Brood size in a polyembryonic parasitoid wasp is affected by relatedness among competing larvae

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Brood size has important implications for the fitness of both parents and offspring. In polyembryonic parasitoid wasps, each egg develops into many genetically identical embryos through clonal division inside the host. Thus, offspring may have the potential to affect brood size by adjusting the degree of embryonic division. In some species, a proportion of embryos develop into soldier larvae, which attack competitors inside the host. This may be another mechanism for offspring to affect final brood size. We investigated the effect of relatedness between competing clones on brood size in the polyembryonic wasp Copidosoma koehleri. We predicted that final brood size would be affected by the number and relatedness between competing clones inside the host. Additionally, we predicted that due to a competitive asymmetry between male and female clones (apparently only female clones produce a soldier larva), this effect would depend on the sex composition of wasps inside the host. We allowed 2 wasp eggs (laid either by 1 female or by different females) to develop in a host and counted the emerging adults. Relatedness between male clones did not affect brood size. However, female-containing broods of related clones were larger than broods of nonrelated clones, suggesting higher aggression of the soldier toward less related individuals. Dissections of hosts parasitized by 2 clones indicate that normally only 1 soldier survives and that it often eliminates unrelated clones. Thus, offspring control over brood size in response to relatedness is probably mediated by soldier aggression and not by clonal division. Key words: brood size, Copidosoma koehleri, polyembryony, relatedness, soldier caste. [Behav Ecol 20:761–767 (2009)]

Brood size has important implications for the fitness of both parents and offspring. Because resources available for the production and care for offspring are often limited, a trade-off exists between brood size and individual fitness. Optimal brood size reflects the resolution of this trade-off (Lack 1947). However, conflict regarding optimal brood size may exist between parents and their offspring. Although parents are selected to maximize the fitness of the whole brood (or several broods), each offspring may benefit from maximizing its individual fitness, even at the expense of its siblings. Thus, optimal brood size is generally larger for parents than for offspring (Trivers 1974).

Although parents control the number of produced offspring, offspring may further alter brood size through competition and siblicide (Roff 1992). Kin selection theory (Hamilton 1963, 1966) predicts that conflict between offspring should increase as the genetic relatedness between them decreases. However, this prediction is difficult to test for several reasons: 1) it is often difficult to separate the effect of parents on brood size from that of offspring; 2) relatedness between offspring normally does not vary extensively; and 3) although relatedness reduces conflict, it is often correlated with resource competition, which increases conflict. For example, when dispersal is limited, both kinship and competition between relatives increase (West et al. 2002).

Polyembryonic parasitoid wasps provide a good system to test the effect of relatedness on the degree of conflict between offspring and as a result on brood size (Godfray 1994). In these wasps, each egg divides clonally to produce a group of genetically identical embryos inside the host. Embryos develop into larvae that actively feed on the host until they consume it completely, pupate inside it, and emerge as adults (Strand 2003). Thus, this system allows us to distinguish between parental and offspring effects on brood size because they are separated in time. Additionally, it provides an opportunity to manipulate the relatedness between offspring, given a relatively constant resource (the host body): if only one wasp egg is laid inside the host, offspring are genetically identical, and thus, no genetic conflict is expected among them or between the offspring and their mother (Godfray 1994). However, if more than 1 egg is laid, a genetic conflict may arise according to the relatedness between competing clones, and a mother–offspring disagreement on optimal brood size is expected. Brood size adjustment in this system may be particularly important because broods that are too small may not be able to emerge from the host. On the other hand, brood size may have a strong negative effect on individual body size and thus on fitness (Ode and Strand 1995).

