

The metabolic enzyme *phosphoglucose isomerase* (*Pgi*) affects the outcome of intra-specific competition in a polyembryonic wasp

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Abstract. 1. Polyembryonic parasitoid wasps in the family Encyrtidae (Hymenoptera) have evolved a caste system consisting of morphologically and functionally distinct larvae called soldiers and reproductives.

2. Two selective pressures are thought to underlie the evolution of the soldier caste: defence against competitors and resolution of the sex ratio conflict. Previous studies also indicate that soldier development time strongly affects the outcome of intra-specific competition in the polyembryonic encyrtid *Copidosoma floridanum* Ashmead. This study builds on prior findings by showing that alleles of the metabolic enzyme *phosphoglucose isomerase* (*Pgi*) differentially affect soldier development time and the outcome of competition.

3. Soldier larvae with the *Pgi* alleles *100* or *120* emerged on average 65 h post-parasitism, whereas soldier larvae with a third allele, *54*, emerged at 67 h. In turn, *C. floridanum* broods homozygous for the *100* and *120* alleles outcompete broods homozygous for the *54* allele.

4. *Pgi* allelic diversity may be maintained through a life-history trade-off affecting female brood sizes with homozygous broods bearing the developmentally disadvantageous *54* allele producing more adult females than broods bearing alternate common alleles.

Key words. Balancing selection, *Copidosoma floridanum*, heterozygote advantage, intra-specific competition, life-history trade-offs, soldier larvae.

Introduction

A distinguishing feature of eusocial animals is the evolution of caste systems whereby some individuals in a group reproduce and others perform helper functions (Robinson, 1992; Queller, 2000). The ecology of insects has favoured the evolution of cooperation in many species, but living in groups can also result in competition for resources and genetic conflicts. These issues are particularly evident among polyembryonic parasitoids in the family Encyrtidae (Hymenoptera). Polyembryonic encyrtids are egg–larval parasitoids whose hosts are primarily Lepidoptera. Each egg a female wasp lays undergoes a protracted period of

clonal development, which results in the formation of multiple, genetically identical embryos (Harvey *et al.*, 2000; Donnell *et al.*, 2004; Smith *et al.*, 2010). Remarkably, these clonal embryos develop into two morphologically and functionally distinct larval castes called soldier and reproductive larvae.

The best-studied polyembryonic encyrtid is *Copidosoma floridanum* Ashmead, which parasitises several species of plusiine moths and has a worldwide distribution (Strand & Grbic, 1997; Donnell *et al.*, 2004; Giron *et al.*, 2007a). Our previous studies of *C. floridanum* populations in North America indicate that adult females always oviposit one male (haploid) egg, one female (diploid) egg, or one male and one female egg into the egg stage of their hosts, which include *Chrysodeixis* (formerly *Pseudoplusia*) *includens* Walker and *Trichoplusia ni* (Hübner) (Strand, 1989; Ode & Strand, 1995). Oviposition of a single egg results in formation of single-sex broods that consist exclusively of male or female wasps, whereas oviposition of both a male and female egg produces mixed-sex broods. *Copidosoma floridanum* eggs clonally propagate themselves during the egg and larval

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stage of the host to produce more than 1000 embryos. In the case of female parasitoid eggs, the first soldier larvae emerge before the host egg hatches and additional soldiers emerge throughout the host's larval stage (Grbic *et al.*, 1992; Giron *et al.*, 2007b). From 20 to as many as 200 soldier larvae ultimately are produced per host (Harvey *et al.*, 2000), whereas the remaining embryos (> 1000) synchronously emerge as reproductive larvae in the final host instar. Reproductive larvae consume the host, pupate inside the remnant host cuticle, and emerge as adults a few days later. In contrast, soldier larvae die after consumption of the host by their reproductive siblings. Male eggs develop similarly, with the exception that they produce fewer soldier larvae, which also do not begin to appear until the larval host molts to its third instar (Grbic *et al.*, 1992; Giron *et al.*, 2007a,b).

Two selective pressures are thought to underlie the evolution of the soldier caste of polyembryonic wasps: (i) defence against competitors (Cruz, 1981; Harvey *et al.*, 2000) and (ii) resolution of the sex ratio conflict of mixed sex broods (Grbic *et al.*, 1992; Gardner *et al.*, 2007). Experimental studies with *C. floridanum* and select other species provide support for both functions (Cruz, 1981; Grbic *et al.*, 1992; Harvey *et al.*, 2000; Giron *et al.*, 2007a; Smith *et al.*, 2010). Studies with *C. floridanum* also indicate that female soldier larvae defend against competitors and the resolve sex ratio conflict, whereas male soldiers exhibit little or no aggressive behaviour (Giron *et al.*, 2007a; Dunn *et al.*, 2014).

