

Competition and brood reduction: testing alternative models of clutch-size evolution in parasitoids

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Competition between siblings occurs in many taxa including parasitoid wasps. Larvae of solitary species eliminate competitors by engaging in aggressive behavior, thus restricting brood size to a single individual. In gregarious species, more than one offspring can develop per host. There are 2 models by which gregariousness can arise in a population of solitary individuals: 1) through a reduction in larval mobility (with the retention of aggressive behavior) or 2) through a reduction in fighting behavior or ability. When more larvae are present than can be supported by available host resources, these 2 models make opposing predictions regarding the process of brood size reduction: Mortality occurring early in larval development under the reduced mobility hypothesis versus mortality occurring throughout larval development under the reduced aggression hypothesis. Here, we measure changes in brood size over the course of larval development of the gregarious parasitoid, *Cotesia flavipes*. Superparasitized hosts contained approximately twice as many *C. flavipes* eggs as hosts parasitized by a single parasitoid female. Brood sizes in superparasitized hosts declined gradually as *C. flavipes* individuals developed, whereas brood sizes remained constant during larval development in singly parasitized hosts. An absence of wounded or destroyed larvae suggested no aggressive behavior. Collectively, these results support the reduced aggression hypothesis. **Key words:** brood reduction, clutch-size evolution, contest competition, *Cotesia flavipes*, parasitic Hymenoptera, scramble competition, sibling rivalry. [*Behav Ecol* 20:403–409 (2009)]

Immature offspring of many animal species are placed in a developmental nursery, which can be characterized as a spatially and structurally discrete location in which progeny complete development (Mock and Parker 1997). A key feature of developmental nurseries is the limited space and food resources available for the successful development of offspring. In many species, juveniles have no ability to move out of the nursery in search of additional resources. Competition for resources is frequently intense in these situations (Mock 1984). As resources become limiting, individuals can compete either by excluding competitors in a contest process, resulting in resource monopolization by one or a few individuals, or alternatively they can compete in a scramble process, in which individuals acquire resources without interfering directly with others (Mock and Parker 1997).

Parasitic wasps (hymenopteran parasitoids) are clear examples of species that use developmental nurseries in that a female parasitoid lays one or more eggs in or on a host, which represents a discrete resource of limited size (Godfray 1994; Quicke 1997). The host organism is usually the immature stage of another insect species. Parasitoid larvae develop to adulthood by feeding on the body of the host, eventually

killing it, and when more than one parasitoid larva develops per host, they are likely to compete for host resources.

Parasitoid species can be classified according to the behaviors exhibited by developing larvae when placed together in or on a host. Larvae of solitary species engage in contest (interference) competition and eliminate rivals during the first instar by physical attack (frequently with enlarged, sclerotized mandibles) or physiological suppression (whereby competitors are dispatched by way of toxins or oxygen deprivation) until only one individual survives (Godfray 1994; Mayhew and van Alphen 1999). In parasitoid wasps, if antagonistic contest competition occurs, it overwhelmingly takes place during the first instar when larvae have enlarged sclerotized mandibles (Salt 1961; Chow and Mackauer 1986; Godfray 1994; Mayhew and van Alphen 1999). In one study where solitary parasitoids were experimentally prevented from developing sclerotized mandibles, multiple individuals developed together presumably because competitors were unable to engage in brood reduction (Khoo et al. 1985). Larvae of gregarious species, in contrast, tend to engage in scramble (exploitative) competition and more than one offspring can successfully develop per host (Godfray 1994). Any reduction in initial clutch size is expected to occur throughout development and, particularly, in later larval stadia when larvae compete intensely for diminishing resources.

The majority of the parasitic Hymenoptera are solitary. The phylogenetic distribution of solitary and gregarious development strongly suggests that gregariousness has independently replaced solitary development in many taxa on numerous occasions (Rosenheim 1993; Mayhew 1998; Mayhew and Hardy 1998; Michel-Salzat and Whitfield 2004; Lopez-Vaamonde et al. 2005), and gregarious development is likely

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Received 29 January 2008; revised 24 October 2008; accepted 30 October 2008.

to be the derived state within the group (le Masurier 1987; Mayhew 1998; Michel-Salzat and Whitfield 2004). This raises challenges, both theoretical and empirical, in understanding the evolutionary transition in the behavior of developing wasps. In particular, the relaxation of sibling rivalry along with the shift from contest to scramble competition presents an evolutionary conundrum because of the advantage of fighting behavior to individual fitness (Godfray 1987; Rosenheim 1993; Ode and Rosenheim 1998; Hoffmeister et al. 2005).