In polyembryonic wasps, offspring may have the potential to affect brood size in 2 different ways: 1) they may control the number of embryos produced during clonal division and 2) they may control the level of aggression between competing clones. Although the first hypothesis has not been tested, there is evidence that the degree of larval aggression depends on the relatedness between the clones. In some species of polyembryonic wasps, a proportion of embryos develop into sterile soldier larvae (Silvestri 1937; Doutt 1952; Cruz et al. 1990) that attack inter- and intraspecific competitors inside the host (Cruz 1981, 1986; Giron, Harvey, et al. 2007). The soldier may represent an extreme case of altruism or spite because it benefits its clone mates while attacking nonrelatives but never matures and reproduces (Gardner et al. 2007). In the best-studied species, Copidosoma floridanum, female soldiers were shown to attack according to relatedness, directing higher levels of
aggression toward less related individuals (Giron and Strand 2004; Giron et al. 2004). However, the effect of this kin discrimination on final brood size has not been demonstrated. We addressed the effect of relatedness on final brood size in the polyembryonic parasitoid wasp Copidosoma koehleri Blanchard (Hymenoptera: Encyrtidae). The study of this species provides an advantage over the study of C. floridanum because of the higher natural variability in relatedness between clones within a host. In C. floridanum, a female normally lays 1 female and 1 male egg per host (Strand 1989b), whereas C. koehleri females lay eggs of different sex combinations and of varying relatedness levels in a host under laboratory conditions (Doutt 1947; Keasar et al. 2006). Additionally, in C. koehleri, only ~40 individuals develop from 1 egg, in comparison to ~1000 or more in C. floridanum, allowing easier determination of brood size.

To ascertain that parasitism by more than 1 clone also occurs under natural conditions, we counted and sexed wasps from a field population and estimated the degree of superparasitism by the proportion of mixed-sex broods (broods originating from at least 1 male egg and 1 female egg). In the laboratory, we manipulated the number of clones inside the host and their relatedness and tested the effect of these manipulations on the size of broods of different sex combinations. We estimated the effect of brood size on offspring fitness by measuring the correlations between brood size, body size, and the longevity of adult wasps. We predicted that final brood size (i.e., the number of wasps that complete development and emerge from the host) would be affected both by the number of competing clones inside the host and by the relatedness between them. Additionally, we predicted that due to the competitive asymmetry between male and female clones (in C. koehleri, apparently only female clones produce a soldier larva, Segoli et al. 2009), the effect of these factors on brood size would depend on the sex composition of wasp clones inside the host.

MATERIALS AND METHODS

Study species

Copidosoma koehleri is a polyembryonic parasitoid wasp that parasitizes the potato tuber moth (PTM) (Phthorimaea operculella Zeller) and is used as a biological control agent of this pest (Horne 1990; Kfir 2003). The adult female lays her eggs into the moth’s egg. Females usually lay 1 egg per oviposition event, but superparasitism by the same or another female is common (Doutt 1947; Keasar et al. 2006). The moth larva hatches and develops, whereas the wasp egg divides clonally to produce many embryos. Female clones often contain more embryos than male clones (40–50 vs. 20–30 individuals, respectively). Dissections along embryonic development suggest that each female clone produces 1 soldier larva, which is already active before the rest of the larvae differentiate. Male clones, on the other hand, do not produce a soldier. Wasp embryos develop into larvae, which consume the host tissues completely before pupation. The wasp life cycle lasts about a month and is highly synchronized with that of the host (Segoli et al. 2009).

A laboratory stock of C. koehleri was used for the laboratory experiments. The stock originated from field-collected individuals from South Africa (courtesy of Dr R. Kfir, Plant Protection Institute, Pretoria). Parasitoids were housed at 27 °C, natural daylight, and fed with honey. A laboratory stock of PTM was housed at 27 °C, natural daylight, and fed with honey and water. PTM eggs were collected daily and were used within 24 h because the eggs’ age is known to influence the parasitoids’ oviposition decisions (Ode and Strand 1995). Adult wasps were used within 3 days after emergence.

Field sample

Larvae of the PTM were collected from potato plants from fields near Pretoria, South Africa. Larvae were provided with potatoes and kept until pupation. Parasitized hosts containing wasp pupae (n = 102) were kept each in a separate vial until the emergence of adult wasps. We counted and sexed wasps from each brood. Additionally, we measured the head width of 5 wasps of each sex from each brood using the integrated Soft Imaging Software (SIS) image analysis package (SIS GmbH, Minster, Germany). Counts and measurements were done at the laboratories of Achva College, Israel, during 2006.