Giron *et al.* (2007b) showed that *C. floridanum* experiences high levels of intra-specific resource competition in the field, with hosts often parasitised by more than one female wasp (superparasitism). This study further showed that mated females usually oviposit eggs that produce mixed-sex or single-sex female broods, that superparasitism results in complete elimination of one brood by the other, and that the winner of this competition depends on which brood produced the first soldier larva. Strikingly, the timing of first soldier emergence correlated with allelic variants of the metabolic enzyme-encoding gene, *phosphoglucose isomerase* (*Pgi*), the product of which is a dimeric enzyme that catalyses the reversible conversion of glucose-6-phosphate into fructose-6-phosphate in the second step of glycolysis (Achari *et al.*, 1981). Metabolic enzymes are among the first genes studied from the perspective of ecology to investigate the responses of organisms to natural selection (Marden, 2013). The role of *Pgi* in affecting fitness-related traits has a long history of study in diverse organisms including fungi (Bidochka *et al.*, 2002), plants (Chojecki & Gale, 1982), nematodes (Vilas *et al.*, 2000), and insects (Dahlhoff & Rank, 2000; Rank *et al.*, 2007; Mitikka & Hanski, 2010; Wheat, 2010). Among adult insects *Pgi* has been shown to affect flight metabolism, running speed, fecundity, and longevity. Molecular population genetic analysis of *Pgi* variation finds strong evidence for historical balancing selection among *Colias* and *Melitaea* butterflies (Wheat *et al.*, 2006, 2010) and directional selection within the copper butterfly, *Lycaena tityrus* (Poda) (Karl *et al.*, 2008). Thus, *Pgi* is a gene that has been implicated in affecting the ecology and life history of a variety of organisms.

In this study, we built upon prior results by first characterising the diversity of *Pgi* alleles present in a population of

C. floridanum from southern Georgia, U.S.A. over 3 years. We then generated homozygous and heterozygous lines for the three most common *Pgi* alleles to assess whether: (i) first-soldier development is affected by *Pgi* genotype, (ii) the outcome of intra-specific competition is also determined by the *Pgi* genotype, and (iii) *Pgi* allelic diversity affects other wasp fitness traits and testing whether heterozygosity also affects fitness. Overall, our results advance our previous results by providing experimental evidence that the *Pgi* genotype significantly affects first-soldier development time and the outcome of intra-specific competition.

Materials and methods

Field sampling and establishment of homozygous *Pgi* lines

Trichoplusia ni larvae were collected on soybean and cotton in six counties across southern Georgia (Tift, Sumter, Decatur, Toombs, Bacon, and Mitchell) in the fall of 2005, 2006, and 2007. Larvae were brought back to the laboratory and reared on an artificial diet until they pupated, died, or formed a mummy containing a *C. floridanum* brood. Wasps that emerged from each mummy were collected and frozen at -80°C . Fifteen clonal female wasps from each all-female ($n=59$) and mixed-sex ($n=82$) brood were pooled and homogenised in 25 μl of a 0.05 M Tris/HCl protein extraction buffer. Electrophoresis of the protein products of the *Pgi* gene [also termed *Glucose-6-phosphate isomerase* (*Gpi*) or *Phosphohexose isomerase*, EC number 5.3.1.9] was conducted in 14% horizontal starch gels for 3.5 h at 75 mA, with banding phenotypes visualised by specific histochemical staining as outlined by Shoemaker *et al.* (1992). PGI protein of *C. floridanum* heterozygotes separates as three bands, with the middle band about twice the intensity of the flanking bands, as expected for a dimeric protein. Six independent lines were created for each of the three most common *Pgi* alleles by mating female wasps homozygous for a given allele to hemizygous males, for a total of 18 lines. The lines, labelled as *Pgi*⁵⁴ A–F, *Pgi*¹⁰⁰ A–F, and *Pgi*¹²⁰ A–F, were maintained using *T. ni* as the host as previously described (Strand, 1989).

First soldier emergence, intra-specific competition, and brood fitness traits

To assay the effect of the *Pgi* genotype on timing of soldier emergence, female wasps from each *Pgi* line parasitised recently laid (<12 h old) *T. ni* eggs. To ensure all hosts used in experiments were parasitised, female wasps were individually observed during oviposition and any host eggs not parasitised were eliminated. As a result, parasitised host eggs for each *Pgi* line contained wasp eggs laid over a 20-min range. Upon oviposition, host eggs were placed in an incubator at 27 $^{\circ}\text{C}$ with a LD 16:8 h. At 61 h post-parasitism, cohorts of 10 parasitised host eggs were dissected (each allelic variant was assayed a total of six times) and examined at hourly intervals for first soldier emergence, as previously outlined (Giron *et al.*, 2007b). To compensate for the 20 min range in parasitism events

that occurred during the experimental setup, the hour at which all host eggs in a cohort contained a soldier was recorded as the hour of soldier emergence. Competition (superparasitism) assays were conducted by allowing a female wasp homozygous for one of the *Pgi* alleles (e.g. female *Pgi*⁵⁴A) to simultaneously parasitise a host egg with another female wasp homozygous for a different *Pgi* allele (e.g. female *Pgi*¹⁰⁰A). Previous studies showed that both wasps in simultaneous superparasitism assays always oviposited (Giron *et al.*, 2007b). After each superparasitism event, the host egg was allowed to hatch and the host larva was then reared on an artificial diet until mummy formation. Wasps emerging from the host were then immediately frozen at -80°C . Twenty emerged wasps from each superparasitised host were assessed for the *Pgi* genotype as described above.