Theory suggests several conditions that favor the transition from solitary to gregarious development. Initial population-genetic models (Godfray 1987) assumed an asymmetry in fighting ability (fighting versus nonfighting phenotypes). If an allele resulting in a nonfighting (tolerant) larval phenotype is to spread in a population, the per capita fitness of an individual tolerant larva developing together with other tolerant larvae has to be greater than that when developing alone. Additional models and empirical observations demonstrated that this criterion can be relaxed when the proportion of single-sexed broods laid increases or when there is a female-biased sex ratio of eggs laid due to the resultant increase in within-brood relatedness (Rosenheim 1993; Ode and Rosenheim 1998).

More recent models, following new experimental evidence (Boivin and van Baaren 2000; Pexton and Mayhew 2001), have instead assumed a reduction in larval searching/mobility rather than a reduction in larval fighting capabilities (Boivin and van Baaren 2000; Pexton et al. 2003). In these models, an allele for "larval immobility" will spread if the fitness of an immobile gregarious brood is higher than the fitness of a solitary, mobile individual, thus substantially reducing the stringency of the criterion for the evolution of gregarious development. Immobile larvae may fight, but direct contact with other individuals may be infrequent.

An important challenge now is to assemble empirical evidence to evaluate the relative importance of reduced mobility and reduced aggression as mechanisms promoting gregarious development. If gregariousness arose after a reduction in larval mobility, one prediction is the existence of gregarious parasitoids that retained the ability to fight as larvae. Contest competition will not ordinarily occur in such species (lethal fighting will be prevented effectively if developing larvae do not physically contact one another during the first larval instar). However, if the number of developing larvae in a host increases, such that the likelihood of coming into direct contact with broodmates increases, we predict that brood reduction would result from density-dependent fighting during the first instar (when larvae possess enlarged mandibles). This is in contrast to solitary species in which the presence of any supernumerary larvae results in systematic lethal fighting between rivals and hence is a form of density-independent contest competition. Alternatively, if gregariousness is the result of reduced fighting behavior or ability, then any competition between developing wasps will be of a scramble nature. Brood size reduction may result from increased competition especially in older, larger larvae that compete for increasingly limited host resources.

Experimental investigation of these differing possibilities is required to test contrasting models (the reduced mobility hypothesis versus the reduced aggression hypothesis) of the evolution of gregariousness and to understand the nature and importance of competition between developing wasps. There are only a few studies examining the competitive abilities of gregarious larvae in the context of the reduced mobility hypothesis (Boivin and van Baaren 2000; Marktl et al. 2002; Pexton and Mayhew 2004). Moreover, the few studies that have been conducted have focused on interspecific competitive interactions between gregarious species and solitary spe-

cies rather than intraspecific competition within a gregarious species.

In this study, we examine intraspecific competitive interactions in a gregarious species in light of predictions made by these 2 models using the gregarious parasitoid, *Cotesia flavipes* Cameron (Hymenoptera: Braconidae). In a study of host discrimination, Potting et al. (1997) observed that naive *C. flavipes* females readily lay clutches into previously parasitized hosts (a phenomenon known as superparasitism). Superparasitizing wasps laid full clutches (between 30 and 40 eggs), but emerging broods were restricted in size to that expected when only a single clutch had been laid (Potting et al. 1997). Using serial dissections to measure changes in parasitoid brood size over the course of larval development of *C. flavipes*, we assess whether brood size reductions are restricted to the first parasitoid instar (suggesting that gregariousness arose as a result of a reduction in larval mobility) or whether brood size reductions occur throughout immature development (suggesting that gregariousness arose as a result of reduced aggression). To support our inferences from the serial dissections regarding the mechanism of clutch-size reduction, we measure larval mobility in vitro of *C. flavipes* and contrast it to larval mobility of the solitary *Cotesia vestalis* (Haliday) and the gregarious *Cotesia congregata* (Say). Our current study fills in an important gap in the understanding of how juvenile behavior influences brood size and, consequently, the mechanisms permitting the evolution of clutch size.