Manipulative experiment

The experiment was conducted at the laboratories of Achva College in 2007. We used females from mixed-sex broods or from female broods that were exposed to males for at least 24 h to allow mating. We used 377 females from 162 broods (maximum 6 females per brood). Each female was allowed to oviposit up to 6 times. Whenever using the same female more than once, we allocated it for different treatments or in combination with different females (in the low relatedness treatment). Around 50% of the hosts did not develop (see Results). Nevertheless, in several cases, 2–3 hosts per treatment that had been exposed to females of the same brood or of the same combination of broods completed their development. Genetic relatedness among brood-mate wasps may render data obtained from these hosts nonindependent. To avoid pseudoreplication (Hurlbert 1984), we retained in the statistical analysis of each treatment data from only one, randomly chosen brood-mate wasp.

At the beginning of each trial, we placed 1 fresh host egg in the center of a petri dish. We then introduced 1 C. koehleri female inside the dish and directed her to the host. Usually, females tried to oviposit into the host after probing it with their antennae. We observed each female under a dissecting microscope until oviposition occurred. As soon as the female pulled her ovipositor out of the host egg, she was separated from the host. If a female did not initiate oviposition during 3 min after the first contact with the host, she was removed. Females that did not oviposit in 3 trials were excluded from the experiment. We then added a slice of potato to each petri dish and incubated the dishes at 27 °C until the emergence of the parasitoids.

We produced hosts parasitized twice by the same female (high relatedness) or parasitized twice by 2 unrelated females (low relatedness). Hosts were randomly assigned to these treatments. The time gap between 2 ovipositions into the same host was approximately 2 h. In addition, we produced a sample of hosts which were parasitized once (one oviposition). This was done in order to compare brood sizes in the high and low relatedness treatments with brood sizes in the absence of interclonal competition.

We documented host mummy mass (including the mass of all wasps that pupated inside it) as wasp puation. After emergence, we sexed and counted the wasps that emerged from each host. We distinguished 3 sex compositions of broods: male broods, which probably originated from 1 or 2 male clones; female broods, which probably originated from 1 or 2 female clones; and mixed-sex broods, which probably originated from 1 female and 1 male clone. We measured the head width of 5 individuals of each sex from each host using the integrated SIS image analysis package. We averaged these measurements to obtain the mean head width for each brood.

Females in the low relatedness and the high relatedness treatments may have differed in their tendency to superparasitize a host, even after inserting their ovipositor. To test for this
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possibility, we dissected a sample of hosts from the experiment and searched under the microscope for wasp eggs. To learn about clonal survival during development, we dissected a sample of hosts on days 10–12 after parasitism (during embryonic division of wasps) and recorded the presence of a soldier and the number of embryonic masses inside the host.

The effect of body size on longevity
To estimate the effect of body size on longevity, we measured head width of up to 5 wasps per sex originating from 11 male broods, 10 female broods, and 9 mixed-sex broods. We placed each individual in a separate tube with a drop of honey, incubated it at 25 °C and recorded survival every second day. Head width and longevity were averaged for 5 wasps of each sex for each host.

Statistical analyses
We used 1-way analysis of variance (ANOVA) to compare brood size and head width among broods of different sex composition (male, female, and mixed) from the field sample. We employed linear regression to test the effect of brood size on head width of adults wasps from the field sample. We used a general linear model (GLM) to test the effect of the interaction between relatedness (high and low) and sex composition (male, female, and mixed) on brood sizes in the experiment. Additionally, we used 1-way ANOVAs, followed by post hoc tests, to compare brood size, mummy mass, and head width between all 3 treatments (one oviposition, high, and low relatedness) within each sex composition. We tested the effect of brood size on mummy mass at pupation and on head width of adult wasps through linear regression. We applied Fisher’s Exact test to compare the frequency of finding 0–1 or 2 wasp eggs in a dissected host egg between the high and low relatedness treatment. Similarly, we employed Fisher’s Exact test to determine whether the frequencies of 1 or 2 wasp embryonic masses inside dissected host larvae containing a soldier depended on their relatedness. We used a GLM to test the effects of sex and head width on longevity. When necessary, data were log transformed to meet the model’s assumptions. In several cases, we failed to determine mummy mass, head width, or brood size, and thus, sample sizes were reduced.

