



Gregarious development in alysiine parasitoids evolved through a reduction in larval aggression

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Population genetic models have suggested that siblicide between the larvae of parasitoid wasps, once gained, can be lost only under stringent conditions, making transitions from solitary to gregarious development rare. However, phylogenetic studies suggest that gregarious development has evolved on numerous occasions, although the mechanisms are largely unknown. We report experiments, on two morphologically similar species of alysiine braconids, directed at an understanding of how gregarious development evolved in one subfamily. We compared the oviposition behaviour and development of *Aphaereta genevensis* and *A. pallipes* in the laboratory, on the host *Drosophila virilis*. *Aphaereta genevensis* usually lays a single egg in each host, and only a single wasp usually develops successfully even when several eggs are laid. However, *A. pallipes* often lays more than one egg in each host, and several offspring often complete development. Dissections of superparasitized hosts showed that this difference is accompanied by differences in larval behaviour: first-instar *A. genevensis* use their sharp mandibles to kill other parasitoid eggs or larvae in the same host. First-instar *A. pallipes* also have sharp mandibles, but do not attack conspecific larvae, suggesting that siblicide might have been lost by a simple change in larval behaviour. *Aphaereta genevensis* shows some features that may have helped select for reduction in larval aggression in the subfamily: a longer development time, multiple egg clutches and incomplete brood reduction. *Aphaereta* spp. show great promise as model systems for studying the evolution of siblicide.

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The behaviour of developing offspring towards their siblings, and its effects on parents, has been the subject of intense research by evolutionary biologists (Cheplick 1992; Elgar & Crespi 1992; Mock & Parker 1997). Current interest stems largely from work by Hamilton (1963, 1964a, b), who showed that selection should act on behaviour towards other individuals according to the direct costs and benefits of the actions themselves, as well as the relatedness of the actor and recipient (see Grafen 1984, 1991; Mock & Parker 1997), defining limits to altruistic and selfish behaviour. The resulting behaviour of family members towards each other may include conflict between offspring (Mock & Parker 1997), and conflict between parents and offspring (Trivers 1974; Godfray 1995).

Some observations that have stimulated theory surrounding this problem concern the oviposition behaviour and development of parasitoid wasps (Hymenoptera),

which lay their eggs on or in a host organism, normally another insect. The developing wasp larvae feed on the still-living body of their host, eventually killing it (Godfray 1994; Quicke 1997). Probably the majority of parasitoid wasp species develop solitarily, meaning that only one offspring develops per host. If more than one egg is laid in a host, the offspring often fight (by physical or physiological means) until only one remains (Salt 1961), behaviour known as siblicide. However, in a number of taxa, wasps develop gregariously: parent wasps may lay several eggs per host, and more than one completes development. Population genetic models have suggested that siblicidal behaviour may evolve in parasitoid populations because, under a range of conditions, it pays offspring to kill their broodmates to gain possession of the entire host (Godfray 1987). One such condition is when offspring develop in a small brood of two or three eggs, which minimizes the inclusive fitness cost of siblicide (Godfray 1987). Once present in a parasitoid population, siblicide has several consequences. One is that parents may be forced to reduce their clutch size to avoid wasting eggs that will inevitably fail to complete development (e.g. Skinner 1985; Waage &

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Godfray 1985; although see Rosenheim & Hongkham 1996). This is thus an example of parent–offspring conflict over clutch size, in which offspring have control (Parker & Mock 1987; Godfray et al. 1991). A second consequence is that it becomes very difficult for non-siblicidal behaviour to invade the population because nonsiblicidal individuals will be killed by their more aggressive broodmates (Godfray 1987). Thus the evolution of siblicide and solitary development may be irreversible (Bull & Charnov 1985; Harvey & Partridge 1987).

