

Presence of soldier larvae determines the outcome of competition in a polyembryonic wasp

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Abstract

Soldier-producing polyembryonic wasps are the only social animals that develop as parasites inside the bodies of other insects. Characterizing the kin composition of broods is central to understanding the evolution of the soldier caste in these unique social insects. Here we studied the role of soldiers in mediating the outcome of competition among clones of the polyembryonic wasp *Copidosoma floridanum*. Soldier-producing female clones usually monopolized host resources, whereas soldierless male clones usually coexisted in hosts. Behavioural experiments further indicated that early-emerging soldiers are specialized to combat intraspecific competitors and later-emerging soldiers are specialized for defence against interspecific competitors. Taken together, our results point to intraspecific competition as a major selective force in the evolution of the soldier caste. Our data also present an evolutionary conundrum: given the benefit of soldiers, why are male clones functionally soldierless?

Introduction

A hallmark of eusociality is the evolution of reproductive altruism, in which some individuals in a group reproduce while others function as nonreproductive helpers (Queller, 2000). Eusociality has evolved in several taxa, but its greatest diversity occurs among insects in the order Hymenoptera (bees, wasps and ants), which exhibit haplo-diploid sex determination and usually produce colonies of related individuals (Alexander *et al.*, 1991; Crozier & Pamilo, 1996; Queller & Strassmann, 1998). Polyembryonic wasps in the family Encyrtidae are unique among social Hymenoptera, as well as all other social animals, because they are obligate parasitoids that clonally produce social groups inside the bodies of other insects (Ivanova-Kasas, 1972; Cruz, 1981; Strand & Grbic, 1997; Strand, 2003). Offspring develop into either reproductive larvae that become adult wasps or sterile soldier larvae that

attack and frequently sacrifice themselves defending clone-mates from competitors in the same host (Grbic *et al.*, 1992; Harvey *et al.*, 2000; Donnell *et al.*, 2004; Giron *et al.*, 2004).

Hamilton's rule specifies that altruism is favoured when $rb-c > 0$, where c is the fitness cost to the altruist, b is the fitness benefit to the recipient, and r is their genetic relatedness (Hamilton, 1964, 1972). Conditions favouring the evolution of a soldier caste by polyembryonic wasps would thus include clonal development in a confined space (high r) and/or substantial benefits, b , of defending a nutrient-rich but limited resource (the host) from competitors relative to the costs, c , in lost reproduction (Harvey *et al.*, 2000; Strand, 2003; Giron *et al.*, 2004). In purely clonal groups, soldier production reflects clone-level allocation to defence over reproduction without genetic conflicts of interest among brood members. In contrast, the coexistence within single hosts of offspring from different clones would reduce genetic relatedness and point toward other factors, such as the benefits of cooperative defense, in the evolution of a soldier caste (Hamilton, 1964, 1972; Giron *et al.*, 2004). Characterizing the kin composition of broods is thus central to understanding the evolution of altruism in these unique social insects.

The polyembryonic wasp *Copidosoma floridanum* parasitizes the egg stage of pluriine moths such as *Trichoplusia ni*

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(Strand & Grbic, 1997; Guerrier & Noyes, 2005). After hatching, the host larva develops to its final (fifth) instar over 14 days. During this period, the *C. floridanum* egg clonally proliferates to produce up to 3000 embryos in an assemblage called a polymorula (Ode & Strand, 1995; Strand & Grbic, 1997; Grbic *et al.*, 1998). Up to 24% of these embryos develop into soldier larvae during the early host instars, whereas the remaining embryos develop into reproductive larvae during the host's final instar (Grbic *et al.*, 1992; Harvey *et al.*, 2000; Giron *et al.*, 2004). Reproductive larvae rapidly consume the host and pupate within the remnant host cuticle to form a 'mummy'. Adult wasps emerge from the mummy 12–14 days later. Soldier larvae, in contrast, die of desiccation following consumption of the host by their reproductive siblings. Development of morphologically and functionally distinct castes is mediated by the asymmetric allocation of germ cells to embryos during clonal proliferation (Donnell *et al.*, 2004; Zhurov *et al.*, 2004). Embryos that inherit the germ line develop into reproductive larvae, while embryos that do not develop into soldiers. Soldiers are thus obligately sterile and constitute extreme altruists with no opportunity for reproduction.