METHODS

Study species and rearing methods

The genus *Cotesia* Cameron contains 400 described species (Shaw and Huddleston 1991) and an estimated total of nearly 1000 species worldwide. As *Cotesia* contains both solitary and gregarious species, and because gregarious development has apparently independently evolved on several occasions within this genus (Michel-Salzat and Whitfield 2004), it represents a promising group with respect to the empirical investigation of the competitive interactions and development modes within the parasitic Hymenoptera. The 3 *Cotesia* species in this study are koinobionts (allowing their hosts to continue development; Askew and Shaw 1986).

Cotesia flavipes and *Diatraea saccharalis*

Cotesia flavipes is a gregarious endoparasitoid of stem-boring moth larvae in graminaceous plants including the sugarcane borer, *Diatraea saccharalis* (Fabricius) (Lepidoptera: Pyralidae) (Ngi-Song et al. 1995; Alleyne and Wiedenmann 2001). Both *C. flavipes* and *D. saccharalis* were obtained from Dr Nancy Beckage (Department of Entomology, University of California—Riverside). Parasitized and unparasitized *D. saccharalis* larvae were reared at 27 °C and a 16L:8D photoperiod in 30-ml plastic soufflé cups (Solo Cup, Highland Park, IL) half filled with an artificial sugarcane borer diet (Southland Products, Lake Village, AR).

Cotesia flavipes colonies were maintained by exposing third and fourth instar sugarcane borer larvae to female wasps until oviposition was observed. Parasitized sugarcane borer larvae were then allowed to continue feeding on artificial diet until wasp larvae emerged and pupated, which was usually during the fifth or sixth instar of the sugarcane borer. On emergence, adult male and female *C. flavipes* were held together in liter plastic containers and given access to 10% honey water.

Cotesia vestalis and *Plutella xylostella*

Cotesia vestalis (= *Cotesia plutellae*) is a solitary endoparasitoid of diamondback moth larvae, *Plutella xylostella* L. (Lepidoptera:

Plutellidae) (Shaw 2003). Approximately 200 *C. vestalis* were obtained from Biofac Crop Care (Mathis, TX) in 2004 and reared on *P. xylostella*, which were collected from cabbage fields near St Paul, Minnesota, United States.

Adult wasps were kept in cages (30 × 30 × 30 cm; Bug-Dorms I, Bioquip, Rancho Dominguez, CA) with access to honey water. Mated females were allowed to oviposit in second or third instar *P. xylostella* host larvae, which were subsequently allowed to complete development on cabbage plants until wasp cocoons were formed.

Cotesia congregata and *Manduca sexta*

Cotesia congregata is a gregarious endoparasitoid of sphingid larvae including *Manduca sexta* L. (Lepidoptera: Sphingidae) (Shenefelt 1972). *Cotesia congregata* was collected from tobacco fields in North Carolina by Dr Clyde Sorenson (Department of Entomology, North Carolina State University), and *M. sexta* were provided by Dr Karen Mesce (Department of Entomology, University of Minnesota). Both *C. congregata* and *M. sexta* were maintained at 25 ± 1 °C and a 16L:8D photoperiod. Both parasitized and unparasitized *M. sexta* larvae were reared in 30-ml plastic soufflé cups (Solo Cup) half filled with an artificial tobacco hornworm diet (Bioserv, Frenchtown, NJ).

Cotesia congregata colonies were maintained by exposing late second instar tobacco hornworm larvae to female wasps until oviposition was observed. Parasitized tobacco hornworm larvae were then allowed to continue feeding on artificial diet until wasp larvae emerged and pupated. On emergence, adult male and female *C. congregata* were held together in liter plastic containers and given access to 10% honey water.

Single parasitism versus superparasitism

Parasitism

To obtain both singly parasitized and superparasitized hosts (controls and experimental treatments, respectively), we performed the following procedure. Mated female *C. flavipes* wasps (up to 24 h old) that had not previously been exposed to hosts were placed individually into a 35 × 10 mm plastic Petri dish with a single host (late third/early fourth instar sugarcane borer larvae). Wasps were observed for up to 3 min and removed from the dish on parasitizing the host. If a female rejected the host, both the female wasp and the host were replaced and the process started again with a new wasp and host. In the superparasitized treatment, a second female was introduced into the dish immediately after the removal of the first parasitizing wasp, observed for up to 3 min, and then removed on parasitizing the host. Naive females readily superparasitized previously parasitized hosts. Hosts were alternately assigned to be parasitized or superparasitized. All parasitized sugarcane borer larvae were placed singly into diet cups containing artificial diet as described above. A total of 180 hosts were singly parasitized and 180 hosts were superparasitized.

