caterpillars were then reared on an artificial diet (Hervet et al. 2016) until parasitoid emergence. Depending upon the lepidopteran species, none, some, or many parasitoids emerged. Caterpillars without parasitoid emergence either died or completed development (see Hervet (2017) for full details).

To better understand the factors that affect parasitoid emergence, we dissected parasitized caterpillars of three species. Dissections were performed up to 11 days after exposure to parasitism. For these dissections, the head and the last rear abdominal segments of each caterpillar were excised and the digestive system removed. Haemolymph was then squeezed from the hemocoel onto a microscope slide, diluted with one drop of saline solution, topped with a coverslip, and examined using a compound light microscope (400×). Some caterpillars dissected immediately after parasitism had large fat bodies that clouded the haemolymph and hindered observations. Thereafter, cater-

pillars were held for 2 days without food prior to dissections. This method allowed for easy viewing of egg and larvae stages (Fig. 1).

The species selected for dissections were cabbage looper (*Trichoplusia ni* (Hübner)) (*n* = 20), true armyworm (*Mythimna unipuncta* (Haworth)) (> 10), and the corn earworm (*Helicoverpa zea* (Boddie)) (Lepidoptera: Noctuidae) (*n* = 17). Our previous experiments identified these species to be 'good', 'medium' and 'poor' hosts, respectively (Hervet 2017). For example, exposure of 30 *T. ni* caterpillars to parasitism resulted in 26 individuals (87%) dying from parasitism and producing an average of 95 F_1 adult *C. vanessae*. The remaining 4 *T. ni* died as larvae without parasitoid emergence. Exposure of 30 *M. unipuncta* caterpillars to parasitism resulted in 6 individuals (20%) dying from parasitism and producing an average of 76 F_1 adult *C. vanessae*. Most (70%) of the remaining *M. unipunctata* caterpillars survived, and some (10%) died as larvae without parasitoid emergence. Exposure of 75 *H. zea* caterpillars to parasitism resulted in three individuals (4%) dying from parasitism and producing an average of 24 F_1 adult *C. vanessae*. Most (75%) of the remaining *H. zea* survived and many (21%) died without completing development.

Our observations on the dissected caterpillars showed both teratocyte production and encapsulation to affect the survival of immature *C. vanessae* (Fig. 1). In each of the three lepidopteran species, eggs were readily detected to document the act of oviposition. Newly laid eggs were similar to those displayed in Yu et al. (2008). Five days after parasitism, teratocytes were observed associated with eggs and neonate larvae in *T. ni* (Fig. 1A, B). Teratocytes are cells produced by the extraembryonic egg membrane and released into the host at the time of egg hatch. They have trophic, immunosuppressive, hormonal, and antimicrobial functions that enhance parasitoid survival (Dahlman 1990). Eggs oviposited into the 'poor host' *H. zea* were encapsulated by host hemocytes within four days (Fig. 1C). Many of these eggs hatched, but the neonate larvae did not develop further; i.e., caterpillars dissected after 11 days contained numerous dead first-instar larvae encapsulated by hemocytes (Fig. 1D). Only one of 17 *H. zea* contained a few live parasitoid larvae in its hemocoel among many encapsulated dead ones. The large size of these surviving larvae indicated that they had successfully evaded host's immune defenses. Encapsulation by the host's hemocytes is a common immune response by lepidopteran larvae to parasitism (Lavine and Strand 2002). Dissection of the 'good host' *T. ni* showed that almost all eggs had hatched within 5 days with no signs of encapsulation. At 11 days there were still a few unhatched eggs, indicating that some eggs failed to hatch, though these eggs were not encapsulated.