C. floridanum produces clonal broods by laying either an unfertilized (haploid) egg that produces males or a fertilized (diploid) egg that produces females (single-sex broods). Hosts containing more than one egg arise in two ways. First, *C. floridanum* females often lay two eggs per oviposition event (Strand, 1989; Grbic *et al.*, 1992; Hardy *et al.*, 1993; Ode & Strand, 1995). When laying two eggs, mated females always lay one fertilized and one unfertilized egg, which results in emergence of both female and male adult wasps (mixed-sex brood). Second, females can oviposit in hosts previously parasitized by another individual (superparasitism).

Female and male eggs produce similar numbers of adult wasps when developing alone in a host (Grbic *et al.*, 1992; Ode & Strand, 1995; Harvey *et al.*, 2000). However, soldier production differs dramatically between the sexes, with female eggs producing large numbers of soldiers throughout host development and male eggs producing only a few soldiers late in host development (Grbic *et al.*, 1992; Ode & Strand, 1995). This effective lack of soldier production by males occurs regardless of whether males develop alone in a host or in the presence of related or unrelated females (Grbic *et al.*, 1992). Its consequence in mixed-sex broods is that almost all soldiers are females, which eliminate many of their brothers to produce a strongly female-biased sex ratio in the emerging adult wasps. Some males from mixed-sex broods also usually survive to mate with their sisters or to disperse and mate with nonrelatives (Grbic *et al.*, 1992; Ode & Strand, 1995).

These data collectively indicate that different kin classes of *C. floridanum* occur in single hosts and that potential intraspecific competitors range from full siblings (brother/sister) to unrelated wasps. Prior studies also

clearly indicate that soldiers distinguish clonemates from nonrelatives. However, it is unclear whether this results in complete or only partial exclusion of competing clones, and if the outcome of competition differs in hosts containing soldier-producing females vs. soldierless males. Soldiers also play an important role in interspecific competition (Cruz, 1981; Harvey *et al.*, 2000), but whether the soldier caste has become specialized for defence against conspecific competitors is unknown. To address these questions, we used genetic markers, manipulative laboratory experiments and field data to assess the role of *C. floridanum* soldiers in host-resource competition. Our results suggest that intraspecific competition is central to the evolution of a soldier caste, while also raising the question of why males are functionally soldierless.

Materials and methods

General methods

A laboratory culture of *C. floridanum* was established from 150 broods collected in Tift County, Georgia in 2003. The culture was maintained as a large, randomly mating population using *T. ni* as the host as previously described (Strand, 1989). We used a single polymorphic allozyme locus (*glucose-phosphate isomerase* [*Gpi*], EC number 5.3.1.9) as a marker to determine the clonal composition of wasp broods. Electrophoresis of the enzyme extracted from individual adult wasps was conducted in 14% horizontal starch gels and GPI banding phenotypes were visualized by histochemical staining as outlined by Shoemaker *et al.* (1992). An initial survey of 71 females, each from a different field-collected brood from Tift County, Georgia, identified six putative *Gpi* alleles, three of which were common (*Gpi*⁵⁴, *Gpi*¹⁰⁰ and *Gpi*¹²⁰; frequencies of 0.246, 0.528 and 0.197, respectively). Mendelian inheritance of the putative allelic products of *Gpi*, a requirement for its use as a marker for clone identification, was supported by: (i) haploid male wasps always produced a single staining band; (ii) heterozygous females always produced three staining bands in a 1 : 2 : 1 intensity ratio (as expected for a dimeric enzyme such as GPI); (iii) bands other than the two expected (see below) were never observed in experimental broods; and (iv) the proportions of putative genotypes in the field sample conformed to proportions expected under Hardy–Weinberg equilibrium (Fisher's exact test, $P = 0.185$). We established inbred isofemale lines from the Tift County population by mating female wasps homozygous for *Gpi*⁵⁴, *Gpi*¹⁰⁰, and *Gpi*¹²⁰ to hemizygous brothers bearing an identical allele.

We determined the number and sex of eggs laid in a host by observing oviposition behaviour (Strand, 1989; Harvey *et al.*, 2000). Mated females usually lay one female egg or two eggs (one female and one male) per oviposition event to produce all-female or mixed-sex broods, respectively, while unmated females lay only

unfertilized eggs that produce all-male broods. By adjusting the number of host/wasp encounters, the brood type (single-sex or mixed-sex) and superparasitism status of any given host was controlled to produce broods of known sex and *Gpi* genotype.

