Using superparasitism by a stem borer parasitoid to infer a host refuge

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Abstract. 1. *Macrocentrus cingulum* Reinhard (Hymenoptera: Braconidae), a parasitoid of the European corn borer, *Ostrinia nubilalis*, and the Asian corn borer, *O. furnacalis* (Lepidoptera: Pyralidae), has high fecundity but has been reported to parasitize a low proportion of host larvae. This was corroborated in field collections: in Hebei (China) and Delaware (U.S.A.), *M. cingulum* parasitized only 15 and 25%, respectively, of hosts collected.

2. Because *M. cingulum* females cannot oviposit through plant tissue, they must parasitize hosts either before they have bored into stalks or while they are near entrance holes, so that at any one time, many *Ostrinia* larvae may be unavailable to *M. cingulum*. This refuge, together with fluctuations in abundance of foraging *M. cingulum* females, may explain why *M. cingulum* parasitizes relatively few *Ostrinia* larvae.

3. To test this hypothesis, levels of superparasitism were measured in the field. Low parasitism resulting from a refuge for host larvae should cause high rates of superparasitism in hosts outside the refuge.

4. Because *M. cingulum* is polyembryonic, the number of parasitoids per host does not indicate the level of superparasitism. Random amplified polymorphic DNA markers were used to determine the number of different genotypes emerging from each host. The resulting frequency distributions were fitted to those expected under random oviposition to estimate the proportion of *Ostrinia* larvae unavailable to *M. cingulum*.

5. In the samples from Hebei and Delaware, the level of superparasitism was much higher than expected by chance if all hosts were available. Fitting the frequencies of genotypes per host to a Poisson distribution, the authors estimated that 74–82% and 69–74% of host larvae were unavailable to *M. cingulum* in these collections, respectively. This means that *M. cingulum* parasitized 60–84% and 82–95% of available hosts in these collections, respectively. These levels of parasitism contrast strongly with the 15–25% found when all hosts were assumed available for oviposition.

6. Genetic distances of *M. cingulum* within and between hosts did not differ, allowing rejection of the hypothesis that high levels of superparasitism resulted from a female laying several eggs in the same host.

7. The hypothesis that *M. cingulum* parasitizes few *Ostrinia* larvae because many larvae are in a refuge explains these data and previously published information better than other hypotheses that have been suggested.

Key words. Biological control, DNA markers, *Macrocentrus cingulum*, *Macrocentrus grandii*, *Ostrinia furnacalis*, *Ostrinia nubilalis*, parasitism, polyembryony, refuge.
Introduction

Macrocentrus cingulum Reinhard (Hymenoptera: Braconidae) (previously Macrocentrus grandit Goidanich; van Achterberg & Haeselbarth, 1983) is a polyembryonic parasitoid of the European corn borer, Ostrinia nubilalis (Hübner) (Lepidoptera: Pyralidae) and the Asian corn borer, O. furnacalis (Guenée), that was introduced into the United States from France and Korea in the late 1920s and early 1930s during extensive importations of parasitoids to control O. nubilalis (Baker et al., 1949). Although Lydella thompsoni Hertig (Diptera: Tachinidae) has been recovered recently in greater numbers in some areas of the U.S.A. (Mason et al., 1994), M. cingulum has been the most abundant parasitoid attacking O. nubilalis in the midwest and the north-east (Peairs & Lilly, 1975; Andreadis, 1982; Romig et al., 1985; Cossentine & Lewis, 1987). Nevertheless, parasitism by M. cingulum is usually patchy and low, only occasionally exceeding 40% (Winnie & Chiang, 1982; Siegel et al., 1987; Anon, 1990; Mason et al., 1994). Each year, parasitism by M. cingulum usually declines in the second generation of O. nubilalis (Siegel et al., 1987), rather than increasing as might be expected. Yet M. cingulum has attributes that would allow it to parasitize many O. nubilalis (Onstad et al., 1991): M. cingulum oviposits in all but first instars of the host, has high fecundity (= 200 eggs/lifetime), and, through polyembryony, each egg can produce many wasps (= 25 progeny/egg) (Parker, 1931). Why does M. cingulum parasitize so few Ostrinia hosts?