Unexpectedly, caterpillars of all three lepidopteran species contained many dead first instar parasitoids that were bisected in the transverse plane (Fig. 1F). Observations of larvae physically attacking each other (Fig. 1G) identify these deaths as an outcome of rival combat. To our knowledge, siblicide in hymenopteran parasitoids previously only has been observed for solitary species (Balduf 1926; Mayhew and van Alphen 1999; Pexton and Mayhew 2002; Salt 1961; Willard 1920; Yu et al. 2008) and for a few species of gregarious Encyrtidae (Bartlett and Ball 1964; Bartlett and Medved 1966; Grbić et al. 1992). Therefore, these observations provide the first evidence of
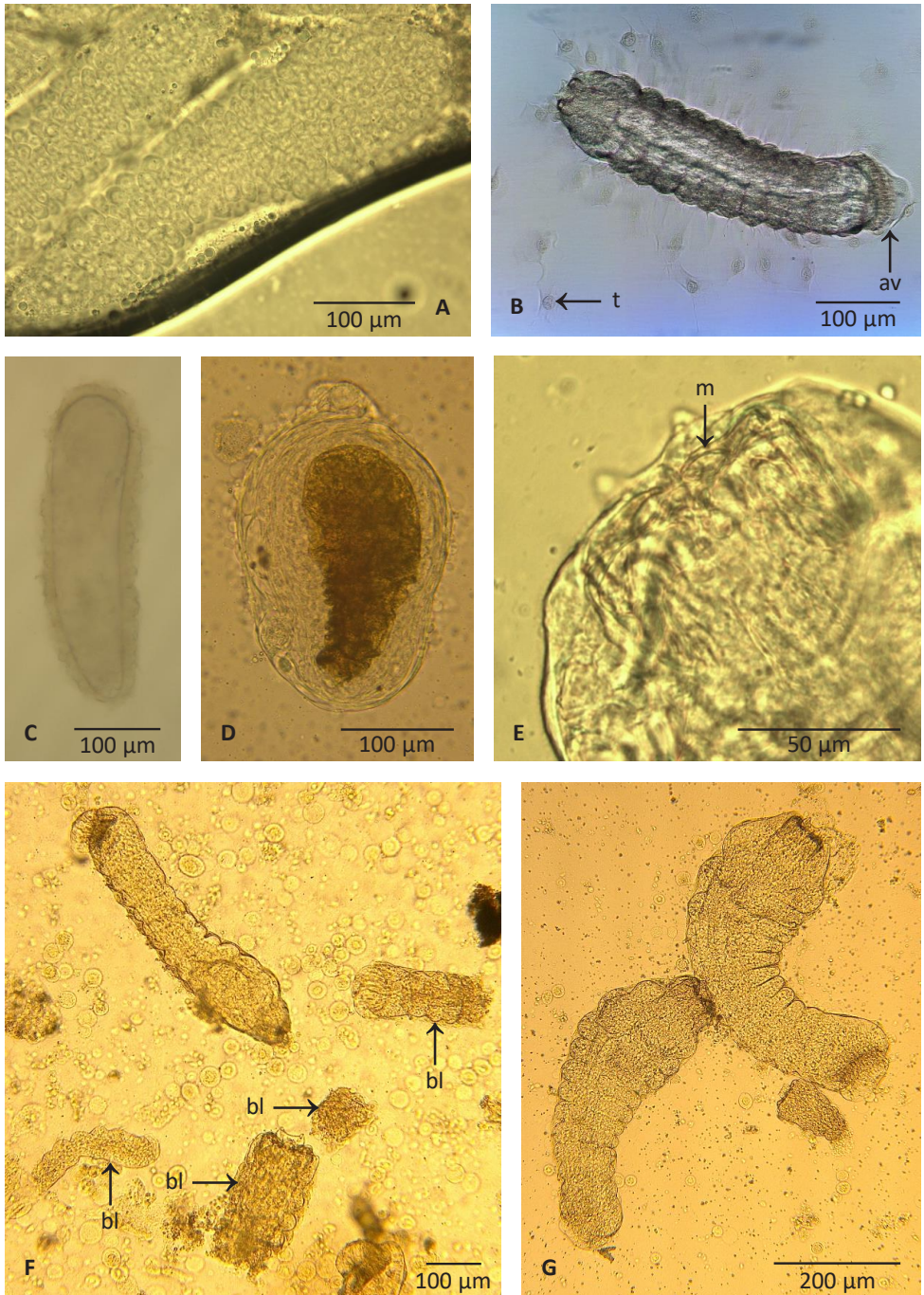
Figure 1. Immature stages of *Cotesia vanessae* in caterpillars of three lepidopteran species. **A** Egg (parts of adjacent eggs visible on left and top sides), with visible extraembryonic membrane made of large cells that will become teratocytes (within *Trichoplusia ni*, five days post-oviposition)

larval siblicide by a gregarious braconid parasitoid. Depending upon whether the larvae of the solitary species use their mandibles to pierce or chew their rivals, rivals are killed by either puncturing or what appears to be messily chewing (Salt 1961; Yu et al. 2008). Larvae of the gregarious *C. vanessae* use their mandibles for chewing, which they seemed to use with an acquired efficiency to neatly transect their rivals. It may be that siblicide is common in other gregarious species of *Cotesia*, but has gone unobserved. Future studies to examine immature parasitoids *in situ* will help clarify this matter. As documented with other gregarious *Cotesia* spp. (Magdaraog et al. 2016), first instar *C. vanessae* have reduced mandibles (Fig. 1E), are vesiculate, and do not have a caudal appendage (Fig. 1).

