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Benefits of self-superparasitism in a polyembryonic parasitoid

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Abstract

Macrocentrus grandii is a polyembryonic parasitoid, with embryos that divide asexually within the host (European corn borer, *Ostrinia nubilalis*) to produce broods of clonal offspring. From a biological control standpoint, polyembryony seems advantageous because each parasitized host yields multiple parasitoids with minimal time and egg investment. When we observed *M. grandii* in the field, however, we found that the parasitoid virtually always invested additional time and, if possible, stings into hosts that it had already stung, apparently reducing some of the advantages of polyembryony. We therefore investigated and found support for two potential benefits that can be gained by self-superparasitism in this system. First, multiple stings allowed production of mixed-sex broods: 27% of multiply-stung versus 0% of singly-stung hosts produced mixed-sex broods. Second, multiple stings increased mean parasitoid progeny produced per host, primarily by reducing the chance of complete brood failure. Our results indicate substantial benefit for a second sting, but little benefit for three or more stings, even though *M. grandii* was sometimes observed to invest more than two stings within a single host. However, we also found that within-host larval competition is prevalent, suggesting that supernumerary stings may pay off in competition against conspecific larvae. Such additional investment within a single host would be particularly beneficial when hosts, rather than eggs, are limiting, but would decrease the overall efficacy of *M. grandii* as a biological control agent.

Keywords

clutch size; foraging behavior; host utilization; intraspecific competition; *Macrocentrus grandii*; *Ostrinia nubilalis*; polyembryony; self-superparasitism

Introduction

Concealed hosts such as the European corn borer (*Ostrinia nubilalis* Hübner [Lepidoptera: Crambidae]) have been difficult targets for biological control (Hawkins et al., 1993), in part because few natural enemies are well-adapted to exploiting the brief windows when European corn borer larvae are vulnerable to attack (White and Andow, 2007). Many

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parasitoids released against the European corn borer failed to establish (Baker et al., 1949), and few established species achieve noticeable levels of parasitism (e.g., Mason et al., 1994; Shanklin et al., 1998). One of the most successful, although still limited, parasitoids of the European corn borer is *Macrocentrus grandii* Goidanich (Hymenoptera: Braconidae; also known as *Macrocentrus gifuensis* Ashmead and *Macrocentrus cingulum* Brischke), which generally achieves low to moderate levels of parasitism (e.g., Onstad et al., 1991; Winnie and Chiang, 1982). One unusual aspect of *M. grandii*'s life history that may contribute to the relative success of this parasitoid is polyembryony.

Polyembryony is a developmental lifestyle in which embryos split during development to produce multiple offspring that are genetically identical to one another, but not to their mother (Craig et al., 1997). Polyembryony has evolved independently in a variety of animal taxa, including four lineages of parasitic hymenoptera, and has been suggested to be a particularly advantageous trait for organisms in which the offspring have more information regarding optimal clutch size than the parents (Craig et al., 1997). For *M. grandii*, polyembryony allows a female to efficiently make use of limited windows of European corn borer vulnerability, producing multiple offspring per host with minimal time and egg investment. Consequently, there would seem to be little reason for *M. grandii* to invest additional effort into a host, once it has been stung. Indeed, based on optimal foraging theory, one would predict that self-superparasitism in polyembryonic species should be discouraged evolutionarily as a waste of time and/or eggs (Stephens and Krebs, 1986; Rosenheim and Hongkam, 1996).

Despite these expectations, preliminary observations of *M. grandii* in the field indicated that this parasitoid does apparently self-superparasitize, as do a number of other polyembryonic species (Strand, 1989a;b; Hoffmeister et al., 2000; Zappalà and Hoy, 2004). In at least one case, the primary purpose of the additional egg appears to be production of mixed-sex broods. *Copidosoma floridanum* lays exactly one or two eggs per host: hosts that receive one egg produce unisexual broods, and the vast majority of hosts that receive two eggs produce mixed-sex broods (Strand, 1989b). Mixed-sex broods are advantageous in species capable of inbreeding, because local brother-sister matings upon emergence help to assure offspring reproductive success (Godfray, 1994). Production of mixed-sex broods requires deposition of only two eggs, however, and *M. grandii* has been reported to sting a restrained host as many as 20 times (Parker, 1931).