In this paper, results concerning an answer to this question are presented. Because M. cingulum females cannot oviposit through plant tissue (Parker, 1931), they must parasitize hosts either before the hosts have bored into stalks or while the hosts are still near the entrances of the holes they have bored. Although dead female M. cingulum do occur in O. nubilalis tunnels, suggesting that parasitoids may occasionally follow hosts into tunnels, the frass plugs produced by polyembryony, each egg can produce many wasps (µ host, has high fecundity (© 1999 Blackwell Science Ltd, Ecological Entomology, 24, 7–12)

Materials and methods

Parasitoid collections

All parasitoids were from parasitized Ostrinia spp. larvae collected from maize plants (Zea mays L.). Ostrinia nubilalis larvae were collected in August 1996 from a field at the University of Delaware. Ostrinia furnacalis larvae were collected in August 1995 from a field in Hebei Province, China. At both locations, host larvae were taken from maize plants in the field and transported to the laboratory in portable coolers. Ostrinia nubilalis larvae were transferred immediately to vials and provided with diet as needed until pupation or parasitoid emergence. Ostrinia furnacalis larvae were shipped to the USDA-ARS quarantine facility at Newark, Delaware, then handled like the Delaware material. All parasitoid cocoons from the same host, referred to as a brood in this paper, were kept together but separate from other broods. Randomly-selected adults from each brood were used to start laboratory colonies, and the remaining adults (eight to thirty-five per brood) were frozen (– 80 °C). From the frozen M. cingulum, twelve were selected from each of a subsample of broods for RAPD analysis. When fewer than twelve were available, all individuals were used. For bisexual broods, the proportion of males to females selected was that which represented the proportion in the total brood most closely.

Conditions for RAPD-PCR

Parasitoid DNA was prepared using Chelex® chelating resin (BIO-RAD Industries, Richmond, California) as described previously (Edwards & Hoy, 1993; Kazmer et al., 1995). Twenty 10-mer primers (kit A, Operon Technologies, Alameda, California) were first screened using five individuals
selected randomly from each laboratory colony. RAPD-PCR was performed in a Perkin-Elmer 9600 thermocycler (Norwalk, Connecticut) using the following reaction conditions: 10 mM Tris-Cl, pH 8.3, 50 mM KCl, 2 mM MgCl₂, 0.16 µM BSA, 0.001% gelatin including 100 µM of each dNTP (Boehringer Mannheim, Indianapolis, Indiana), 0.2 µM primer, and 0.5 units of Taq DNA polymerase (Boehringer Mannheim) in a total volume of 25 µl. Amplifications were run for 45 cycles consisting of 15 s at 94 °C, 15 s at 38 °C, and 1.5 min at 72 °C with maximum ramp speeds between set points.

PCR products (7.5–10 µl) were electrophoresed in a tris-borate-EDTA agarose gel (0.5% SeaKem, 1.0% NuSieve, FMC Bioproducts, Rockland, Maine) for 3 h at 60 mA. The DNA was stained with ethidium bromide, and the bands visualized on a UV transilluminator (Fotodyne Inc., New Berlin, Wisconsin). Digitized images of stained gels were obtained using a CCD camera (VIDICHIP II, Javelin Electronics, Torrance, California) (Kazmer et al., 1995).

From the twenty primers screened initially, four were used in the testing of broods: A13, A10, A03, and A02. All of these primers produced profiles consisting of both monomorphic and polymorphic bands. Monomorphic bands were important in assessing the quality of the amplifications; polymorphic bands were necessary to differentiate broods. Genotype identifications were accepted only if supported by the results of two different primers.

**Estimating the proportion of hosts available**

For all host larvae, the observed number of genotypes per host was measured using differences in sex and RAPD banding patterns. With the assumption that the distribution of eggs among available (unrefuged) hosts was random, a series of expected distributions for number of genotypes per host was generated given various values for the number of hosts available. The expected distribution that best fitted the observed distribution indicated the number of hosts most likely to have been available to *M. cingulum*. To generate each expected distribution, λ (mean genotypes per host) was solved for using the probability density function for the Poisson distribution when the number of genotypes per host was set to 0 (i.e. the probability of escaping parasitism):

\[
p(x = 0) = \frac{\lambda^x e^{-\lambda}}{x!} = e^{-\lambda} = 1 - \frac{n_p}{n_a}
\]

\[
\lambda = -\ln\left(1 - \frac{n_p}{n_a}\right)
\]

where \(n_p\) is the number of hosts found parasitized in each field collection and \(n_a\) is the hypothesized number of hosts available to *M. cingulum*. The frequencies of parasitized hosts expected to have one, two, three, and four or more genotypes under the Poisson distribution were then calculated. Expected and observed frequencies of genotypes were compared using the chi-square statistic. Values of this statistic that were less than the value from the chi-square distribution for 3 d.f. and 95% confidence indicated that the observed distribution was not significantly different from the Poisson distribution.

This model assumes that each host is either accessible or inaccessible. Although all hosts probably experience periods of varying accessibility, only the accessibility of hosts when adult *M. cingulum* are present need be considered. Because *M. cingulum* eggs laid in instars 2–4 do not develop until instar 5 (Parker, 1931), development takes longer when earlier instars are attacked (Dittrick & Chiang, 1982). This variation in development time tends to synchronize adult emergence, and field collections indicate that the majority of *M. cingulum* adults in each generation emerge during a 4- to 5-day period. In addition, under laboratory rearing, adults live < 1 week and field longevity is likely to be less than that in the laboratory. Synchronized development and short adult life mean that *M. cingulum* females are likely to be present foraging for host larvae for only a part of each host generation. For this reason, accessibility of the host population has been treated as a snapshot in time, with a fixed proportion of the hosts in enemy-free space.