Serial dissections

We dissected both singly parasitized and superparasitized hosts, starting 3 days postparasitism, in order to quantify the developmental trajectory and mortality of developing wasps. Preliminary observations revealed that the eggs of *C. flavipes* are extremely small and translucent on oviposition and thus difficult to count reliably. However, they rapidly swell, and by day 3 eggs are easily visible with a light microscope and by day 4 almost all wasp eggs hatch into first instars. By day 6, all larvae had molted to the second instar. Hence, we started our dissections at day 3 continuing daily through to day 12 ($n = 15$ per day, for both treatments; 300 total dissections) which is when wasp larvae typically stop feeding, exit their hosts to spin cocoons and complete development into adults

outside of the host remains. Hosts were dissected in Ringer's solution and the number of wasp eggs and larvae recorded, and larvae were examined for signs of direct combat (obviously injured or destroyed larvae).

Adult development

We allowed wasp broods from the remaining 30 singly parasitized and 30 superparasitized broods to complete their development and recorded developmental time (both to exiting the host and to adult eclosion) along with emerging brood size and the sex ratio of emerging broods (proportion of males among adult offspring). Differences in adult sex ratio between singly parasitized and superparasitized hosts would indicate sex-specific differences in developmental mortality.

Larval mobility

We measured the mobility of first instar *C. flavipes* and compared it with mobility exhibited by the solitary *C. vestalis* and the gregarious *C. congregata*. First instars of *C. flavipes*, *C. vestalis*, and *C. congregata* were dissected from their host larvae (*D. saccharalis*, *P. xylostella*, and *M. sexta*, respectively) 4 days after parasitism. Larval movement was recorded as described in Pexton and Mayhew (2004). *Cotesia* larvae were dissected and held in a drop of Ringer's solution for 5 min. The number of folds (vertical bending) and twists (lateral torsion) was recorded in 5 consecutive 1-min blocks using a stereomicroscope at ×50 magnification. Comparing larval movement across the five 1-min time periods allowed us to determine whether larval movement drops as a consequence of being held outside the host for this period of time.

Statistical analyses

Data analysis was performed using SAS 9.1.3 (SAS Institute, 2006). An analysis of covariance (ANCOVA) was performed to compare the number of eggs and/or larvae present in singly and superparasitized hosts from days 3 through 12 (day postparasitism was treated as the covariate). Although these data consisted of counts, the means were high and the residuals were normally distributed. Two-sample *t*-tests were used to compare the number of adults, the development time from parasitism until larval emergence from the host, and the development time from parasitism until adult emergence between singly parasitized and superparasitized hosts. The effect of number of females contributing offspring to a host (single vs. superparasitism) on the sex ratio of adult offspring (number of males/total number of adults) was analyzed using logistic regression. Mobility (twists and folds) data across the five 1-min intervals were analyzed with a repeated measures analysis of variance separately for each species.

RESULTS

Serial dissections

The numbers of *C. flavipes* eggs and larvae present across days 3 through 12 differed significantly between superparasitized and singly parasitized hosts (ANCOVA, $F_{3,296} = 139.24$, $P < 0.0001$; Figure 1). On dissection 3 days postparasitism, superparasitized hosts contained nearly twice as many eggs on average (mean ± standard error [SE]: 68.60 ± 3.34 eggs) than did singly parasitized (36.27 ± 1.75 eggs) hosts (2-sample *t*-test, $t_{28} = 8.59$, $P < 0.0001$), indicating that additional eggs had been laid by superparasitizing females. Whereas the number of *C. flavipes* offspring in superparasitized hosts significantly declined from day 3 to day 12 (slope = -4.04 offspring/day, *t*-statistic from ANCOVA = -10.26,