Of what possible advantage are these additional stings? Two possibilities were suggested by Parker (1931). He inferred that a single egg was only capable of producing 8-10 offspring, and that multiple ovipositions were therefore needed to fully utilize the host and produce broods that averaged 20 individuals. This conclusion, however, was highly speculative because it was based on a distribution of brood sizes that emerged from hosts where the underlying number of stings per host was unknown. When the number of stings was controlled (J. White, unpublished data; present study), we found that a single oviposition was sufficient to produce large broods capable of fully using the host.

An alternative possibility stems from Parker's observations. He dissected numerous recently-stung European corn borer larvae, and when he was able to find *M. grandii* eggs,

they were located indiscriminately throughout the host's body, suggesting that some fraction of eggs may be laid in inappropriate places (e.g., the gut) and fail to produce any offspring at all. Other parasitoid species have been noted to have low rates of success per egg for this reason (Martin, 1914, as cited in Hoffmeister et al., 2000). In addition, eggs can be destroyed by host immune response (van Alphen and Visser, 1990), some proportion of stings may fail to result in egg deposition, and/or some proportion of eggs may be inviable. In any of these situations, multiple stings into the same host would be favored as insurance that progeny are produced, particularly if multiple stings depress the host's immune response, and/or the costs of additional stings are minimal.

The purpose of the present study was two-fold. First, we confirmed the presence of multiple stings by *M. grandii* under field and laboratory conditions into unrestrained hosts, quantifying the cost to the parasitoid in terms of time and presumed egg investment. Second, we evaluated the potential benefit of multiple stings to the same host by manipulating the number of stings per host and quantifying progeny production.

Methods

Study system

The European corn borer feeds solitarily in concealed locations within the host plant and is only intermittently accessible to *M. grandii*, which cannot enter corn borer tunnels or penetrate plant material with its ovipositor (White and Andow, 2007). *Macrocentrus grandii* is attracted to volatile chemicals associated with corn borer feeding (Ding et al., 1989; Udayagiri and Jones, 1992), but due to the inaccessibility of the host, the proportion of these patch visits that result in successful oviposition for the parasitoid is relatively low. We have defined a "sting" within this system as ovipositor contact with a host, followed by a distinctive cocking behavior (Parker, 1931), similar to that described for *Venturia canescens* (Rogers, 1972). Note that while a sting likely coincides with an oviposition, the two are not necessarily equivalent. We have found that the ovipositor cocking behavior is a necessary indicator of oviposition (we never observed parasitoid progeny when the cocking behavior was absent; J. White, unpublished data), but cannot absolutely verify that it is a sufficient indicator of egg deposition. *M. grandii* eggs are very small and deposited indiscriminately within the much larger host (Parker, 1931), so dissecting the host to quantify parasitoid eggs is not feasible. We therefore have restricted our study to an interpretation of stinging behavior, with the explicit realization that some stings may not have resulted in ovipositions. If lack of oviposition were the driving factor in multiple stings, then an interpretation of "self-superparasitism" would clearly be unwarranted. However, the results of our study are highly suggestive that additional post-sting investment into the host is not driven by failed ovipositions, support the supposition that a sting coincides with an oviposition, and indicate that most, if not all, multiple stings are multiple ovipositions, and therefore self-superparasitism.

Post-sting investment by *Macrocentrus grandii*

We evaluated post-sting time investment and self-superparasitism by *Macrocentrus grandii* in both the field and in the laboratory. All wasps used in these experiments either originated

from wild-collected broods or were laboratory-propagated for no more than 2 generations, to minimize the selective influence of laboratory conditions on wasp behavior. Prior to experimental use, wasps were maintained in 0.03m³ cages at relatively low density (10-100 wasps per cage) at ~22°C, 75% RH and 16:8 photoperiod, with constant access to honey and water, but without access to hosts. All wasps were at least 4 days old at the time of experimental use, and had had opportunity to mate.