**Within- vs. between-brood genetic distances**

If *M. cingulum* females laid multiple eggs in the same host, this would inflate the observed level of superparasitism above that expected from random distribution of eggs among hosts, independently of restrictions in the number of hosts accessible. One way to test whether females laid multiple eggs per host would be to compare genetic distance between individuals within and between broods. If females laid several eggs per host, genetic distances would be lower within than between broods. Therefore, genetic distances were compared within and between broods using RAPD markers.

To avoid problems of multiple counting arising from polyembryony, genetically identical individuals were excluded from the genetic distance calculations. This means that only one parasitoid individual of each genotype from each brood was used. It also means that distances between genetically identical individuals from different broods were not used, so that the analysis was conservative for rejecting the hypothesis that individuals from the same brood but different eggs were more closely related than individuals from different broods. Superparasitism was not frequent enough in the Chinese collection to provide a sufficient number of within-brood comparisons to perform this analysis, so only the data from the Delaware collection were analysed. For each individual in the analysis, the presence (1) or absence (0) of all RAPD markers produced by primers A-13 (six bands) and A-03 (five bands) was scored. A genetic distance matrix was constructed using the algorithm of Apostol et al. (1993) and the RAPDistance computer program (Armstrong et al., 1994). A new data set was then constructed consisting of all the pairwise comparisons, classified by two factors: the
Owain R. Edwards and Keith R. Hopper

Fig. 1. Frequencies of *M. cingulum* broods with various numbers of genotypes. Observed bars are for hosts collected in (a) Hebei, China, and (b) Delaware, U.S.A. Expected bars are frequencies expected with random distribution of eggs among hosts and all hosts assumed to be available. Best fit bars are frequencies expected with random distribution of eggs among hosts and the proportion of hosts available that gave the least difference between expected and observed frequencies.

Sex of the individuals in the comparison (male–male, male–female, female–female) and whether the individuals emerged from the same host (within-host, between-host). The sexes of the individuals in each comparison had to be considered because dominance in RAPD markers together with haplodiploidy in Hymenoptera cause females to appear more closely related than males. *ANOVA* was used to test the fixed effects of sex, host, and their interaction on genetic distance (SAS Institute, 1990). Genetic distance values were arcsin-transformed to achieve homogeneity of variance.

Results

Field collections

Of the 1019 *O. furnacalis* larvae imported successfully into quarantine from Hebei, China, 378 died of disease or unidentified causes. *Macrocentrus cingulum* emerged from 98 (15.2%) of the remaining 641 larvae. Of the 784 *O. nubilalis* larvae collected in Delaware, 327 died of disease or unidentified causes. *Macrocentrus cingulum* emerged from 115 (25.2%) of the remaining 457. From the Chinese and Delaware collections, forty-seven and thirty-two broods, respectively, were chosen for RAPD analysis.

Superparasitism and the proportion of hosts available

In the Chinese sample, twenty-four of forty-seven broods were from superparasitized larvae (Fig. 1a). The frequency of hosts with more than one genotype was greater than that expected by chance when all larvae are available ($\chi^2 = 36.9$, d.f. = 1, $P < 0.001$). The observed frequency distribution of genotypes among hosts fitted a Poisson distribution (95% confidence) with 18–26% (117–164 out of 641) of host larvae available to *M. cingulum* and the rest unavailable (Fig. 2). If only 18–26% of host larvae were available, 60–84% (98 out of 117–164) of those available were parasitized by *M. cingulum*.

In the Delaware sample, twenty-five of thirty-two broods were superparasitized (Fig. 1b). The frequency of hosts with more than one genotype was greater than that expected by chance when all larvae are available ($\chi^2 = 20.1$, d.f. = 1, $P < 0.001$). The observed frequency distribution of genotypes among hosts fitted a Poisson distribution (95% confidence) with 26–31% (121–141 out of 457) of host larvae available to *M. cingulum* and the rest unavailable (Fig. 2). If only 26–31% of host larvae were available, 82–95% (115 out of 121–141) of those available were parasitized by *M. cingulum*.

Within- vs. between-brood genetic distances

Genetic distances within broods did not differ from that between broods ($F = 0.78$, d.f. = 2,1892, $P = 0.38$; Table 1).
This indicates that female *M. cingulum* did not lay multiple eggs in the same host so that such behaviour could not have caused the excess superparasitism observed in field collections. As expected from dominance in RAPD markers together with haplodiploidy in Hymenoptera, genetic distances between males were greater than between females (*F* = 64.6, d.f. = 2,1892, *P* < 0.001; Table 1). Sex (male–male, female–female, female–male) and host (within vs. between hosts) did not interact in their effects on genetic distance (*F* = 0.46, d.f. = 11892, *P* = 0.63).