Some empirical evidence supports these theoretical predictions. *Apanteles* species (sensu Nixon 1965) attack both small hosts, which provide resources for only one offspring, and a range of larger hosts which provide enough resources for several offspring. However, broods of 2–12 eggs are rare and many solitary species attack hosts large enough to support gregarious broods (le Masurier 1987). This suggests that siblicide has evolved in small gregarious broods and that solitary development has taken its place, just as Godfray's (1987) model predicts. However, such observations do not hold for many other parasitoid taxa in which small gregarious broods are apparently stable (Rosenheim 1993; Mayhew 1998a, b; Mayhew & Hardy 1998). In addition, the phylogenetic distribution of solitary and gregarious development suggests that solitary development is ancestral to most if not all families and that gregarious development has evolved on numerous occasions (current data suggest at least 43 times), contrary to crude theoretical expectation (Rosenheim 1993; Mayhew 1998a).

The latter observations beg the question of how non-siblicidal behaviour can have invaded and replaced siblicidal behaviour. Theory already suggests several (albeit stringent) conditions under which this might occur (Godfray 1987; Rosenheim 1993; Ode & Rosenheim 1998), and the biggest challenge now is to assemble empirical evidence to evaluate these possible routes to gregarious development (Mayhew et al. 1998). The first step is to identify taxa in which larval behaviour and brood size have changed and to characterize the selective pressures and constraints operating on adult oviposition behaviour and larval behaviour in these systems (Mayhew et al. 1998). Such observations can then be used as the basis for more refined theoretical treatments of the problem, and eventually to conduct experimental or comparative tests of theory (Mayhew et al. 1998).

We conducted laboratory experiments on two species in the braconid genus *Aphaereta* to identify how brood size may change in parasitoids. The genus *Aphaereta* is nearly cosmopolitan and is best known in the Holarctic region (Wharton 1980). In the New World at least 16 species occur (Wharton 1977). *Aphaereta* belongs to the subfamily Alysiinae, containing over 1000 described species in about 70 genera (Shaw & Huddleston 1991), where it occupies a derived position with the genera *Asobara* and *Phaenocarpa* (Wharton 1980; Gimeno et al. 1997). The Alysiinae itself forms a monophyletic group with the subfamily Opiinae (containing over 1300 species; Quicke & van Achterberg 1990; Quicke 1993; Gimeno et al. 1997; Belshaw et al. 1998), and all members of this clade are endoparasitoids of cyclorrhaphous

Diptera, and do not permanently parasitize their hosts, hence are termed koinobionts (Shaw & Huddleston 1991). With the exception of a recently described species of *Phaenocarpa* (van Achterberg 1998), *Aphaereta* is the only genus in both subfamilies known to contain gregarious species (Wharton 1977, 1980, 1984; Shaw & Huddleston 1991). Together, this suggests that at least one transition from solitary to gregarious development has occurred within the *Phaenocarpa*–*Aphaereta*–*Asobara* lineage.

The gregarious nature of some *Aphaereta* species is well established from rearing records, experimental studies of clutch size and developmental studies (Evans 1933; Salkeld 1959; Houser & Wingo 1967; Hughes & Woolcock 1976; Vet et al. 1993, 1994; Visser 1994, 1996). However, the solitary nature of other alysiine parasitoids is less well established: although rearing records show that only one wasp develops from each host, the number of eggs laid in each host and the nature of larval competition is unknown except in a few cases. Reports of solitary development in *Aphaereta* species derive only from rearing records (e.g. Cole & Streams 1970; Wharton 1977). Thus, it is not known if the probable transition to gregarious development stems from parallel changes in larval behaviour within the genus, or if such species are simply facultatively solitary through the use of small hosts (e.g. Vet et al. 1993) with changes in larval behaviour having occurred deeper in the phylogeny. Evans (1933) reported that *Alysia manducator* has obligate solitary development, with supernumerary larvae being consumed only once the host contents have been fully depleted. The obligatory solitary development of *Asobara tabida* is well established from superparasitism experiments (e.g. van Alphen & Nell 1982). However, although Carton et al. (1986) reported that physical attack is involved, precise details of the brood reduction mechanism are lacking.