RESULTS
Field sample
Forty of the hosts collected from the field contained male wasps only, 23 contained females only, and the remaining 39 contained both males and females. Thus, the minimal frequency of superparasitism in the sample, estimated by the frequency of mixed-sex broods, was 38%. The number of emerging wasps was larger for male and mixed broods compared with female broods. Head width of wasps also differed between brood types; it was larger for wasps emerging from male broods than for wasps emerging from male or mixed broods (Table 1). There was a negative effect of the number of wasps per brood on individual size (linear regression, N = 101, R² = 0.28, P < 0.0001). Sex ratio in mixed-sex broods was 0.90 ± 0.17, 0.03–0.68 (mean ± standard deviation [SD], range).

Manipulative experiment
Proportions of developing parasitoids and their sex
Out of 429 hosts, 214 did not complete development (the host larva did not hatch or died during development) and 77 developed into adult moths. *Cepidonas koehleri* emerged from the remaining 138 hosts. After the removal of pseudoreplicates (see Materials and methods), the sample contained 65 male broods (22 of the one oviposition, 21 of the high, and 22 of the low relatedness treatment), 51 female broods (13 of the one oviposition, 15 of the high, and 23 of the low relatedness treatment), and 13 mixed-sex broods (1 of the one oviposition, 6 of the high, and 6 of the low relatedness treatment). Additionally, we removed 1 host from the one oviposition treatment that contained a mixed-sex brood (indicating that it was parasitized twice) because we could not determine the source of the second brood.

Brood size
The interaction between relatedness (high or low) and sex composition had a significant effect on brood size (GLM, F[2,86] = 4.87, P = 0.01). When considering each sex composition separately, the treatment (one oviposition, high relatedness, or low relatedness) had a significant effect on brood size of male broods (Figure 1A; ANOVA, F[2,60] = 13.4, P < 0.001). A Tukey post hoc test revealed that mean brood size of the low relatedness treatment did not differ from that of the high relatedness treatment (P = 0.83) but was significantly larger than mean brood size in the 1 oviposition treatment (P < 0.001). Female broods were also affected by treatment (Figure 1B; ANOVA, F[2,48] = 9.73, P < 0.001). Broods in the low relatedness treatment were significantly smaller than those in the high relatedness treatment (P = 0.005) but did not differ from broods of the one oviposition treatment (P = 0.34). Mixed-sex broods of the high relatedness treatment tended to be larger than those of the low relatedness treatment, but this result was only marginally significant (Figure 1C; ANOVA, F[1,10] = 4.3, P = 0.065). Sex ratio (proportion of males in mixed-sex broods) was 0.24 ± 0.14, 0.05–0.49 (mean ± SD, range). Sex ratio did not differ between the high and low relatedness treatments (ANOVA, F[1,10] = 0.3, P = 0.6).

The effects of brood size and treatment on mass at pupation and on head width of wasps
Total mass at pupation was positively related to brood size, as reflected in linear regression analyses. This result was significant when considering all brood types (N = 115, R² = 0.50, P < 0.001), male broods only (N = 59, R² = 0.46, P < 0.001), or female broods only (N = 45, R² = 0.46, P < 0.001), but not in mixed-sex broods (N = 11, R² = 0.23, P = 0.076). Head width was negatively affected by brood size, as reflected in linear regression analyses. This result was significant when considering all brood types (N = 126, R² = 0.37, P < 0.001).

| Table 1 |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Number of wasps emerging from field-collected hosts and their average head width |
|                | Male broods     | Female broods   | Mixed-sex broods | ANOVA test      |
| Brood size of wasps (average ± SD) | 52.5 ± 26.3a | 37.0 ± 10.0b | 48.9 ± 15.6a | F[2,99] = 4.7, P = 0.011 |
| Head width of wasps (average ± SD) | 412.0 ± 37.6a | 439.6 ± 23.4b | 411.3 ± 22.2a | F[2,98] = 8.0, P = 0.001 |