Experiment 1: Outcome of competition in simultaneously superparasitized hosts

Our first experiment compared the outcome of competition in hosts simultaneously parasitized by two wasps of different genotype that laid either one female and one male egg, two male eggs, or two female eggs. After parasitism, hosts were reared on artificial diet and the emerging wasps were immediately frozen at -80°C . Thirty randomly selected individuals were then genotyped from most broods with 400 wasps per brood genotyped in selected instances. We summarized the skew in clonal membership of the wasps in each brood by calculating a monopolization index, Q , as outlined in Ruzzante *et al.* (1996). Q ranged from zero, when each female contributed equally to the parentage of adult wasps that emerged from a brood, to one, when all such emerging wasps were from only one of the two possible clones.

Experiment 2: Outcome of competition in sequentially superparasitized hosts

To assess the effect of residency on the outcome of competition, hosts were parasitized by one wasp followed by superparasitism by a second wasp of different genotype 12 or 24 h later. In these trials, both the resident and intruder clones were females. Wasps were allowed to emerge from each host and 30 randomly selected individuals were genotyped to estimate Q as outlined for Experiment 1.

Experiment 3: Genotyping of field-collected broods

We compared the genetic composition of broods from the field to broods in our controlled laboratory experiments by collecting *T. ni* larvae from soybean and cotton fields in Tift County, Georgia during August–November, 2004. Hosts were returned to the laboratory and reared on artificial diet, with those that produced *C. floridanum* mummies maintained until wasp emergence. We then genotyped 30 randomly selected wasps at *Gpi* from 17 all-female and 15 all-male broods and calculated monopolization indices as described above. Unlike our laboratory studies, these Q estimates measured only the variance in *Gpi* genotypes and not necessarily the variance in abundance of different wasp clones. This is because different clones of the same sex could have the same genotype despite having different parents (nondetection error; Pedersen & Boomsma, 1999).

Experiment 4: Effects of *Gpi* genotype on timing of soldier emergence

Prior studies indicated that soldiers eclose throughout development of female broods such that the number of soldiers progressively increases as the host caterpillar grows (Strand & Grbic, 1997; Harvey *et al.*, 2000; Corley *et al.*, 2005). Precisely when the first soldier(s) ecloses, however, is unknown. To address this question and its possible impact on intraspecific competition, female wasps of different *Gpi* genotype were allowed to oviposit one female egg into host eggs that were 0 h (newly laid), 12 h, or 24 h old, and cohorts of these host eggs ($n = 10$) were dissected thereafter at specific intervals. We then determined the location of the eggs laid by different wasps in superparasitized hosts by feeding newly emerged wasps 0.001% acridine orange (AO) in a 10% sucrose solution. This dye fluorescently labels eggs in the wasp's ovaries that can then be seen after oviposition into the host (Strand *et al.*, 1990). Two AO-labeled wasps of different *Gpi* genotype were allowed to simultaneously oviposit in a newly laid host egg. The location of each parasitoid egg was then determined immediately after oviposition and 60 h later when the host embryo was within 12 h of hatching. Images were captured using a Q imaging camera mounted on a Leica epifluorescent microscope.

Experiment 5: Behavioural assays using first and later-emerging soldiers

To determine whether soldiers recognize and attack competitors, we conducted previously developed *in vitro* assays (Giron *et al.*, 2004; Giron & Strand, 2004). In no-choice assays, a newly emerged first soldier of known genotype was placed in the assay well with a conspecific, unrelated clonal embryo mass. The number of soldiers that attacked the unrelated clone after a 1 h period was then recorded. In choice assays, first soldiers ($n = 47$) and soldiers from second instar hosts ($n = 50$) were placed into the bioassay chamber with an unrelated embryo mass (conspecific competitor) and an egg from the parasitoid wasp *Microplitis demolitor* (heterospecific competitor). The numbers of soldiers that attacked the two types of competitors after a 1 h period were then recorded. The body lengths and head capsule widths of first soldiers and soldiers collected from second instar hosts (both derived from female clones) were measured using an ocular micrometer on a Leica stereomicroscope.