In the field, we observed the on-patch behavior of 265 individual wasps over the course of two field seasons (2003 and 2004) as described in detail elsewhere (White, 2005; White and Andow, 2007), of which 25 wasps (9.4%) stung a host; these datapoints were considered to be censored observations for the purposes of previous studies (White, 2005; White and Andow, 2007). For the present study, we continued to observe these wasps following the sting to investigate 1) how much additional time they would invest in hosts they have already stung and 2) how many stings they would invest in a single host individual. We used survival analysis to calculate mean post-sting time investments and 95% confidence intervals (Cox and Oakes, 1984), and calculated Pearson's correlation coefficient to determine whether there was any relationship between the pre-sting and post-sting patch tenure of individual wasps.

In the laboratory, we evaluated post-sting behavior by observing individual wasps on small (1.6 cm diameter by 0.4cm deep) patches of corn grit diet (7% w/v corn grits, 2% w/v agar) upon which one 4th or early 5th instar corn borer larva had been feeding for one day. Larvae in these stages are highly suitable for *M. grandii* (J. White, unpublished data). Each host larva was accessible from the surface of the diet at the time of each trial. Each wasp was placed on her patch and observed continuously following the initial host sting. We observed the wasp until she chose to leave the patch (as indicated by flying or walking away from the patch), quantifying the number of stings and total time invested on the patch following the initial sting. In cases where the observation was terminated before the wasp left the patch (n=10/67 observations) we considered the datapoint to be censored. We again used survival analysis to calculate post-sting time investments and 95% confidence intervals (Cox and Oakes, 1984). Following the laboratory trial, a subset of the host larvae were reared out on artificial diet (Andow and Stodola, 2001) to determine the sexual composition of the resulting parasitoid brood.

Brood outcome as a function of stings per host

To evaluate brood outcome as a function of number of stings per host, we allowed *M. grandii* to oviposit into hand-held corn borer hosts. In this way we were able to 1) allow the parasitoid easy access to the host, 2) sufficiently restrain the host to avoid damaging the wasp and 3) decouple number of stings per host from wasp choice by removing hosts before multiple stings could occur or re-presenting the same host for more stings than the wasp might otherwise choose to deposit. We presented a wasp with a series of 4th-5th instar hosts, each of which was randomly assigned to a sting number category (1, 2, 3, 4, 5, or 10). We presented each host to the wasp as often as necessary to achieve the desired number of stings, replaced the host, and continued to acquire stings in the new host. We continued this procedure for as long as we were able with each wasp, up to a maximum of 30 stings. The

wasps often flew away from us at various points during the sequence, but could usually be lured back to hand by re-presenting hosts to them. When a wasp completely lost interest in stinging behavior and/or flew beyond our reach, we terminated her sequence of hosts, categorizing the final host larva based on the number of stings it received. We repeated this procedure with 19 *M. grandii*, for a total of 337 stings in 133 hosts (range = 1 to 11 hosts per wasp). Because we were able to obtain relatively few hosts with 10 stings ($n=7$) we lumped this category together with the 5 sting category. If there was any ambiguity about the number of stings a host received ($n=4$ hosts), the host was eliminated from the experiment. We reared out all host on artificial diet (Andow and Stodola, 2001), categorized the outcome per host as brood, pupa, or deceased, and quantified the total number of adult parasitoid offspring per brood. We then calculated offspring production per host parasitized and per sting invested. Due to the large number of zeros representing failed broods, the resulting data distribution was bimodal and not suitable for parametric analysis. We therefore compared treatments using Kruskal-Wallis one-way ANOVA, followed by Dunn's multiple contrasts to separate means (Siegel and Castellan, 1988). This analysis can not incorporate wasp as a blocking factor, and therefore runs the risk of being unduly influenced by individual wasps, and the data matrix was too sparse to conduct a 2-way Friedman nonparametric ANOVA. Inspection of the data indicated that the major source of inter-female variation was whether or not a female experienced brood failure, and brood failures were spread relatively evenly among the experimental wasps. Ten out of 19 wasps experienced at least one brood failure, and no wasp had all of her broods fail. We therefore feel confident that the Kruskal-Wallis one-way ANOVA provides robust results.