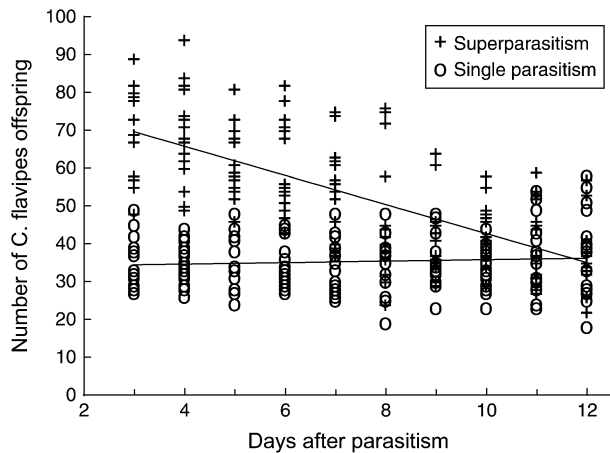


Figure 1

Number of *Cotesia flavipes* eggs and larvae observed from dissected superparasitized (plus symbols) and singly parasitized (open circles) sugarcane borer larvae (per day) over the course of endoparasitic development ($n = 15$ per day in both treatments). The regression relationships between the number of *C. flavipes* offspring and day postparasitism differed significantly between superparasitized and singly parasitized broods ($F_{3,296} = 139.24$, $P < 0.0001$; superparasitized broods: offspring = $81.11 - 3.84$ [day]; singly parasitized broods: offspring = $33.86 + 0.19$ [day]). Whereas the number of *C. flavipes* larvae declined linearly from day 3 through day 12 in superparasitized broods (t value = -10.26 , $P < 0.0001$), the number of larvae remained constant throughout the same development period (t value = 0.69 , $P = 0.49$).

$P < 0.0001$), the number of *C. flavipes* offspring in singly parasitized hosts did not change from day 3 to 12 (slope = 0.19 offspring/day, t -statistic from ANCOVA = 0.69 , $P = 0.49$) (Figure 1). By day 12, however, there were no significant differences in the number of larvae present in superparasitized (37.33 ± 2.28) or singly parasitized (38.73 ± 3.09) hosts (2-sample t -test, $t_{28} = 1.09$, $P = 0.28$).

Dissections of both singly parasitized and superparasitized hosts revealed similar developmental trajectories: On day 3, swollen eggs about to hatch were observed in both types of hosts; on days 4 and 5 postparasitism, first instars were present in both singly parasitized and superparasitized hosts. By day 6, all larvae had molted into the second instar in both types of hosts. In none of the 300 dissections did we find evidence of wounded or destroyed larvae. Indeed, in superparasitized hosts, first instar *C. flavipes* were observed to be clustered and in direct contact with broodmates within the host and yet did not appear to be engaging in aggressive activity (Figure 2A). Despite possessing visible mandibles (Figure 2B), first instar *C. flavipes* shows no evidence of engaging in fighting behavior such as the presence of dead competitors with puncture wounds. Second and third instar *C. flavipes* did not possess visible mandibles. Instead, larvae in superparasitized hosts are variable in size (Figure 2A), suggesting that scramble competition occurs between developing larvae for host resources.

Adult development

The average number (\pm SE) of adult wasps emerging from superparasitized and singly parasitized hosts did not differ significantly (36.2 ± 1.23 adults from superparasitized hosts and 34.29 ± 1.28 from singly parasitized hosts; $t_{58} = -0.73$, $P = 0.47$) indicating high levels of developmental mortality within superparasitized hosts. The average number of days from parasitism to larval emergence (\pm SE) from the host did not differ significantly in broods from either superparasitized or singly