Results

Experiment 1: Soldier-producing female clones monopolize host resources

As previously reported (Grbic *et al.*, 1992; Ode & Strand, 1995), simultaneously parasitized hosts containing one

female and one male egg (mixed-sex brood) and two male eggs produced similar numbers of adult wasps (>1000 per brood) (data not presented). However, almost all wasps emerging from mixed-sex broods were females, whereas near equal proportions of male wasps of each genotype emerged from most all-male broods. Accordingly, monopolization indices (Q) for mixed-sex broods clustered about 1.0 (Fig. 1a), while all-male broods included a majority of estimates <0.5 (Fig. 1b), with distributions of Q values differing significantly between the two brood types (one-tailed permutation test with 1000 data resamplings; $P < 0.0001$). These outcomes indicated that soldier-producing female clones always outcompeted soldierless males, whereas male clones usually coexisted.

We then examined the outcome of competition in hosts containing two soldier-producing female clones.

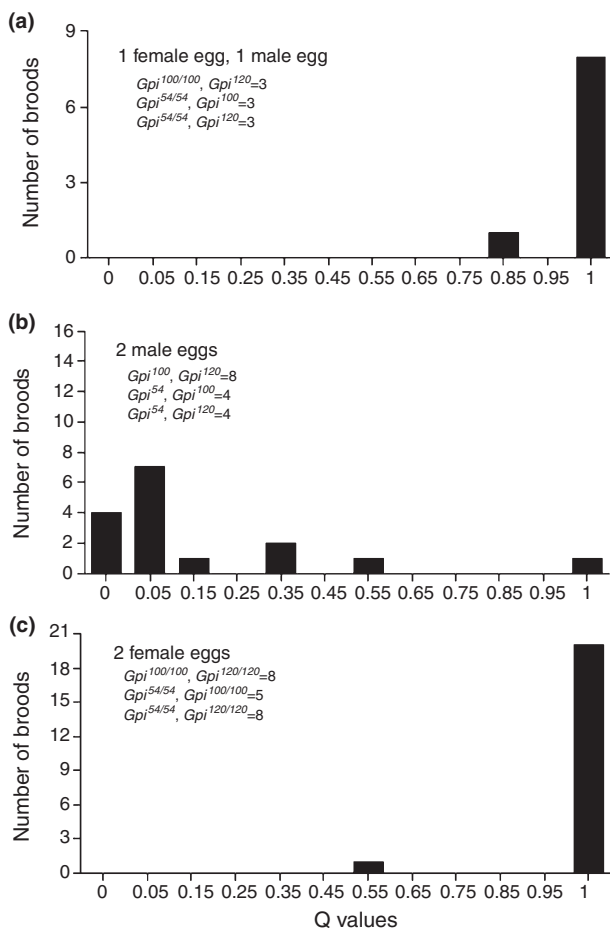


Fig. 1 Monopolization indices, Q , calculated for superparasitized hosts containing one female and one male egg (a), two male eggs (b), or two female eggs (c) produced by allowing two wasps of different Gpi genotype to simultaneously oviposit. The number of broods analysed is shown for each combination of genotypes in each treatment.

Again, more than 1000 wasps emerged per brood but, surprisingly, most broods contained only a single genotype, resulting in a preponderance of estimated Q values of 1.0 (Fig. 1c). This indicated that even though both clones could potentially produce soldiers, one clone usually completely outcompeted the other.

Analysis of these data in relation to genotype of the competitors revealed a second remarkable pattern. Competition between homozygous Gpi^{100} and Gpi^{120} clones resulted in half the broods producing only $Gpi^{100/100}$ wasps and the other half producing only $Gpi^{120/120}$ wasps, corresponding with the expectation that clones of these two genotypes had an equal chance of monopolizing the host (two-tailed binomial test; $P = 0.27$). In contrast, competition between homozygous Gpi^{54} and Gpi^{120} clones resulted in 75% of the broods producing only $Gpi^{120/120}$ offspring and competition between homozygous Gpi^{54} and Gpi^{100} clones resulted in 80% of the broods producing only $Gpi^{100/100}$ offspring, a disparity in survivorship that was significant when $Gpi^{54/54}$ clones were compared with the other genotypes pooled (two-tailed binomial test; $P = 0.035$). Thus, $Gpi^{54/54}$ clones were at a disadvantage in direct competition with the other two genotypes.