### Discussion

Several reasons have been suggested for low levels of parasitism by *M. cingulum*, including infection by *Nosema pyrausta* (Siegel *et al.*, 1987; Onstad *et al.*, 1991), foraging using a spreading of risk strategy (Onstad *et al.*, 1991), and refuge hosts (Onstad *et al.*, 1991). However, there are arguments to reject all but the last as the principal factor limiting parasitism.

Onstad *et al.* (1991) and Siegel *et al.* (1987) suggested that the microsporidian *N. pyrausta* may limit parasitism by *M. cingulum*, based principally on the observations that there are increases in *N. pyrausta* infection and decreases in *M. cingulum* parasitism between first and second generations of the host (Siegel *et al.*, 1986, 1987; Onstad *et al.*, 1991). Infection by *N. pyrausta* decreases eclosion, longevity, and fecundity of adult *M. cingulum* (Cossentine & Lewis, 1987). When parasitism is measured by the emergence of larvae from the host (Siegel *et al.*, 1986, 1987) or by dissection, it should be unaffected by *N. pyrausta* infection in the same host generation. If anything, the high levels of *N. pyrausta* infection seen in second generation parasitoids should limit parasitism in the first generation the following year, which is not the observed pattern. Furthermore, *N. pyrausta* infection as the principal factor limiting *M. cingulum* parasitism would not explain high levels of superparasitism, as found in this study. These points argue against *N. pyrausta* as the principal factor limiting *M. cingulum* parasitism.

Spatial spreading of risk is only likely to develop in organisms with small populations (<100) (Gillespie, 1974; Courtney, 1986). Furthermore, spatial spreading of risk among host patches cannot explain disproportional superparasitism. In the field populations of *M. cingulum* in Hebei, China, and Delaware, U.S.A., parasitism was low yet superparasitism was high. The laying of multiple eggs in the same host by a single female cannot have given rise to high levels of superparasitism because *M. cingulum* emerging from different eggs in the same host were no more closely related than those emerging from different hosts, which indicates that *M. cingulum* females did not lay multiple eggs in the same host.

The results described here support the hypothesis that *M. cingulum* parasitism of *Ostrinia* spp. is low because some host larvae are in a refuge from parasitism. If only a small fraction of hosts is accessible to ovipositing females, these hosts are likely to be stung by several females. The frequency distributions of genotypes per host fitted a model of chance encounter assuming that 69–82% of host larvae were unavailable. Given that it is unlikely that *M. cingulum* oviposition is completely random, and that host accessibility probably changes during the *M. cingulum* oviposition period, the actual percentage of hosts in refuges may differ from the estimates of the model. Nonetheless, these results suggest clearly that a large proportion of potential hosts is protected from parasitism by *M. cingulum*, although other factors may also contribute to low parasitism by *M. cingulum*.

A more complete understanding of the impact of host refuge on *M. cingulum* parasitism rates could be obtained by comparing superparasitism between host generations and among host plants. Host refuge may explain lower parasitism rates in second generation hosts, when larger hosts would provide greater refuge. Superparasitism may be lower in plants with narrower stems, such as cotton and *Artemisia* spp. Host plant refuge should be considered when releasing parasitoids as part of biological control programmes, especially when the host plant differs in the collection and release areas or when the level of refuge may change during the season.

*Macrocentrus cingulum* can (1) parasitize several larval instars (2nd to 5th), (2) develop polyembryonically, allowing maximum use of each host parasitized, and (3) develop gregariously with offspring from several eggs emerging from a single host. These attributes allow *M. cingulum* to exploit well the hosts available to it. However, because *M. cingulum* populations appear to parasitize most of the available hosts,
increasing the number of *M. cingulum* adults in the field is unlikely to increase the impact on *O. nubilalis*. On the other hand, it may be possible to increase the impact of *M. cingulum* on *O. nubilalis* by increasing the period of *M. cingulum* foraging during each host generation, e.g. by releasing parasitoids at times when *M. cingulum* is rare or to increase adult longevity by supplemental feeding.

**Acknowledgements**

We thank Charles E. Mason (University of Delaware) for providing *Macrocentrus cingulum* from China, Jeffrey S. Sopa (USDA-ARS, Newark, Delaware) for technical assistance and Fred L. Gould (North Carolina State University), Jay A. Rosenheim (University of California, Davis), Michael R. Strand (University of Wisconsin), and two anonymous reviewers for their comments on the manuscript.

**References**


Accepted 22 May 1998