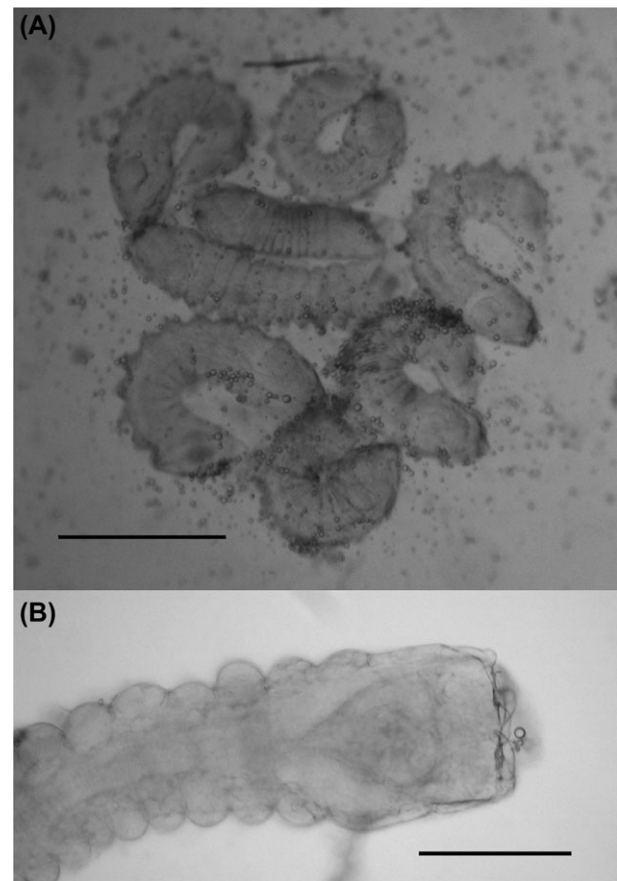


Figure 2

Photomicrographs of *Cotesia* first stadia larvae: (A) cluster of *Cotesia flavipes* larvae from a recently dissected host, *Diatraea saccharalis*, (B) reduced mandibles of first instar *C. flavipes*. Length of black scale bar is 0.5 mm for (A) and 0.15 mm for (B).

parasitized hosts (13.47 ± 0.40 days in superparasitized hosts; 13.23 ± 0.35 days in singly parasitized hosts; $t_{58} = 0.43$, $P = 0.67$). The average number of days from parasitism to adult eclosion (\pm SE) did not significantly differ in broods from either superparasitized or singly parasitized hosts (19.77 ± 0.49 days in superparasitized hosts and 18.93 ± 0.47 in singly parasitized hosts; $t_{58} = 1.22$, $P = 0.23$). The sex ratio at emergence of adults did not significantly differ from broods emerging from either superparasitized or singly parasitized hosts (33.91% male from singly parasitized hosts, 35.67% male from superparasitized hosts; Wald chi-square = 0.6265 , $P = 0.4286$).

Larval mobility

Over the 5-min observation period, *C. flavipes* larvae exhibited significantly more movement, both in terms of number of folds and twists, than larvae of the gregarious *C. congregata*. On the other hand, *C. flavipes* larvae did not exhibit as much movement as larvae of the solitary *C. vestalis* (Figure 3). In all 3 species, the number of twists and folds exhibited by first instars were consistent over the five 1-min time periods (Figure 4A–C).

DISCUSSION

We found that brood sizes of superparasitized hosts gradually declined as *C. flavipes* individuals developed. Furthermore, we

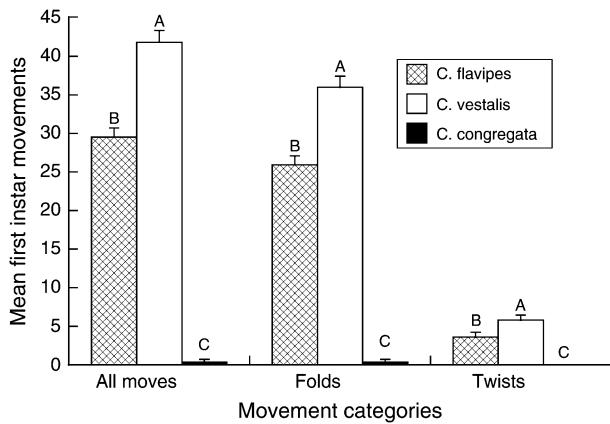


Figure 3 Mean number of first instar movements (total moves, folds, twists) of *Cotesia flavipes*, *Cotesia vestalis*, and *Cotesia congregata* observed over 5 min. Combined movements (folds and twists) differed significantly among the 3 *Cotesia* species ($F_{2,42} = 705.05, P < 0.0001$). Similarly, the number of folds ($F_{2,42} = 537.58, P < 0.0001$) and twists ($F_{2,42} = 108.02, P < 0.0001$) differed significantly among the 3 species.

found no indication of wounded or destroyed larvae in any dissections that would typically result from aggressive behavior (Mayhew and van Alphen 1999) despite developing in close proximity to broodmates, possessing visibly sclerotized mandibles, and being highly mobile. The reduced mobility hypothesis predicts that if the proximate mechanism allowing gregarious development is reduced larval searching/mobility (with fighting ability retained), then most mortality should occur during the first instar as a result of physical aggression under crowded conditions (i.e., superparasitism) when immobile larvae are forced in contact with one another and little, if any, mortality in older instars. However, first instar *C. flavipes* exhibited mobility levels approaching those found in *C. vestalis* first instars, a species whose larvae are known to fight, rather than lack larval mobility similar to that found in another gregarious species, *C. congregata*. One possible explanation for this pattern is that gregariousness arose more recently in *C. flavipes* compared with *C. congregata*; however, our data set is too limited to make any definitive conclusions. In contrast, under the reduced aggression hypothesis, if any brood reduction occurs under crowded conditions in gregarious species, mortality should occur throughout development and should be a consequence of starvation. Taken together, the results of our study are consistent with the reduced aggression hypothesis and not consistent with the reduced mobility hypothesis.