Given the large size of *C. floridanum* broods, we considered the possibility that offspring of both female clones actually emerged from some hosts in this experiment but that our sample size of 30 wasps was inadequate to detect a low level of survival of the inferior competitor (mostly $Gpi^{54/54}$ clones). Thus, we genotyped 400 wasps from each of two broods containing the clonal combinations $Gpi^{100/100}$ and $Gpi^{120/120}$, $Gpi^{100/100}$ and $Gpi^{54/54}$, and $Gpi^{120/120}$ and $Gpi^{54/54}$. In every case, all 400 wasps were the same genotype, and in no case was this genotype $Gpi^{54/54}$.

Experiment 2: Resident female clones outcompete intruders

A total of 14 broods were analysed from hosts parasitized by a wasp that laid one female egg (resident clone) followed by a wasp of different genotype that laid a second female egg (intruder clone) 12 h (seven broods) or 24 h (seven broods) later. In all cases, the wasps that emerged were from the resident clone. The resident clones for six of these broods were $Gpi^{54/54}$, while the resident clones for the other eight were $Gpi^{100/100}$ ($n = 4$) and $Gpi^{120/120}$ ($n = 4$). This indicated that a temporal advantage shifted the outcome of competition completely in favour of the resident clone regardless of genotype.

Experiment 3: Female and male broods from the field also exhibit competitive asymmetries

Our laboratory experiments indicated that soldier-producing female clones almost always monopolize hosts, whereas male clones usually can coexist within single

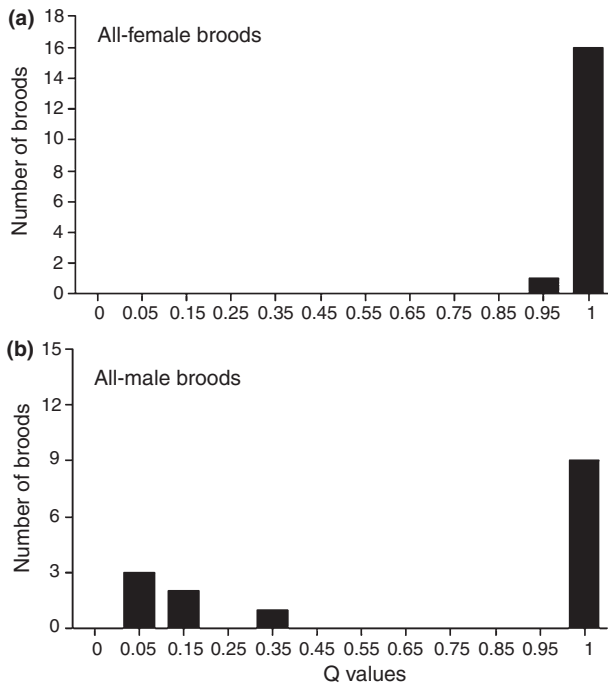


Fig. 2 Monopolization indices, Q , for all-female (a) and all-male (b) broods collected from the field, based on *Gpi* genotypes.

hosts. We found a similar dichotomy in the field. Almost all female broods (96%) produced offspring of one genotype, whereas only 60% of male broods did (Fig. 2). The remaining 40% of male broods produced wasps of two genotypes in relatively even proportions. The resulting distributions of Q values for female and male broods differed significantly (one-tailed permutation test with 1000 data resamplings; $P < 0.001$).

Experiment 4: Timing of first soldier emergence varies by *Gpi* genotype

Given that *C. floridanum* produces thousands of embryos over a 14 day period, we reasoned that total elimination of a competing clone was more likely possible early in development, when only a small number of competitor embryos were present, than later, when large numbers of embryos and soldiers have distributed themselves throughout the host (Grbic *et al.*, 1996; Strand & Grbic, 1997; Donnell *et al.*, 2004). If so, the timing of first soldier emergence from a clone is likely critical in determining the outcome of competition. Dissection of hosts parasitized at 0, 12, and 24 h by wasps of unknown *Gpi* genotype revealed that all host eggs parasitized at 0 h contained a single soldier by 74 h, while all hosts parasitized at 12 and 24 h contained a single soldier at 86 and 96 h, respectively (all times are intervals from host oviposition; see Fig. 3a). This indicated that female clones produce a single soldier in 70–75 h regardless of