Mated female wasps were collected from mixed-sex broods produced from each homozygous *Pgi* line (*Pgi*⁵⁴A–F, *Pgi*¹⁰⁰A–F, *Pgi*¹²⁰A–F) then allowed to parasitise newly laid *T. ni* eggs, followed by rearing of larvae as described above. Date of emergence and brood type were recorded for each sample, and total developmental period was recorded as the number of days from oviposition to emergence of adult wasps (*Pgi*⁵⁴, $n=296$; *Pgi*¹⁰⁰, $n=137$; and *Pgi*¹²⁰, $n=132$). Subsamples of each of these broods were frozen in a -80°C freezer. Brood sizes were determined by counting the total number of adult wasps produced from all female broods (*Pgi*⁵⁴, $n=11$; *Pgi*¹⁰⁰, $n=12$; *Pgi*¹²⁰, and $n=13$), all male broods (*Pgi*⁵⁴, $n=12$; *Pgi*¹⁰⁰, $n=12$; and *Pgi*¹²⁰, $n=12$) and mixed-sex broods (*Pgi*⁵⁴, $n=12$; *Pgi*¹⁰⁰, $n=12$; and *Pgi*¹²⁰, $n=12$).

First soldier emergence and intra-specific competition using heterozygous *Pgi* lines

Many studies of *Pgi* variation find evidence for balancing selection via a heterozygote advantage (e.g. Wheat, 2010). To test whether a heterozygote advantage might exist for *Pgi* in *C. floridanum*, we mated female wasps homozygous for the 54 allele (i.e. females 54B, 54D, 54E, and 54F) with single male wasps hemizygous for the 100 allele (i.e. males 100B, 100D, 100E, and 100F). We chose this combination on the basis of our competition experiments and the disparity found between broods homozygous for the 54 and 100 alleles, respectively (see section Results). In contrast, the outcome of our competition studies showed that individuals homozygous for the 100 and 120 alleles behaved similarly. Matings between 54 allele females and 100 allele males resulted in four heterozygous lines (*Pgi*^{54B/100B}, *Pgi*^{54D/100D}, *Pgi*^{54E/100E}, and *Pgi*^{54F/100F}). We measured the timing of first soldier emergence for these lines as described above. We then assessed the performance of these heterozygous lines in competition assays by simultaneously parasitising a host with one heterozygous female and one homozygous female in the following pairings: *Pgi*^{54B/100B} with *Pgi*^{100E/100E}, *Pgi*^{54D/100D} with *Pgi*^{100C/100C}, *Pgi*^{54E/100E} with *Pgi*^{100B/100B}, *Pgi*^{54F/100F} with *Pgi*^{100A/100A}, *Pgi*^{54D/100D} with *Pgi*^{54C/54C}, and *Pgi*^{54F/100F} with *Pgi*^{54A/54A}. After superparasitism, each host was reared and the genotype of the emerging wasp offspring was determined as described above.

Statistical analysis

Initial statistical analyses were carried out using JMP version 9.0 (SAS Institute, Inc., Cary, NC, U.S.A.). The Kruskal–Wallis (KW) test was used to determine if timing of soldier emergence varied with *Pgi* genotype, with specific pair-wise differences between genotypes determined using the Bonferroni–Dunn multiple comparisons test. Fisher's exact test was used to test the outcome of intra-specific competition between broods homozygous and heterozygous for particular *Pgi* alleles.

Further statistical analyses of total developmental time of the reproductive caste and brood size were conducted by means of resampling tests coupled with paired-sample *t*-tests (the latter conducted using JMP). Resampling procedures were necessary because use of multiple replicate broods from each of the 18 homozygous *Pgi* lines meant that all replicates were not independent, thus violating a basic assumption of most standard statistical tests. Each homozygous genotype was compared with each of the other genotypes for the two traits in each brood type (all-female, all-male, and mixed-sex) in the following way. For each test, a replicate of each genotype was randomly selected and the difference in developmental time or brood size values between the genotypes was recorded. We then randomly selected another replicate of either of the two focal genotypes from a different line, and the difference between the two same-genotype replicate values was recorded. This procedure was performed 1000 times (with replacement), and the paired between-genotype and within-genotype differences were analysed with paired-sample *t*-tests. The sample sizes for calculating the *t*-test statistic and for the degrees of freedom after resampling potentially range from the number of independent lines (most conservative) to the number of replicates (most liberal) used in the comparison. We chose to use the number of lines as well as 2× the number of lines in our analyses; for the brood size experiments the latter also equals the total number of replicates, whereas for the developmental time experiments it averages 18% of the number of replicates. Because the conclusions were very similar using either sample size, we report only those based on 2× the number of lines.