Data for head width are based on measurements of 5 wasps per sex per host containing male wasps only (male broods), female wasps only (female broods), or both males and females (mixed-sex broods). Letters represent results of Tukey post hoc tests.
male broods only ($N = 63, R^2 = 0.51, P < 0.001$), female broods only ($N = 51, R^2 = 0.14, P < 0.001$), and mixed-sex broods ($N = 12, R^2 = 0.31, P = 0.035$).

Treatment had a significant effect on mass at pupation for male broods (Figure 2A; ANOVA, $F_{2,42} = 4.07, P = 0.024$) and female broods (Figure 2B; ANOVA, $F_{2,57} = 5.6, P = 0.006$), but not for mixed broods (Figure 2C; ANOVA, $F_{1,9} = 0.79, P = 0.39$). In addition, treatment had a significant effect of the opposite direction on head width of wasps for male (Figure 3A; ANOVA, $F_{2,62} = 4.21, P = 0.02$) and female (Figure 3B; ANOVA, $F_{2,48} = 5.17, P = 0.01$), but not for mixed broods (Figure 3C; ANOVA, $F_{1,10} = 1.31, P = 0.28$).

**Dissections of host eggs after oviposition**

We found 2 wasp eggs in 10 out of 14 host eggs of the high relatedness treatment, a single egg in 3 hosts, and no eggs in the remaining host. We found 2 wasp eggs in 16 out of 23 host eggs of the low relatedness treatment and 1 wasp egg in the remaining 7 hosts. In comparison, we found 1 wasp egg in 12 out of 14 hosts of the one oviposition treatment and no wasp egg in the remaining hosts. The number of wasp eggs found inside host eggs did not differ between hosts from the high relatedness treatment and hosts from the low relatedness treatment (Fisher’s Exact test comparing the frequency of cases with 0–1 wasp eggs and cases with 2 eggs between high and low relatedness treatments, $P = 1$).

**Dissections of host larvae during development**

In 23 out of 112 dissected host larvae, we did not find any sign of developing wasps, 43 hosts contained wasp embryos but did not contain a soldier, and 45 contained a single soldier. In addition, 1 host from the high relatedness treatment contained 2 soldiers, one of which was immature. After the removal of pseudoreplication (see Materials and methods), the sample of hosts containing 1 or 2 soldiers was reduced to 40. Most of the hosts (10 out of 15) from the high relatedness treatment contained only one embryonic mass, indicating that 2 clones were developing inside the host. In contrast, the majority of hosts (13 out of 14) from the low relatedness treatment contained only one embryonic mass, indicating that 1 clone was probably eliminated. For comparison, a single clone was found in all 11 hosts of the one oviposition treatment that contained a soldier. The proportion of hosts containing a soldier with 1 or 2 embryonic masses differed significantly between the high and low relatedness treatments (Figure 4; Fisher’s Exact test, $P = 0.002$).

**The effect of body size on longevity**

Sex of wasps had no significant effect on longevity. Head width had a positive effect on wasp longevity (Figure 5; GLM, $N = 34$; sex: $F_{1,31} = 2.31, P = 0.14$; head width: $F_{1,31} = 7.37, P = 0.01$).
DISCUSSION

We studied the effect of the number and relatedness between competing clones on final brood size in the polyembryonic parasitoid wasp *C. koehleri*. In accordance with our predictions, brood size was affected by relatedness, depending on the sex combinations of the competing clones. Brood size was larger when the relatedness between competing clones was higher but only if a female clone was present inside the host, probably due to the presence of a female soldier. These results suggest that brood size is regulated by soldier aggression rather than by the number of embryonic divisions.