We also evaluated the within-host competitive environment by comparing pre-emergence brood size as a function of number of stings. *Macrocentrus grandii* larvae feed internally for the first 3 instars, emerging and feeding externally for the 4th and final instar (Parker, 1931). Any parasitoid larvae that fail to emerge in synchrony with their broodmates are consumed along with the remaining host contents, and would not be evident in final brood size. We once again used a hand-sting protocol to generate hosts that received either 1 or 4 stings (46 parasitized hosts from 25 different wasps). After 2 wk of rearing on artificial diet, we categorized each host as a pupa, deceased, or still a larva; larval hosts were then dissected and the developing wasp larvae within each host were counted. We compared pre-emergence brood size between treatments using a t-test on log-transformed values. The data on outcome per host (brood, pupa, or deceased) was combined with the previous experiment, and brood failure was compared among treatments using χ^2 analysis. Because we knew the order in which hosts were parasitized by individual wasps, we also used χ^2 analysis to test whether wasp oviposition order influenced host outcome. We compared the outcome of singly-stung hosts depending on whether they were the first host parasitized by a naïve wasp or later within the sequence of hosts parasitized by a wasp. Only one singly-stung host per wasp was used in this analysis: if the wasp produced a singly-stung host first, then that was the host included in the analysis ($n = 22$). If the wasp produced singly stung hosts later in her stinging sequence, then one singly stung host per wasp was chosen at random for inclusion in the analysis ($n = 19$).

Results

Post sting investment by Macrocentrus grandii

Confirming our initial observations, we found that *M. grandii* often attempts self-superparasitism in the field. Of 25 wasps observed to parasitize corn borer larvae in the field, 22 (88%) persisted at the host infestation following the initial sting, and 8 (32 %) were observed to sting the same host individual again. Usually the wasp was only observed to sting the host twice, but in one instance, the wasp proceeded to sting a host larva 12 times. On average, post-sting time investment at a host infestation was comparable to time invested to make the first sting (post-sting=23.0 min, 95% CI = 16.1 < $\times$ < 35.5 min; pre-sting = 20.2 min, 95% CI = 14.1 < $\times$ < 31.2 min), indicating that wasps doubled their temporal cost by persisting on host infestations following a sting. There was no relationship between the pre-sting and post-sting tenure of individual wasps ($r = 0.23$, $n = 25$, $P = 0.266$).

In the laboratory, virtually all wasps given access to readily-accessible European corn borer larvae persisted on their patches following a sting (64/67 = 96%). Of these, 48 wasps (72%) were observed to sting the host again, 37 (55%) persisted beyond the second sting, and 11 (16%) were observed to sting 3 or more times, with a maximum of 7 stings in one host. The mean $\pm$ 1 S.E. stings per host was 2.15 ± 0.15 . The mean post-sting time investment was 20.46 min (95% CI = 16.04 < $\times$ < 27.01 min), ~4X longer than the mean time invested to make the first sting (5.8 min, 95% C.I. = 4.6 < $\times$ < 7.5 min).

Of the 26 self-superparasitized hosts whose outcomes were monitored, seven resulted in mixed-sex broods, and 19 resulted in male broods. In all of our experiments, we never observed a singly-stung larva to produce a mixed-sex brood ($n = 38$ broods), indicating a high probability that multiple stings are needed to produce mixed-sex broods, and corroborating the assumption that no more than one egg is laid per sting (Parker, 1931).

Brood outcome as a function of number of stings

Offspring production per host differed significantly depending on sting number ($\chi^2 = 13.03$, $d.f. = 4$, $P = 0.011$; Fig. 1); offspring number per host was doubled by investing a second sting, but diminished with additional stings such that these treatments did not differ significantly from singly-stung hosts. When these per-host offspring production values were converted to a per sting basis, (presumably reflecting the number of eggs invested), we found that offspring production per sting was equivalent for singly and doubly-stung hosts, but dropped off in hosts that received additional stings ($\chi^2 = 16.22$, $d.f. = 4$, $P = 0.003$; Fig. 1).