Results

We assessed *Pgi* allelic diversity in *C. floridanum* from southern Georgia by collecting a total of 141 broods and scoring the genotypes of 15 females (pooled) from each brood. A total of seven *Pgi* alleles were identified (see Giron *et al.*, 2007b), with *Pgi*¹⁰⁰, *Pgi*⁵⁴, and *Pgi*¹²⁰ being the most common at frequencies of 0.655, 0.169, and 0.116, respectively. No indication of any secondary genotypes was found for any of these broods, suggesting that each was composed entirely of clonemates.

Time to first soldier emergence in *C. floridanum* all-female broods varies with *Pgi* genotype

First soldiers produced from the *Pgi*¹⁰⁰ and *Pgi*¹²⁰ lines emerged on average at 65 h, whereas soldiers homozygous for *Pgi*⁵⁴ averaged 67 h (Fig. 1). The timing of first soldier

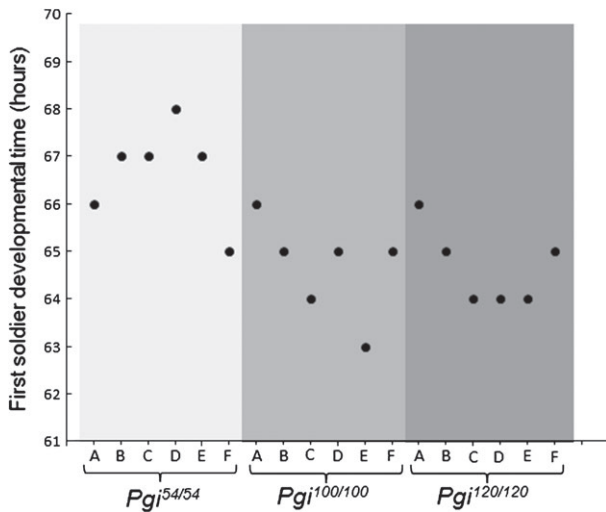


Fig. 1. Time from oviposition into a host egg by a female wasp until first soldier emergence for six homozygous lines (named A–F, $n = 1$ for each line) established in the laboratory for the three most common *Pgi* alleles (Pgi^{54} , $n = 6$; Pgi^{100} , $n = 6$; and Pgi^{120} , $n = 6$) detected in field populations of *C. floridanum*.

emergence differed significantly among the three lines (KW test, $n = 18$; $P = 0.0132$), with Pgi^{100} and Pgi^{120} soldiers emerging at statistically indistinguishable times (Bonferroni–Dunn test, $P = 1.0$) and Pgi^{54} soldiers emerging later than soldiers of either alternate genotype (Bonferroni–Dunn test, both $P = 0.003$).

Earlier first soldier emergence confers a competitive advantage in competition assays

The differences in first soldier emergence times for the different *Pgi* genotypes strongly affected the outcome of competition in our superparasitism assays (Fig. 2). To increase the power of the exact tests, at least 10 assays were conducted for each of the 18 possible competitive pairings. All emerged wasps exhibited only one *Pgi* genotype, that is, there was never an instance of individuals from multiple broods emerging from a superparasitised host. Fisher's exact test for each pairing of hosts parasitised by homozygous $Pgi^{54/54}$ and $Pgi^{100/100}$ females indicated a large disadvantage for the Pgi^{54} homozygote ($P < 0.001$ for five of the six pairings of this type), with the great majority of all emerged wasps in such pairings being Pgi^{100} homozygotes. The same disadvantage for Pgi^{54} homozygotes was observed when paired against homozygous $Pgi^{120/120}$ females ($P < 0.05$ for all pairings), whereas simultaneous superparasitism of hosts by $Pgi^{100/100}$ and $Pgi^{120/120}$ homozygous females resulted in a nearly equal chance of either genotype outcompeting the other.

Pgi genotype affects brood size but not developmental period of the reproductive caste

For the 18 homozygous lines, we produced a total of 296 broods (all-male, all-female, or mixed-sex) homozygous for the 54 allele, 137 broods homozygous for the 100 allele, and 132