Here we show that two species of *Aphaereta*, which are almost indistinguishable in adult morphology (and hence are probably close evolutionary relatives), have very different oviposition strategies and larval behaviour when developing in the same host species (i.e. one is normally solitary and the other gregarious), suggesting that brood size changed through a reduction in larval aggression. We also identify other differences in biology which may help explain the different oviposition and developmental strategies of the two species. These observations allow us to begin to construct more informed scenarios about how clutch size and sibling rivalry evolve in parasitoids and hence stimulate theoretical developments.

METHODS

Cultures

We studied laboratory populations of *A. genevensis* and *A. pallipes*. The *A. genevensis* culture was initiated from a single female captured walking on milk-cap fungi on 4 September 1996 on the North Shore of Long Island, New York. The *A. pallipes* culture was also initiated from a single female, caught on 1 October 1995 in Queens County, New York city, on a compost pile. Both species

were initially reared on *Drosophila repleta* but with deteriorating success, after which they were reared on *D. virilis* for 1 year prior to the experiments, which began in January 1998. *Drosophila* were reared in glass bottles on standard yeast-based medium. Wasps were reared in glass pots 5 cm in diameter with foam stoppers. A 2-cm layer of agar was poured into the base of the pot and allowed to cool. We added several 5–8-day-old *Drosophila* larvae, together with a dab of viscous yeast medium. Then, we added 2–10 1–4-day-old mated female wasps with no prior experience of hosts and left them until they died. Cultures were maintained at 20°C, 70% relative humidity, and 16:8 h light:dark photoperiod. The two wasp species were maintained in separate rooms to prevent contamination (adult female wasps can be distinguished only by small (overlapping) differences in the relative length of the ovipositor; males are indistinguishable at present, Wharton 1977). Emerging wasps were aspirated out of the pots daily and stored at 14°C in glass pots with an agar base and foam stoppers supplied with a drop of honey.

Experiments

The experiments were maintained under the same environmental conditions as the cultures, except that for valid comparison experimental material of both wasp species was reared in a single environment chamber (to prevent contamination, we did not open culture containers containing adult wasps until they were dead). For each species, we carried out a single parasitism and a superparasitism treatment, and measured the number of eggs laid per host and offspring emerging per host in each treatment. In the single parasitism treatment, two to four mated female wasps of variable age ($\bar{X} \pm \text{SE} = 4.33 \pm 0.81$ days in *A. genevensis*, 6.75 ± 2.57 days in *A. pallipes*) and with no prior experience of hosts were removed from storage and placed in 5-cm petri dishes with an agar base, on the surface of which were placed at least 100 third-instar *D. virilis* larvae also of variable age (6.06 ± 0.53 days exposed to *A. genevensis*, 5.30 ± 0.47 days exposed to *A. pallipes*). We observed wasps under a binocular dissection microscope until they attempted to oviposit. An oviposition attempt was recognized by probing of the ovipositor, temporary paralysis of the host and rhythmic vibration of the tip of the abdomen and ovipositor sheaths which probably represented the movement of an egg down the ovipositor (Vet et al. 1993). When the female had finished oviposition and moved away from the host, we removed the host, with a mounted needle, and placed it in a rearing tube. The wasp was also removed so that she could not oviposit in two hosts. Sometimes a female appeared to finish oviposition but did not move away from the host, and then probed the same host again and resumed oviposition. We counted this as a single oviposition visit (sensu Rosenheim & Hongkham 1996) and included these data in our analyses, but we do not know if eggs were successfully laid on both or only one of the oviposition probes. All hosts parasitized by each wasp species on a particular day's observation period ($\bar{X} \pm \text{SE} = 19.54 \pm 1.67$ for *A. genevensis*, 8.95 ± 2.04 for *A. pallipes*) were placed together in a glass

rearing tube 2 cm in diameter ($N=25$ for *A. genevensis*, $N=20$ for *A. pallipes*), containing a 2-cm layer of agar at the base, a 2-mm layer of viscous yeast medium, and capped with a foam stopper. It was necessary to rear hosts together because those reared alone often died.