Our results are consistent with a study on *C. floridanum* showing that female soldiers are more aggressive toward nonrelatives compared with siblings (Giron and Strand 2004; Giron et al. 2004). In our study, we further demonstrated a possible effect of such behavioral plasticity on brood size and on individual fitness. Results from dissection of hosts further suggested that the soldier is often active at a relatively early stage of brood development, before the completion of embryonic divisions. This may explain the ability of the soldier to eliminate a large proportion of the competing embryos. The near absence of cases in which 2 soldiers were found in 1 host suggests that 1 soldier (possibly the first one to develop) eliminates the other soldier and attacks the competing clone according to relatedness. In agreement with this interpretation, Giron, Ross, and Strand (2007) found that in *C. floridanum*, the timing of soldier development determines the outcome of competition between 2 unrelated clones.

Mummy mass at pupation may represent the host carrying capacity because at this stage all the host resources are allocated to the wasps. The positive relationship between brood size and mummy mass may be explained by the ability of wasp embryos to adjust their numbers to the host carrying capacity. However, in *C. koehleri*, most of the growth of the host occurs after embryonic division is completed (Segoli M, unpublished data). Alternatively, wasps may affect host growth according to their numbers. Increased growth by parasitized versus unparasitized larvae is a general phenomenon in gregarious parasitoids (Slansky 1986) and was reported for several *Copidosoma* species (e.g., *C. bakeri*, Byers et al. 1993; *C. floridanum*, Strand 1989a; *C. koehleri*, Segoli et al. 2009). Increased host growth may result from either direct manipulation by the parasitoids or an indirect response of the host to the parasitoids’ activity. These effects may be stronger as the number of parasitoids inside the host increases (Slansky 1986). This is likely to be the case in our experiment, as treatment had a significant effect on mummy mass at wasp pupation.

In spite of the positive effect of brood size on mass at pupation, brood size had a strong negative effect on individual body size, suggesting that the increase in host mass did not fully when the relatedness between competing clones was higher but only if a female clone was present inside the host, probably due to the presence of a female soldier. These results suggest that brood size is regulated by soldier aggression rather than by the number of embryonic divisions.

Figure 3
Average AD head width (microns) of wasps (each data point is the average of 5 wasps per sex per host) in the one oviposition treatment (1 oviposition per host), high relatedness treatment (2 ovipositions by the same female), and low relatedness treatment (2 ovipositions by 2 unrelated females). Head width was compared separately between treatments in male broods (A), female broods (B), and mixed-sex broods (C). Letters above columns represent results of Tukey post hoc test.

Figure 4
Percentage of host larvae that were dissected during development from the one oviposition treatment (1 oviposition per host), high relatedness treatment (2 ovipositions by the same female), and low relatedness treatment (2 ovipositions by 2 unrelated females) that contained a soldier and either 2 (black sections of bars) or 1 (white sections of bars) embryonic masses.

Figure 5
Average longevity (day) per host versus average head width (microns) per host of male and female wasps.
compensate for the number of developing wasps. Negative relationships between the number of offspring and individual size are known from other organisms (Messina and Fox 2001) and may be especially strong in polyembryonic wasps, where offspring consume the host tissues completely (Grbic et al. 1998; Strand 2003; Zhurov et al. 2007). Body size is known to have a strong effect on individual fitness (Roff 1992). In this study, we found a positive relationship between body size and longevity under laboratory conditions. Body size may further affect reproductive success of parasitoid wasps in the field (Visser 1994; Kazmer and Luck 1995; West et al. 1996; Ellers et al. 1998, 2001; Kollikar-Ott et al. 2003). Thus, by attacking and killing competitors, the female soldier may expose its genetically identical clone members to reduced resource competition and allow them to attain a larger body size.