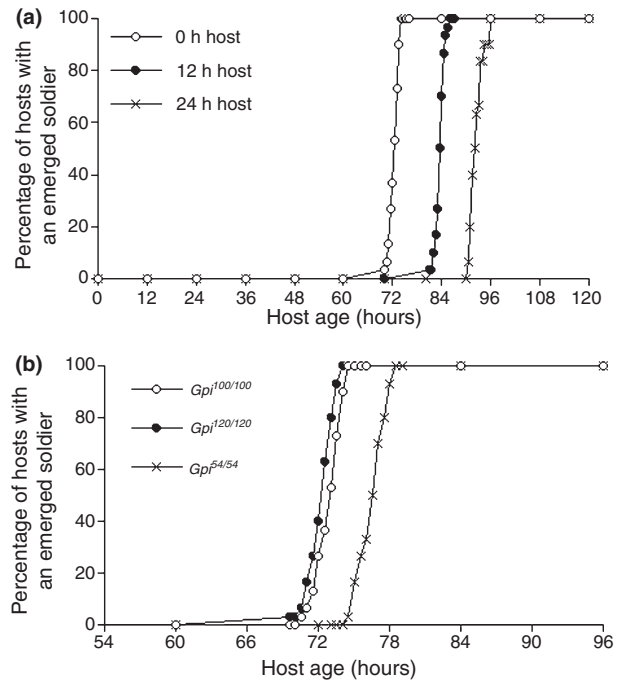


Fig. 3 First soldier emergence during host development. (a) Emergence of first soldiers from female clones of unspecified *Gpi* genotype oviposited into 0 h (newly laid), 12 h, or 24 h old host eggs. (b) Emergence of first soldiers from female clones with the genotypes *Gpi*^{100/100}, *Gpi*^{120/120}, or *Gpi*^{54/54} oviposited into a 0 h old host egg.

the age of the host egg at oviposition. When 0 h old host eggs were parasitized by wasps from the homozygous *Gpi* lines, all hosts containing *Gpi*^{100/100} and *Gpi*^{120/120} clones produced a first soldier by 74 h, whereas hosts containing a *Gpi*^{54/54} clone produced soldiers by 76.5 h (Fig. 3b). A Kruskal–Wallis test indicated that timing of first soldier emergence differed significantly with *Gpi* genotype ($n = 30$; $P < 0.0001$). A subsequent Dunn's multiple comparisons test further indicated that first soldiers emerged at similar times from *Gpi*^{100/100} and *Gpi*^{120/120} clones ($P > 0.09$), but soldiers emerged significantly later from *Gpi*^{54/54} clones compared with the other two genotypes (both $P < 0.0001$). Oviposition experiments using AO-labelled *C. floridanum* revealed that wasps usually laid eggs in different locations in superparasitized hosts (Fig. 4a). As the host embryo developed, however, wasp eggs almost always co-localized to the host's prothorax (Fig. 4b). This localization occurred regardless of wasp genotype and resulted in wasp clones being in close proximity to one another at the time that a first soldier emerged.

Experiment 5: First soldiers preferentially attack conspecific competitors

We compared the attack behaviour of 60 newly emerged (<1 h) first soldiers from *Gpi*^{100/100}, *Gpi*^{120/120} and *Gpi*^{54/54}