We found that naive *C. flavipes* females readily superparasitized hosts and laid an additional clutch of similar size to those expected in singly parasitized hosts (approximately doubling the number of eggs present). Despite this, the final brood size from superparasitized hosts was similar to that from singly parasitized hosts. This indicates that there is intense competition within superparasitized hosts resulting in substantial mortality among developing wasps. Although there was significant parasitoid mortality within superparasitized hosts, there was not a significant decrease in larval numbers during the first instar nor was there any difference between the numbers of eggs and first instars within superparasitized larvae. Similar numbers of eggs and first instars suggest that physical aggression with enlarged mandibles is not responsible for clutch-size reduction. Instead, within superparasitized hosts, the number of *C. flavipes* larvae de-

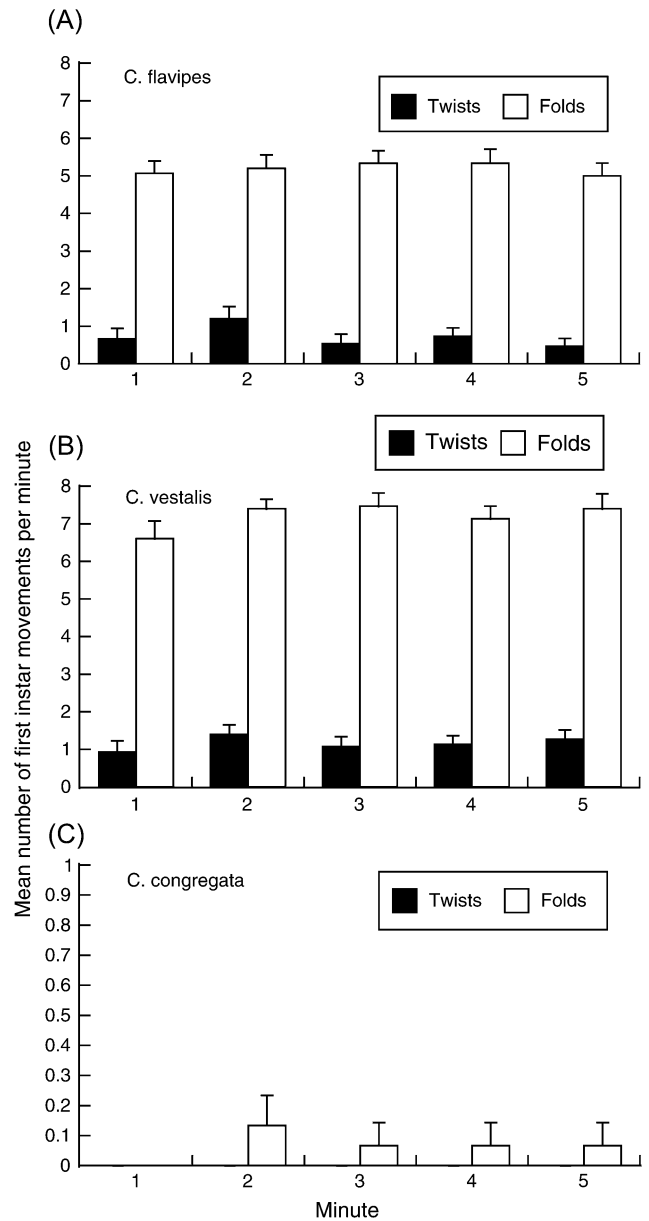


Figure 4 Mean number of folds (white bars) and twists (black bars) observed per minute in (A) *Cotesia flavipes*, (B) *Cotesia vestalis*, and (C) *Cotesia congregata*. The number of movements (folds or twists) did not differ over time for *C. flavipes* (folds: Wilks' $\lambda = 0.79, F_{4,11} = 0.74, P = 0.59$; twists: Wilks' $\lambda = 0.75, F_{4,11} = 0.94, P = 0.48$), *C. vestalis* (folds: Wilks' $\lambda = 0.66, F_{4,11} = 1.44, P = 0.28$; twists: Wilks' $\lambda = 0.80, F_{4,11} = 0.69, P = 0.61$), or *C. congregata* (folds: Wilks' $\lambda = 0.73, F_{4,11} = 1.00, P = 0.49$; no twists were observed). In panel C, note the difference in the scale of the y axis and that no twisting movements were observed during the 5-min observation period in any *C. congregata* larvae.