To estimate the primary clutch size, after 4 days, we removed about half the surviving hosts from each rearing tube and dissected them in a drop of ringer solution by removing the head with a mounted needle and gently squeezing out the host's contents. Immediately after oviposition, parasitoid eggs are translucent, very narrow and difficult to observe amongst the host's organs. However, after 4 days they swell to a spherical shape ca. 0.5 mm in diameter and are easy to count (e.g. Evans 1933). Many of the hosts exposed to single parasitism attempts failed to contain any eggs upon dissection, and we ascribed such cases either to failed oviposition attempts or to the eggs dying early in development. The data on egg number and surviving adults per host were entirely consistent with this view, since the proportion of hosts producing emerged wasps was always lower than the proportion of hosts from which eggs were dissected, suggesting that we were not missing or destroying large numbers of eggs in our dissections (see Results). Upon pupation, puparia were washed gently out of the tube with lukewarm water and placed individually in small glass tubes with cotton-wool plugs at the base. We checked tubes daily for emerging wasps until 50 days after the host was parasitized. Any emerging wasps were left to die of starvation in the tube. We examined every tube 63–110 days after the host was parasitized, and dissected the host's remains to record the fate of the host and any wasps. The sex and hindtibia lengths of all emerged wasps were recorded.

The superparasitism treatment was identical to the single parasitism treatment except as described below. To infect flies with wasp eggs, we placed 10 third-instar fly larvae directly into rearing tubes as above, added 10 female wasps and left them for 24 h before removing them. This exposed each fly larva to several oviposition attempts. Subsequent treatment was exactly as above ($N=15$ tubes for *A. genevensis* and 14 tubes for *A. pallipes*). We dissected the larvae to count eggs 4 days after the wasps were removed (i.e. 4–5 days post parasitism) because eggs had not yet hatched at this stage but had swelled sufficiently to be easily observed; the vast majority of hosts were found to contain several eggs.

To observe the development and behaviour of wasp larvae towards conspecific larvae in the same host more directly, we infected more tubes exactly as in the superparasitism treatment but used these entirely for dissection purposes. Hosts were dissected on the fifth day after we removed the wasps (i.e. 5–6 days after parasitism) and on subsequent days up until the 14th day after we removed the wasps (14–15 days after parasitism; dissections on each day after infection were made from $N=11$ to 22 tubes per species). This covered most of the prepupal development of the wasps. Upon dissection, the number, developmental stage, length and width, and health (classified as healthy or unhealthy) of each wasp egg larva or pupa were noted. Wasp larvae were classified as

unhealthy if they both failed to move after dissection and were either extremely small or showed direct signs of damage such as extrusion of body contents or loss of body parts. Otherwise they were classified as healthy. Wasp eggs were rarely misshapen or damaged or contained malformed larvae; these were additionally classed as unhealthy.

We estimated the body size of emerging wasps by their hind tibia length. However, because the relative size of morphological parameters may be taxon and sex dependent, we considered the dry weight of the emerged wasp to be a more relevant measure of body size across species and sexes. To estimate dry weight, we constructed statistical models of the relationship between hind tibia length and dry weight for both sexes of each species, and used this model to estimate dry weight from the hind tibia measurements taken above. We constructed the model in the following way. Starved emerged wasps of known hind tibia length ($N=20$ for each sex of each species) were placed individually in small glass tubes with cotton wool stoppers. The tubes were placed in an oven at 70°C for 4 days and then weighed on a microbalance accurate to 10^{-4} mg. Subsequent analysis using GLIM (see below) resulted in the following minimum adequate model: dry weight, $\text{mg} = -0.1791 + (0.368 \times \text{hind tibia length, mm}) + (0.02035, \text{ females only}) - (0.02028, A. pallipes \text{ only})$, explaining 71.68% of the variance in wasp dry weight. Interaction terms were not significant, and a nonlinear parameter (tibia length²) failed to improve the fit of the model.