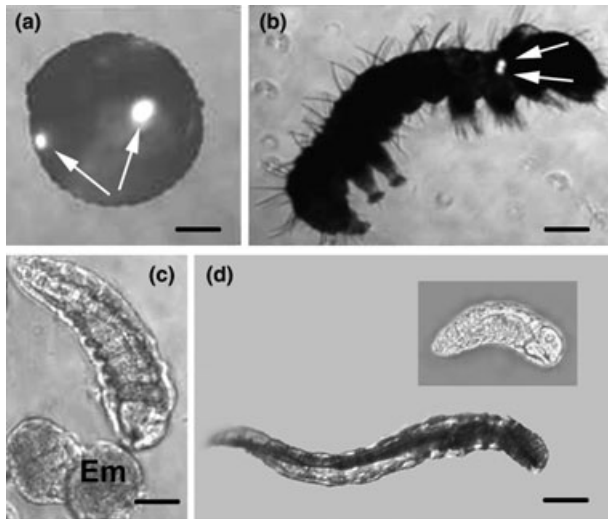


Fig. 4 Locations of *Copidosoma floridanum* eggs in hosts, and soldier behavior and morphology. (a) Superparasitized host egg containing two newly laid *C. floridanum* eggs labelled with acridine orange (arrows). Scale bar = 40 μm . (b) Superparasitized host embryo at 60 h post-parasitism. Note that the two wasp eggs have now localized to the prothorax of the host (arrows). The host embryo was removed from the chorion for ease of visualization. Scale bar = 100 μm . (c) Newly emerged first soldier attacking *C. floridanum* embryos (Em) from an unrelated clone during an *in vitro* assay. Scale bar = 70 μm . (d) First soldier that emerged from a female clone (inset) compared to larger soldier that emerged later in host larval development. Scale bar = 100 μm .

clones toward nonkin using an *in vitro* bioassay. Approximately 75% of the soldiers of each genotype attacked the competitor clone within the 1 h bioassay period, with no significant differences among genotypes ($\chi^2_2 = 0.001$; n.s.) (see Fig. 4c). Given that: (i) female resident clones always outcompete intruders derived from later ovipositions; (ii) first soldiers of each genotype exhibit the same attack behaviour; (iii) $Gpi^{54/54}$ first soldiers emerge later than soldiers from $Gpi^{100/100}$ and $Gpi^{120/120}$ clones; and (iv) $Gpi^{54/54}$ clones are usually outcompeted by $Gpi^{100/100}$ and $Gpi^{120/120}$ clones in simultaneously parasitized hosts, these results strongly suggest that timing of oviposition and first soldier emergence strongly influence the outcome of intraspecific competition.

Many other parasitoid species also parasitize the plusiine hosts attacked by *C. floridanum* (see Lee & Heimpel, 2005). The majority of these heterospecific competitors, like the braconid wasp *Microplitis demolitor*, are solitary endoparasitoids that oviposit in host larvae and produce aggressive larvae that readily fight for host resources (Price, 1974; Askew & Shaw, 1986; Harvey *et al.*, 2000; Strand, 2000). Given that *C. floridanum* produces a single soldier prior to hatching of the host egg as well as many soldiers during later development, we asked whether the soldiers produced at different

times have become specialized for intra- or interspecific defence. Morphological measurements revealed that first soldiers ($n = 47$) were significantly shorter (0.29 ± 0.06 mm) than soldiers that emerged from second instar hosts ($n = 50$) (1.25 ± 0.04 mm) (*t*-test; $t = 13.6$, $P < 0.05$), although head capsule widths were similar between the two soldier types (73 ± 3 μm vs. 72 ± 2 μm , $t = 0.3$, n.s.) (Fig. 4d). In choice assays between an unrelated *C. floridanum* clone and an *M. demolitor* egg, a significantly larger proportion of first soldiers attacked the conspecific competitor ($n = 50$; two-tailed binomial test, $P < 0.01$). In contrast, later-emerging soldiers exhibited a slight but significant preference for attacking the heterospecific competitor ($n = 50$; two-tailed binomial test, $P = 0.049$).

Discussion

Although kin selection theory is central to the modern study of social evolution (Foster *et al.*, 2006), comparative studies indicate that no simple relationship exists between levels of within-group relatedness and the evolution of altruistic helper castes (Alexander *et al.*, 1991; Queller & Strassmann, 1998; Queller, 2000; Perrin & Lehmann, 2001). Clonal development in a confined space combined with the ability of soldiers to distinguish clone-mates from nonrelatives suggest a central role for kin selection in the evolution of a sterile soldier caste by polyembryonic wasps (Giron *et al.*, 2004; Giron & Strand, 2004). However, polyembryonic wasps also share similarities with social species in which kin and nonkin coexist in confined domiciles and benefit mutually from cooperative defence (Stern & Foster, 1997; Abbot *et al.*, 2001; Cooney, 2002; Duffy *et al.*, 2002; Roux & Korb, 2004). Distinguishing between the relative importance of kin selection and mutualism in the social evolution of polyembryonic wasps thus requires knowledge of the genetic composition of broods.