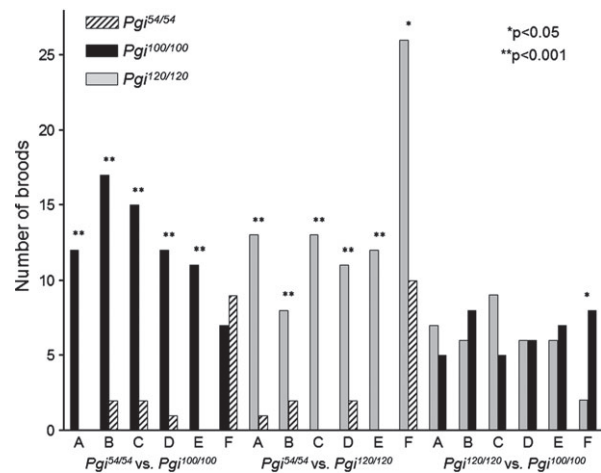


Fig. 2. Outcome of intra-specific competition when adult females from homozygous lines for each of the three most common *Pgi* alleles superparasitised hosts. Letters along the x-axis correspond to the homozygous lines for each allele used in pairwise assays (Pgi^{54A} vs. Pgi^{100A} $n = 12$, Pgi^{54B} vs. Pgi^{100B} $n = 19$, Pgi^{54C} vs. Pgi^{100C} $n = 17$, Pgi^{54D} vs. Pgi^{100D} $n = 13$, Pgi^{54E} vs. Pgi^{100E} $n = 11$, Pgi^{54F} vs. Pgi^{100F} $n = 16$, Pgi^{54A} vs. Pgi^{120A} $n = 14$, Pgi^{54B} vs. Pgi^{120B} $n = 10$, Pgi^{54C} vs. Pgi^{120C} $n = 13$, Pgi^{54D} vs. Pgi^{120D} $n = 13$, Pgi^{54E} vs. Pgi^{120E} $n = 12$, Pgi^{54F} vs. Pgi^{120F} $n = 36$, Pgi^{120A} vs. Pgi^{100A} $n = 12$, Pgi^{120B} vs. Pgi^{100B} $n = 14$, Pgi^{120C} vs. Pgi^{100C} $n = 14$, Pgi^{120D} vs. Pgi^{100D} $n = 12$, Pgi^{120E} vs. Pgi^{100E} $n = 13$, and Pgi^{120F} vs. Pgi^{100F} $n = 10$). Significant differences between numbers of broods of each genotype that emerged from superparasitised hosts from each pairing are marked with asterisks. For each replicate, a host egg was simultaneously superparasitised by a single adult female from the two competing homozygous lines. All broods produced in these experiments contained exclusively one genotype.

broods homozygous for the 120 allele. We then assessed whether the *Pgi* genotype affected the developmental time or the number of reproductive caste offspring produced in the three types of broods. Total developmental time from oviposition to emergence of reproductive caste offspring as adults ranged from 27.6 to 28.6 days, with *Pgi* genotype having no effect on this period for male, female or mixed-sex broods (paired *t*-tests, all $P > 0.1$). In contrast, the *Pgi* genotype did affect brood size, with female broods homozygous for the 54 allele larger than female broods homozygous for the 100 or 120 allele (Fig. 3; paired *t*-tests, both $P < 0.025$). The *Pgi* genotype had no significant effect on male brood size (paired *t*-tests, all $P > 0.1$) but, surprisingly, mixed-sex broods showed a pattern opposite to that found for female broods, with mixed-sex broods homozygous for the 100 and 120 alleles being larger than broods homozygous for the 54 allele (Fig. 3; paired *t*-tests, both $P < 0.01$).

Heterozygous *Pgi* lines exhibit semidominance

We assessed the effects of heterozygosity at *Pgi* by generating four lines heterozygous for the Pgi^{54} and Pgi^{100} alleles, and then comparing the timing of first soldier emergence between these and the homozygous parental lines from which they were derived. Three of the heterozygous lines exhibited first soldier

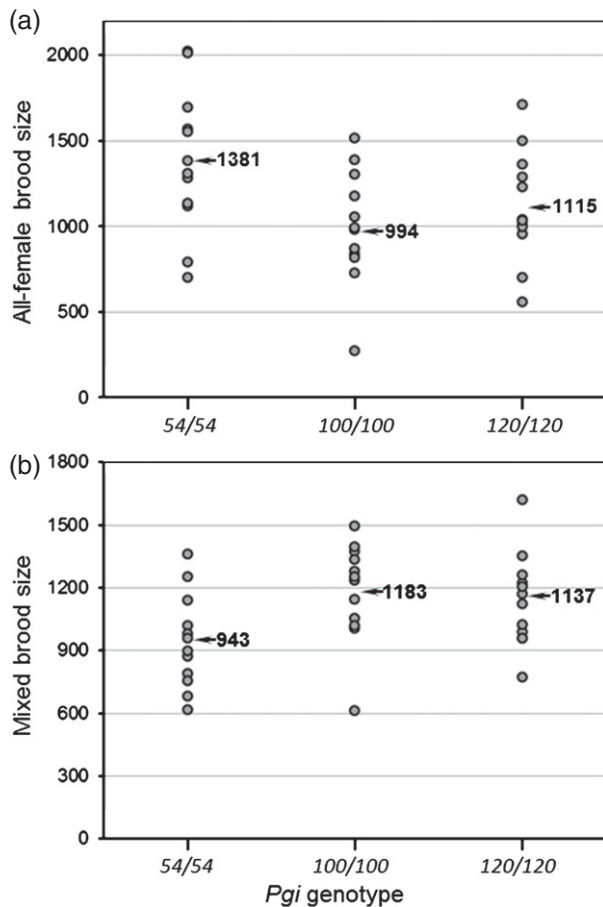


Fig. 3. (a,b) Number of reproductive caste offspring produced in all-female (Pgi^{54} , $n=11$; Pgi^{100} , $n=12$; and Pgi^{120} , $n=13$) and mixed broods (Pgi^{54} , $n=12$; Pgi^{100} , $n=12$; and Pgi^{120} , $n=12$) from homozygous lines for each of the three most common *Pgi* alleles. Means are shown for each line.