clined gradually as hosts (and parasitoid larvae) increased in age. Furthermore, although not quantified in our study, the size of developing *C. flavipes* larvae appeared more variable in superparasitized hosts. Our observations corroborate the findings of a previous study of *C. flavipes* developing in another stemborer, *Chilo partellus* (Swinhoe) (Lepidoptera: Pyralidae), which found that adult male and female *C. flavipes* were smaller when developing on a superparasitized host suggesting scramble competition for limiting host resources

(Potting et al. 1997). Both the previous experimental and our observational evidence strongly suggest that contest competition does not occur within *C. flavipes*, or if it does, it is much less important than scramble competition. Taken together, these findings are consistent with the reduced aggression hypothesis associated with gregariousness in this species.

Interspecific studies involving members of the genus *Cotesia* also support the reduced aggression model. Interspecific competition has been investigated in the gregarious *Cotesia glomerata* (L.) and the solitary *Cotesia rubecula* (Marshall), both of which attack a common host, *Pieris rapae* (Lepidoptera: Pieridae) (Laing and Corrigan 1987). When both species are present in the same host, *C. rubecula* always successfully survived if it had been the first egg oviposited; *C. rubecula* won (via physical combat) in more than 90% of hosts when it had been the secondarily oviposited egg (Laing and Corrigan 1987). During the first instar, *C. rubecula* has highly developed mandibles, whereas *C. glomerata* has comparatively poorly developed mandibles. These observations would, prima facie, support the reduced aggression model based on fighting/nonfighting larval phenotypes. In addition, observations of *C. rubecula* first instar larvae in host hemocoel demonstrated them to be highly mobile. *Cotesia rubecula* larvae appeared to actively search or "hunt" for other larvae (Laing and Corrigan 1987). These observations are suggestive of the reduced aggression hypothesis and that the solitary lifestyle requires high levels of movement and searching, along with the ability to fight within the genus *Cotesia*.

In contrast, in studies of interspecific competition between gregarious species and their solitary sister species (*Anaphes listronoti* Huber and *Anaphes victus* Huber [Mymaridae] [Boivin and van Baaren 2000]; *Aphaereta pallipes* (Say) and *Aphaereta genevensis* Fischer [Braconidae] [Mayhew and van Alphen 1999; Pexton and Mayhew 2004]), the first instars of the gregarious species retain similar, if not identical mandibular morphologies compared with their solitary sister species. Findings in both of these systems support the reduced mobility hypothesis.

In *C. flavipes*, competition for host resources under superparasitism restricts final brood size to that expected in singly parasitized hosts. The pattern of mortality in developing *C. flavipes* wasps is consistent with scramble competition, thus the evolution of gregariousness appears, in this species, to conform much more closely to the reduced aggression hypothesis (fighting/nonfighting behavioral phenotypes) as opposed to the reduced mobility hypothesis (searching/nonsearching behavioral phenotypes). This study is, to our knowledge, the first explicit comparison of the reduced aggression hypothesis with the reduced mobility hypothesis for a gregarious parasitoid wasp. However, further study of larval behavior and competitive interaction in other microgastrine genera that contain both solitary and gregarious species (e.g., *Apanteles*, other species of *Cotesia*, *Diolcogaster*, *Glyptapanteles*, and *Microplitis*) is required to ascertain whether the proximate mechanism for gregariousness varies within and among these genera and to understand further other possible factors (e.g., patterns of within-brood relatedness) influencing the evolutionary transition away from solitary development.

FUNDING

United States National Science Foundation (0344665 to P.J.O.; 0344131 to G.E.H. and L.E.M.V.; and 0344829 to J.B.W.).

We thank Karen Mesce for providing *M. sexta*, Chris Asmundson, Dan Barta, Virginia Howick, Lynn Knutson, Beth Sandagar, and Laura Stone for assistance rearing insect colonies and 2 anonymous reviewers for their insightful comments.

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