The difference in offspring production between singly- and doubly-stung hosts was primarily attributable to greater probability of brood failure in the singly-stung larvae. The proportion of singly-stung larvae that failed to produce broods was ~28%, two or more times greater than the proportion of multiply-stung larvae that failed to produce broods ($\chi^2 = 11.89$, $d.f. = 4$, $P = 0.018$; Fig. 2). This difference among treatments primarily arose from host pupation, which was much lower in multiply-stung than singly-stung hosts; premature host death was relatively low (<8%) across all treatments. Host presentation order did not influence the probability of brood failure. We found that 22.7% of singly-stung hosts that

were the first sting by a naïve wasp failed to produce a brood, whereas 31.5% of singly-stung hosts parasitized by more experienced wasps failed to produce a brood ($\chi^2 = 0.40$, *d.f.* = 1, $P = 0.53$).

In terms of pre-emergence brood size, hosts that received 4 stings contained nearly twice as many wasp larvae as singly-stung hosts ($t_{23} = 3.73$, $P = 0.001$, Fig. 3), even though adult wasp production was relatively comparable for these two treatments (Fig. 1), suggesting that within-host competition was responsible for reduced offspring production at higher sting numbers.

Discussion

Consistent with previous reports by Parker (1931), the polyembryonic species *M. grandii* exhibits self-superparasitism. Under field conditions where host accessibility was low, females usually persisted at host patches following a sting (88% of wasps), but only occasionally were able to sting the host again (32% of wasps). In contrast, a majority of wasps succeeded in multiple stings in the laboratory (72%), where host accessibility was engineered to be higher than field conditions. These results suggest that host availability, rather than wasp propensity, limits self-superparasitism in this system: given opportunity, *M. grandii* will sting European corn borer individuals more than once. Indeed, a majority of the laboratory wasps persisted on a patch beyond their second sting (55%), and when possible stung the host three or more times.

Our data indicate two potential advantages for a second sting. First, deposition of a second egg allows production of a mixed-sex brood. No singly-stung individual ever yielded a mixed-sex brood, whereas 27% of multiply-stung hosts gave rise to mixed-sex broods. Because *M. grandii* broods generally emerge synchronously, are capable of mating immediately after eclosion, and show no signs of inbreeding depression (J. White unpublished data), mixed-sex broods could be advantageous by allowing local brother-sister matings (Godfray, 1994). However, the percentage of multiply-stung hosts that yielded mixed-sex broods was relatively low, possibly because these mothers were unmated: unisexual broods arising from self-superparasitized hosts were inevitably all male. Despite their presumed unmated status, however, these mothers proceeded to invest extra stings, suggesting that there is likely additional motivation for self-superparasitism.

The second, and likely more important, advantage to a second sting in the same host is increased progeny production on a per host basis. We found that a second sting doubled progeny production, on average, relative to singly-stung hosts. Most of this difference can be attributed to reduced probability of brood failure in the doubly-stung hosts: only 9% of doubly stung hosts managed to pupate despite their parasitism, whereas more than 20% of singly-stung hosts pupated. The second sting may therefore act as “insurance” (Rosenheim and Hongkam 1996) against failure of the first sting (Parker and Courtney 1984), either due to host immune response (van Alphen and Visser, 1990), misplaced eggs (Parker 1931), inviable eggs, or failure of egg deposition. It should be borne in mind, however, that host larvae that received only a single sting produced parasitoid broods more than 70% of the time. Given that virtually all parasitoids attempted multiple stings, this result strongly

suggests that multiple stings are not restricted to incidences of failed oviposition as perceived by the parasitoid, and are genuinely acts of self-superparasitism.