emergence times intermediate to those of the corresponding homozygous lines (Fig. 4), demonstrating semi-dominance of these two most common alleles with respect to this trait. Semi-dominance also is not inconsistent with the results for the fourth line, $Pgi^{54F/100F}$, because first soldier emergence times were virtually identical between the homozygous parental lines and the heterozygote line derived from them (Fig. 4, see also Fig. 1).

We next conducted superparasitism bioassays in which females from a $Pgi^{54/100}$ heterozygous line were competed against females from a line homozygous for one of these same two alleles. If the outcome of competition is determined primarily by the timing of first soldier emergence, then the brood whose first soldier emerges earliest should outcompete the other. Based on our data for first soldier developmental periods for the different genotypes (see Figs 1 and 4), we predicted that $Pgi^{54/100}$ clones would usually lose in competition to $Pgi^{100/100}$ clones but usually win in competition with $Pgi^{54/54}$ clones. This prediction was largely upheld in the six different assays we conducted (Fig. 5). The $Pgi^{100/100}$ competitors in assays (1–3)

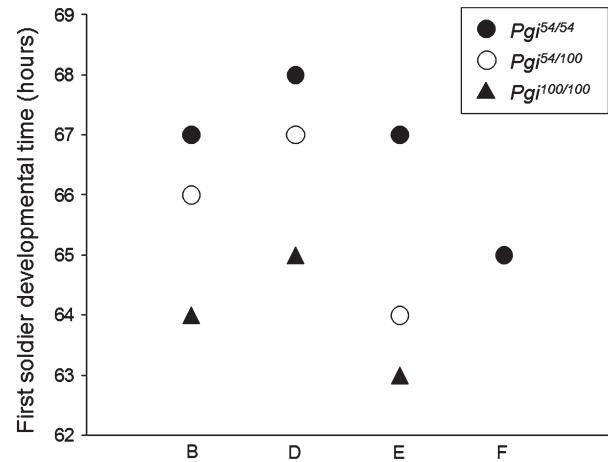


Fig. 4. Time from oviposition into a host egg by a female wasp until first soldier emergence for heterozygotes and homozygotes for the Pgi^{54} and Pgi^{100} alleles. Letters along the x-axis correspond to the lines of homozygotes used to create the heterozygotes [e.g. homozygote lines 54B, D, E, F (closed circles) were crossed with homozygote lines 100B, D, E, F (triangles) to create 54B/100B, 54D/100D, 54E/100E and 54F/100F heterozygote lines (open circles)]. Note that only a closed circle is shown for 54F/100F heterozygotes and 54F and 100F homozygotes because first soldier emergence times overlapped.

produced a first soldier that emerged earlier post-oviposition than the $Pgi^{54/100}$ competitor and in each case the resulting broods of reproductive caste offspring all derived from the homozygous competitor. In the fourth assay, a majority of the broods derived from the $Pgi^{54/100}$ competitor, which was consistent with first soldiers emerging from the $Pgi^{54F/100F}$ line emerging slightly earlier than from the $Pgi^{100B/100B}$ line. $Pgi^{54/100}$ clones also generally outcompeted the homozygous $Pgi^{54/54}$ clones in the two assays (5, 6) where these genotypes were paired.

Discussion

Competition for hosts is a major factor in parasitoid ecology and evolution, but little is known about how specific traits affect the performance of individuals in securing host resources for the development of offspring. *Copidosoma floridanum* and other polyembryonic wasps in the family Encyrtidae are fascinating because they have evolved a caste system in which soldiers mediate competition within hosts between offspring from different females (superparasitism) and the sex ratio of mixed-sex broods (Cruz, 1981; Grbic *et al.*, 1992; Harvey *et al.*, 2000; Giron *et al.*, 2004, 2007b; Smith *et al.*, 2010). Previous studies showed that *C. floridanum* experiences high levels of intra-specific resource competition (superparasitism) in the field (Giron *et al.*, 2007b). Previous studies also show this high level of competition is due in large measure to the narrow temporal window that host eggs can be parasitised, which results in different females superparasitising hosts within only a few hours of one another (Ode & Strand, 1995; Giron *et al.*, 2007b). Thus, a major question is what factors determine the outcome of