Our laboratory experiments show that intruder offspring almost never survive in hosts already occupied by soldier-producing females, strongly suggesting a substantial role for kin selection in soldier evolution and a minimal role for mutualism. This conclusion is also supported by our field data, which almost always identified only a single genotype in female broods. The coexistence of clones of different genotype in a significant proportion of all-male (soldierless) field broods suggests that superparasitism occurs and that intraspecific competition for hosts in the wild is potentially intense.

Consistent with intraspecific competition being a major force in the evolution of a soldier caste in polyembryonic wasps is our finding that early emergence of soldiers is also critical to the outcome of competition. Yet, continued risks from interspecific competitors as the size and value of the hosts increases apparently also has favoured the production of additional, larger soldiers that have become specialized as executioners of other parasitoids.

Our serendipitous finding that *Gpi*^{100/100} and *Gpi*^{120/120} clones produce first soldiers significantly earlier than *Gpi*^{54/54} clones, and that clones with the former two genotypes generally outcompete those with the latter genotype, suggests that soldier developmental rates have a strong genetic basis, including one or few genes of major effect, and that these rates are likely under selection. While the benefits of early soldier production in intraspecific competition are clear, these benefits may be outweighed in some circumstances by costs such as decreased numbers of reproductive larvae. Evolutionary biologists have long debated the adaptive significance of allelic enzyme polymorphisms in natural populations, with some allozymes appearing neutral to natural selection and others differing functionally in ways that can affect fitness (Avisé, 1994). Prior studies of other insects and invertebrates have frequently identified *Gpi* as an example of the latter, with different genotypes exhibiting variation in enzyme kinetic and thermal stability properties that correlate with adaptation to particular microhabitats (Watt, 1994; Dahlhoff & Rank, 2000; Rand *et al.*, 2002). As a metabolic enzyme, it is possible that *Gpi* could directly affect developmental processes like soldier developmental time and that it could also have quite distinct pleiotropic effects in different castes and life stages (e.g. flight performance of adults; Watt, 1983).

Given the benefits of soldiers, perhaps the most perplexing aspect of social evolution in *C. floridanum* is the lack of soldier production by males. The phylogenetic relationships of polyembryonic encyrtids are poorly defined, but current evidence indicates that soldier-producing species form a monophyletic assemblage of approximately 200 described species (Zolnerowich, 1995; Guerrier & Noyes, 2005). The sexual asymmetry in soldier production observed in *C. floridanum* could thus reflect a secondary loss of male soldier production or conditions that prevent or limit soldier production in all polyembryonic encyrtids. Distinguishing between these possibilities obviously requires comparative data, but these will be difficult to obtain because the hosts parasitized by most species either are unknown or cannot be reared in the laboratory. Intriguingly though, recent results indicate that female clones of *Copidosoma koehleri* and *C. sosares* produce soldiers while male clones do not (Kearse *et al.*, 2006; P. J. Ode and M. R. Strand, unpublished). A more general constraint against male soldier development may thus underlie the tendency of mated *C. floridanum* females to produce mixed-sex broods and only infrequently oviposit single male eggs into hosts (Hardy *et al.*, 1993; Ode & Strand, 1995). We also note that soldier/worker castes are exclusively female in other eusocial Hymenoptera. This has traditionally been explained by kin selection under haplo-diploidy, as there is no relatedness asymmetry that would favour the evolution of worker-like altruism in males (Hamilton, 1972). Others, however, have suggested that male worker castes have not evolved because developmental

constraints, like the absence of a sting, render males ill-suited for defence and other worker functions (West-Eberhard, 1975; Charlesworth, 1978; Starr, 1985).

The alternative to developmental constraint is that the absence of male soldiers reflects a functional adaptation. One possibility in this regard is that soldier production evolved to regulate conflicts over sex ratio in mixed-sex broods rather than for defence against competitors (I. C. W. Hardy and A. Gardner, personal communication). Any female that dies represents a loss of a potential mate to males and, therefore, is a cost, whereas the death of a male is not necessarily a cost to females, because only a few males are needed to inseminate all of their female broodmates. However, many species of polyembryonic wasps, like *C. sosares*, predominantly produce single-sex broods where sex ratio regulation is unnecessary, yet males still do not appear to develop into soldiers. Resolving this evolutionary conundrum is a key goal for future study.

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