It also seems likely that total progeny produced per host may be increased by a second sting, above and beyond compensation for brood failure. For non-zero broods, we found that offspring production tended to be higher in doubly-stung hosts than singly stung hosts. For example, the maximum brood size was higher in the doubly stung category (80 wasps) than in the singly stung category (65 wasps). Note, however, that progeny production in the singly stung category was not limited to a relatively low number (as suggested by Parker, 1931), indicating that a single egg can produce substantial numbers of offspring. Nor is it clear that increased progeny production necessarily increases the mother's fitness, given the usual tradeoffs between offspring number and size when developing on a finite resource, and frequent evidence that smaller individuals have reduced fitness (Godfray 1994). Despite this caveat, production of more offspring, even if they are smaller, is certainly beneficial when contrasted with complete brood failure, and therefore investment in a second sting should be favored evolutionarily if the cost of such investment is relatively low.

We found that the temporal cost of a second sting for *M. grandii* was relatively small. Reproduction in parasitoid wasps can be limited either by time or eggs, or a complex combination of the two factors over the parasitoid's lifetime (Heimpel and Rosenheim, 1998). For *M. grandii*, in which accessible hosts are rare, and eggload is large (Parker, 1931; Olson et al., 2000), a strong argument can be made that time, rather than eggs, is usually the most important currency. The time invested in attempting a second sting was comparable to time needed for the first sting (~20 minutes), and is short relative to the lifespan of this parasitoid (median = 17 days in the lab: Olson et al., 2000), or the time needed to locate another accessible host under all but the highest host densities (White and Andow, 2007). It therefore seems that *M. grandii* has little to lose and much to gain by attempting a second sting into the same host.

But why should *M. grandii* attempt third and subsequent stings, for which little is apparently gained? There is no additional benefit for production of mixed-sex broods, and if anything, progeny production tends to decrease beyond the second sting. We suggest that additional stings may provide an advantage in intraspecific competition with conspecific females. Under field conditions, Edwards and Hopper (1999) found evidence for high levels of superparasitism by *M. grandii*, suggesting strong intraspecific competition for accessible hosts. Our dissection results indicated that more wasp larvae develop within multiply-stung hosts than singly-stung hosts (Fig. 3), but only a comparable number of wasp larvae are able to emerge and complete development (Fig. 1), suggesting that competition within a host may be intense. Multiple stings by a wasp would be favored if they increased the proportion of the successful brood that descended from her rather than her competitors. A similar phenomenon has been demonstrated in solitary parasitoids, particularly when parasitoid density is high (Rosenheim and Hongkham, 1996). It should be noted that if *M. grandii* bases self-superparasitism decisions on perceived competitive environment, then mass rearing in the laboratory (even at the relatively low densities used in these experiments) probably increases the tendency toward self-superparasitism. However, the gregarious

nature of this species means that, even under natural field conditions, encounter with conspecifics and associated behavioral modification is likely common.

In conclusion, our results suggest that self-superparasitism by *M. grandii* may be adaptive in a competitive environment where few hosts are accessible to the foraging parasitoids. We found that *Macrocentrus grandii* rarely passes up an opportunity to sting an accessible host, even if the host has been stung before. As a consequence, the parasitoid population likely concentrates a disproportionate number of stings in the small proportion of the host population that is most accessible (Edwards and Hopper, 1999; White and Andow, 2007). This tendency therefore undercuts *M. grandii*'s abilities as a biological control agent, because intraspecific competition for the most accessible hosts will diminish the effort invested in less accessible hosts, and likely decrease the overall proportion of the host population that is parasitized.

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Fig. 1. Mean ($\pm$ SEM) offspring produced by *Macrocentrus grandii* per host and per sting, as a function of number of stings per host. Among treatments, letters above each column denote significant differences per host, and letters within each column denote significant differences per sting. For each measure, columns that share a letter were not significantly different at $\alpha = 0.05$.

Figure 2.

Proportion of hosts that failed to develop into parasitoid broods as a function of number of stings per host ($\chi^2 = 11.89$, $d.f. = 4$, $P = 0.018$). All hosts developed into broods in the 3 sting treatment.

Figure 3. Mean ($\pm$ SEM) pre-emergence offspring developing within a host, as determined by host dissection. These treatments differed significantly at $\alpha = 0.05$.