Gregariousness has evolved multiple times from solitary ancestors within the genus *Cotesia* and in other parasitoid taxa, typically accompanied by a loss of antagonistic behaviour toward siblings and a loss of motility (Mayhew 1998; Michel-Salzat and Whitfield 2004). This negative association between antagonistic behaviour toward siblings and gregariousness is common across parasitoid taxa (Dorn and Beckage 2007), and likely reflects kin selection for tolerance among close relatives (Godfray 1987). The apparent exception to this general rule exhibited by *C. vanessae* therefore seems particularly noteworthy.

Motility of first instar solitary species is typically enabled by a caudal appendage (Harvey et al. 2013). These larvae need to move within the host to find contestants in order to kill them because the hosts of these species generally do not have sufficient resources to enable the development of multiple individuals. The lack of a caudal appendage in first instar *C. vanessae* (Fig. 1) suggests that gregarious development in this species was enabled by a loss of motility. Indeed, previous work suggests that the evolution from solitary to gregarious development in hymenopteran parasitoids is more likely to evolve through reduced larval motility than through reduced sibling rivalry, because the loss of larval motility is less stringent than that for tolerance of other larvae (Boivin and Van Baaren 2000; Pexton et al. 2003).

The laying of supernumerary eggs combined with reduced mobility of antagonistic first instars means that for *C. vanessae* it is not the mother wasp but its offspring that adjust brood size according to the available resources. The existence of this parent-offspring conflict could mean that *C. vanessae* larvae still have to evolve kin tolerance, which would enable female wasps to lay fewer eggs into hosts, all of which could survive to maturity,

Figure 1. *Continued.* **B** Neonate larva with teratocytes (t) (within *T. ni*, five days post-oviposition). Head on the left, anal vesicle (av) on the right, thoracic and first 7 abdominal segments each partly surrounded on their dorsal and lateral sides by a row of cuticular spines projecting backward **C** Egg becoming encapsulated by hemocytes (within *Helicoverpa zea*, four days post-oviposition) **D** Encapsulated first-instar larva (within *H. zea*, eleven days post-oviposition) **E** Front of head (within *T. ni*, eight days post-oviposition). Microscope focused on mandibles (m) **F** First-instar larva (on its side), with four pieces of bisected larvae (bl) nearby (within *Mythimna unipuncta*, seven days post-parasitism) **G** Larva biting a sibling, with fore-half of bisected larva nearby (centre right) (within *M. unipuncta*, seven days post-oviposition). (Photo credit: Photo B by S. Harris, Agriculture and Agri-Food Canada, Saskatoon, SK; all other photos by V.A.D. Hervet.)

but it may also be that this strategy confers an advantage to this species. Laying hundreds of eggs in the same individual in one oviposition bout will generally optimize the use of available resources, regardless of host size. On one hand, large clutches enable the development of large broods in those hosts that allow for it. On the other hand, siblicide reduces scramble competition and acts as a safety mechanism to prevent the death of the whole brood in hosts that do not have sufficient resources for the development of all eggs laid (Hardy et al. 1992). A large number of fighting larvae may further enhance the ability of the female's progeny to competitively exclude the larvae of conspecific females or larvae of other parasitoid species co-occurring in the host (Giron et al. 2004; Harvey et al. 2000). In addition, a greater number of parasitoids within a host may increase their ability to overwhelm the host's immune system (van Alphen and Visser 1990), therefore enabling larvae to complete development in otherwise marginal host species.

It is interesting to speculate on the factors that could trigger larval siblicide. Presumably these include physical or chemical cues associated with the immature parasitoids. Arguably, the likelihood of physical contact or the concentration of chemical cues would be greater in a 'good' host (e.g., *T. ni*) than in a 'poor' host (e.g., *H. zea*); the immune system of the latter killing many first instars. We also note that our observations are based on a parthenogenetic population of *C. vanessae* for which all immatures within the host are essentially clones. Comparison of siblicide behaviour between parthenogenetic versus bisexual populations might prove enlightening. For example, what are the implications for larval siblicide on adult sex ratios in host species of different quality?

Cotesia vanessae has a broad range of lepidopteran hosts within the Noctuidae and Nymphalidae, including many species that are important economic pests of crops (Hervet 2017). Understanding features of host-parasitoid interactions, including larval siblicide within the host, has practical implication for potential use of *C. vanessae* as a biocontrol agent to control these pests. Thus, our unanticipated discovery of siblicidal behaviour is not only relevant for studying basic questions of life history, but also has consequences for applied ecology.

Acknowledgements

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