Brood size had a negative affect on body size of wasps from both male and female broods; thus, it is difficult to explain why male clones did not evolve a soldier to reduce resource competition as well. In the wasp *C. floridanum*, male clones produce soldiers but fewer and later during development compared with females (Grbic et al. 1992, 1997), and male soldiers are apparently nonaggressive (Göricke, Hoeney, et al. 2007). The production of soldiers in other species of *Copidosoma* is yet unknown, but observations of female-biased sex ratios from mixed-sex broods of additional species (Patterson 1919; Stoner and Weeks 1975; Walter and Clarke 1992) indicate that soldiers originate most commonly from female clones. One possible explanation is that brood size has a stronger negative effect on the fitness of females than that of males (Odle and Strand 1995). A second explanation may be that soldiers are produced to mediate a conflict over sex ratio in mixed-sex broods. In this case, theory predicts that females should invest more heavily in soldier production than males (Gardner et al. 2007). Studying the intensity of superparasitism, sex composition and relatedness between competing clones in relation to soldier production and function, within and among species, may help gain insight into the relative importance of these explanations.

The proportion of mixed broods in our experiment (0.1) was significantly lower than expected under random sex allocation (0.5) (Fisher’s Exact test, *P* < 0.001). This result was retained even when taking into account that ovipositor insertions with no egg laying (~0.15 of the cases, as calculated from the data of dissections of host eggs) reduce the expected proportion of mixed-sex broods to ~0.35. The low proportion of mixed-sex broods may suggest that sex allocation was not random, that some of the eggs did not develop, or that in some cases a female soldier completely eliminated a competing male clone. The uncertainty regarding the original number and sex of eggs laid in some of the hosts might have increased the variance in our results. However, given the significant effect of the treatment on brood size and on the number of developing clones within a host, but not on the number of wasp eggs found in dissected host eggs, we believe that this uncertainty should not affect our interpretation.

According to Giron et al. (2004), sex ratios are expected to be more female biased in mixed broods of the low relatedness than in those of the high relatedness treatment. Contrary to this prediction, we did not find a significant difference in sex ratio between treatments. The lack of effect of relatedness on sex ratios in our experiment may be attributed to the small samples of mixed broods. Alternatively, it may reflect our inability to distinguish female broods that originated from 2 female eggs from those originating from 1 male and 1 female egg, in which the soldier totally eliminated the male clone.

Brood size regulation via soldier aggression makes evolutionary sense only if parasitism by more than 1 clone is common under natural conditions. The results from the field study provide evidence for this effect. Mixed-sex broods comprised ~40% of the parasitized broods collected from the field. This is probably an underestimation of superparasitism levels because it does not include broods containing several clones of the same sex. A similar proportion of mixed broods were observed in the laboratory under conditions of high parasitism level (e.g., high wasp density or long exposure to hosts, Keasar et al. 2006). Further evidence is brood size of male clones relative to female clones. Whereas female broods are larger than male broods under conditions of single or low parasitism level (Keasar et al. 2006; Segoli et al. 2009), the opposite is true under conditions of high parasitism level (Keasar et al. 2006). This is possibly due to soldier activity in female-containing broods. We found that male broods from the field were larger than female broods and that brood sizes were similar to those observed under high levels of parasitism in the laboratory (Keasar et al. 2006).

In this study, we experimentally manipulated the number and relatedness of eggs inside the host. In nature, these variables are likely to be affected by female oviposition decisions. In *C. floridanum*, for example, a female normally lays 1 male and 1 female egg in each host, inducing competition between sisters and brothers. When host availability is high, however, a female may switch to laying a single egg per host (Hardy et al. 1993). In *C. koehleri*, on the other hand, females normally lay 1 egg per oviposition, but superparasitism is common. It would be interesting to study female preferences for hosts according to their parasitism status (previously parasitized or not), the sex and relatedness of a wasp egg from previous parasitism, and females’ previous experience. It would be especially interesting to test whether *C. koehleri* females more often avoid hosts parasitized by unrelated female eggs in which the survival of their offspring is expected to be low. Such studies are expected to illuminate the interplay between parental and offspring effects on brood size.

**FUNDING**

Israel Science Foundation (grant No. 184/06).

We thank Rami Kfir, Ori Becher, Sara Baranes, Adi Sadeh, Daphna Gottlieb, Snir Yahuda, Shalhevat Azriel, Ittai Malka, Na’ama Morag, Moran Sidi, Michael Strand, David Giron, and Jay Rosenheim for assistance and discussions. We thank Stuart West and an anonymous referee for useful comments on the manuscript.

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