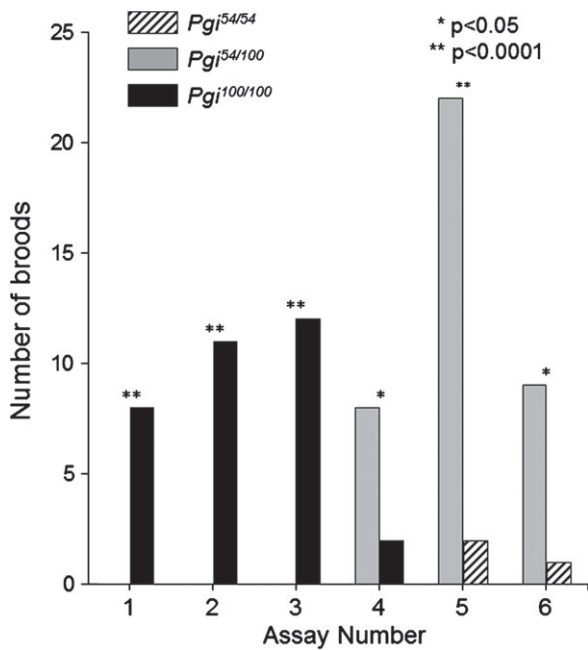


Fig. 5. Outcome of intra-specific competition when adult females from a given homozygous line for the *Pgi*⁵⁴ and *Pgi*¹⁰⁰ alleles were paired with a heterozygous female and superparasitised hosts. Significant differences between numbers of broods of each genotype in a pairing are marked with asterisks (each brood contained exclusively one genotype). Numbers along the x-axis correspond to the following superparasitism combinations: (1) *Pgi*^{100E/100E} versus *Pgi*^{54B/100B}, (2) *Pgi*^{100C/100C} versus *Pgi*^{54D/100D}, (3) *Pgi*^{100B/100B} versus *Pgi*^{54E/100E}, (4) *Pgi*^{100A/100A} versus *Pgi*^{54F/100F}, (5) *Pgi*^{54D/100D} versus *Pgi*^{54C/54C}, and (6) *Pgi*^{54F/100F} versus *Pgi*^{54A/54A}. Significant differences are marked with asterisks. All broods produced in these experiments also contained exclusively one genotype as found in Fig. 2.

competition? Given that *C. floridanum* produces broods comprised of thousands of offspring, the results of Giron *et al.* (2007b) were surprising because they showed that superparasitism always results in the offspring of one female fully outcompeting those of another, and that this outcome correlates with soldier developmental time and genotype of *Pgi*. The purpose of this study was to estimate the number of *Pgi* alleles present in field populations of *C. floridanum* and experimentally assess whether *Pgi* is a gene of likely major effect in determining the outcome of intra-specific competition.

Our multiyear field survey identified several *Pgi* alleles but only three of these exist at high frequency. By establishing isofemale lines from multiple individuals and then using these lines in competition experiments, we show that female wasps homozygous for the *Pgi*¹⁰⁰ and *Pgi*¹²⁰ alleles produce all-female or mixed-sex broods in which the first soldier emerges sooner than is the case for wasps homozygous for the *Pgi*⁵⁴ allele. Because these homozygous lines were inbred, all daughters in a clonal brood were also homozygotes for the same allele as their mother. The consequence of more rapid development for the first soldier produced by *Pgi*¹⁰⁰ and *Pgi*¹²⁰ broods is that wasps homozygous for these alleles usually outcompete *Pgi*⁵⁴ broods in superparasitism assays.

We were initially surprised that a difference of only 2–3 h in the time from oviposition to first soldier larva emergence can translate into the progeny of one wasp completely outcompeting the progeny of another when a host is superparasitised. However, this occurs because competing wasps oviposit their eggs in very close proximity to one another in the host egg and the first soldier that emerges kills the embryo(s) of the competing brood almost immediately (Giron *et al.*, 2007b). As a result, the potential competitor brood is eliminated before the host egg hatches, and well before the extensive clonal propagation during the host larval stage that produces the large number of reproductive caste larvae that develop into adult wasps. Earlier studies further show that *C. floridanum* strongly prefers to oviposit in host eggs that are less than 12 h old (Ode & Strand, 1995), which results in a narrow temporal window that host eggs can be parasitised or superparasitised. Under conditions of high host abundance and rapid encounters with non-parasitised and parasitised hosts, *C. floridanum* females do discriminate against parasitised hosts and avoid superparasitism (M. R. Strand, unpublished), but under conditions of low encounter rates females almost always superparasitise hosts (Giron *et al.*, 2007b).

This tendency likely reflects two features of host and wasp biology. First, the plusiine moth hosts of *C. floridanum* lay eggs singly on plants that are widely separated from one another. As a result, *C. floridanum* females, which are approximately 1 mm in length, probably experience relatively long intervals between successive host encounters. Second, *C. floridanum* adults have short life spans of only a few days, which together with low host encounter rates results in wasps rarely being egg limited, which collectively favours oviposition into hosts whether already parasitised or not (Godfray, 1994; Ode & Strand, 1995). However, these features can also result in high levels of intra-specific competition between offspring in agricultural ecosystems where *C. floridanum* populations relative to host availability sometimes reach high levels (Giron *et al.*, 2007b). Thus, any advantage by offspring in one brood to outcompete the other, such as more rapid first soldier development, will be selectively favoured. Results of this study overall indicate that offspring from the first wasp that parasitises a host are at a competitive advantage over eggs from a second female. The strength of this competitive advantage, however, is affected by the *Pgi* genotype with the 100 and 120 alleles outperforming wasps with the 54 allele. Future experiments to determine whether the order of oviposition by wasps of different genotypes could reveal other affects on brood fitness not identified in this study.

A series of classic studies by Watt *et al.* (1983, 1985, 1996, 2003) demonstrated that allelic variants of *Pgi* significantly affect flight performance and fitness in *Colias* butterflies. *Pgi* similarly has been implicated in affecting fitness-related traits of European map (Mitikka & Hanski, 2010) and Glanville fritillary butterflies (Haag *et al.*, 2005; Hanski & Saccheri, 2006). Insect flight is energetically demanding (Suarez, 2000), and it has been suggested in these systems that enzymes of the central metabolic pathway, including *phosphoglucose isomerase*, are essential in the production of energy (ATP) for flight muscles such that variants of the enzyme differentially affect pathway flux and the resulting phenotypes. We hypothesize that similar energetic demands in association with embryogenesis could underlie the

effects of the different *Pgi* genotypes on soldier developmental periods in *C. floridanum*. We also recognise though that *Pgi* may simply be a marker gene for one or more other genes that could affect first soldier developmental time, and with which it is in complete linkage disequilibrium (e.g., Wang *et al.*, 2013).

Unlike the other *Pgi*⁵⁴ homozygous lines we generated, line 54F produced broods that resembled those of the *Pgi*¹⁰⁰ and *Pgi*¹²⁰ lines in first soldier developmental time and in competitive ability in our superparasitism assays. One possible explanation is that there is 'cryptic' synonymous or non-synonymous variation in the *Pgi* allele of line 54F that functionally distinguishes it from the alleles of the other *Pgi*⁵⁴ lines, even though the charge of its enzyme product makes it electrophoretically indistinguishable. Although synonymous substitutions are often considered silent because the amino acid is not modified, they can affect transcription, splicing, mRNA transport, and translation, any of which could alter phenotype. Molecular studies on *Pgi* in the fall webworm [*Hyphantria cunea* (Drury)] found five non-synonymous mutation sites, one of which changed the hydrophobicity of the glycosaminoglycan attachment site (Luo *et al.*, 2012). A similar scenario could be occurring in the 54F line of *C. floridanum*, impacting enzyme activity but not altering enzyme charge, our only way of detecting allelic variation. Alternatively, if *Pgi* is simply a marker, the activity of other tightly-linked genes of major effect bearing variant alleles could underlie the outcomes seen in our experiments with the 54F line. DNA sequencing and functional studies of *Pgi* variants in *C. floridanum* will be needed to distinguish these possibilities. Overall although, these results are significant because they identify *Pgi* as a trait of potential importance in determining a significant ecological outcome in parasitoid biology: resolution of competition for host resources.

That wasps homozygous for the 54 allele usually develop first soldiers more slowly and perform more poorly in competition assays obviously raises the question of why this allele is maintained at high frequency (0.169) in the field. Among the many possible mechanisms that could be responsible, heterozygote advantage is appealing because previous studies with Lepidoptera have revealed overdominance at *Pgi* with respect to crucial fitness-related traits (Watt, 1983; Haag *et al.*, 2005; Saastamoinen, 2007; Hedrick, 2012). However, we found little evidence for such an advantage in *C. floridanum*. Our studies with heterozygous lines indicate that the 54 allele and the most common *Pgi* allele (100) are semidominant, with heterozygotes exhibiting first soldier developmental times and competitive abilities intermediate to those of homozygotes for these alleles. This resulted in 54 allele heterozygotes still losing in superparasitism assays when competing against 100 allele homozygotes that have distinctly earlier first soldier emergence times. However, 54 allele heterozygotes did have a competitive advantage against 54 allele homozygotes. Our results also show that wasps homozygous for the 54 allele produce larger all-female broods than wasps homozygous for the 100 allele, a potential life-history trade-off between brood size and developmental rate in 54 homozygotes that could be important in the maintenance of the allele. Our study focused on only a few fitness-related traits (soldier developmental period, brood

size, egg-to-adult reproductive developmental period), yet *Pgi* has been shown to affect many other important traits in insects including larval growth rate, adult longevity, and fecundity (Dahlhoff & Rank, 2000, 2007; Rank & Dahlhoff, 2002; Neargarder *et al.*, 2003; McMillan *et al.*, 2005; Rank *et al.*, 2007; Karl *et al.*, 2008). Additionally, our survey of *Pgi* alleles was conducted over a relatively narrow geographical region relative to the large range across North America where *C. floridanum* has been collected (Ode & Strand, 1995). Future surveys of *C. floridanum* sampled over larger areas of its natural range coupled with studies of additional fitness traits will be needed to shed light on the maintenance of *Pgi* allelic diversity in this parasitoid.

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