Analysis

We used general linear modelling in the GLIM statistical package (see Crawley 1993). We initially assumed Poisson error variance for count data and binomial error variance for proportion data. Statistical models were built by stepwise subtraction from a full model including all potential explanatory variables for which we had data, starting with the least significant terms. Significance was assessed by the change in deviance when a variable was removed from the model, under normal errors by an F test, and under Poisson and binomial errors by a chi-square test. Only significant terms were allowed to remain in the model, which is then termed the minimum adequate model. The appropriateness of Poisson or binomial errors was assessed by a heterogeneity factor, equal to the residual deviance divided by the residual degrees of freedom. If the heterogeneity factor was greater than 1.3, indicating overdispersion, the model was rescaled using the value of Pearson's χ^2/df (see Crawley 1993). We have presented means of untransformed proportion and count data, in Table 1 and elsewhere, in preference to less intuitive statistics such as the back-transformed means of logit transformed data. Because of this, the standard errors presented here are symmetrical; in our data these symmetrical standard errors did not yield impossible values, such as a sex ratio of less than zero.

Because hosts reared together in the same tube had experienced very similar environments, which might have been different from those in other rearing tubes

(i.e. date and time of oviposition, quality of food and humidity in tube), results would be biased according to these particular conditions if individual hosts or wasps were treated as independent replicates. To avoid this, we treated each rearing tube as an individual replicate, taking mean figures for each tube as our data for analysis. Where several measurements were made from each host in a tube (i.e. the size of wasps where several wasps emerged from each host), the mean figures from each host were first taken, and then finally the average of those means over the whole tube. This results in a loss of degrees of freedom in our analyses at the gain of greater test validity.

To test whether variances in the brood sex ratio were significantly different from binomial, we used the Meelis test (see Nagelkerke & Sabelis 1991). In the Meelis test, R is the variance ratio, calculated as the observed sex ratio variance over that expected under a binomial distribution. Therefore $R=1$ when variance is binomial, $R<1$ when sex ratios are underdispersed (precise sex ratios, with broods having similar sexual composition) and $R>1$ when sex ratios are overdispersed (when individuals of the same sex tend to be associated together in broods). The test statistic U allows us to judge the significance of any deviation from binomial variance. The Meelis test requires both that the raw data are whole numbers (which prevents us from using tube averages) and that each brood is an independent replicate, which is problematic when raw brood data reared under different conditions are unequally represented. We went ahead regardless of this possible flaw, because sex ratio variance is usually considered a rather fixed species property (see Hardy et al. 1998), relative to other variables in our analysis, such as mean sex ratio (see Godfray 1994). However, this assumption should be borne in mind when evaluating the results.

RESULTS

Single Parasitism

We dissected eggs from a greater proportion of hosts exposed to *A. pallipes* than from hosts exposed to *A. genevensis* ($\chi^2_1=24.06$, $P<0.001$; Table 1). From those hosts containing eggs, the vast majority parasitized by *A. genevensis* contained only a single egg, although a few contained up to four. Hosts parasitized by *A. pallipes* contained significantly more eggs on average than those parasitized by *A. genevensis*, ranging from one to seven eggs per host ($\chi^2_1=30.29$, $P<0.001$; Table 1). Egg length, width and volume (estimated from an ovoid model; Blackburn 1991) did not differ significantly between species (length: $F_{1,31}=3.326$, NS; width: $F_{1,31}=0.617$, NS; volume: $F_{1,31}=2.116$, NS).

Of those hosts that pupated successfully, a significantly greater proportion produced emerged adults of *A. pallipes* than of *A. genevensis* ($\chi^2_1=11.36$, $P<0.001$; Table 1). From those hosts where at least one wasp emerged successfully, significantly more emerged per host in *A. pallipes* than in *A. genevensis* ($\chi^2_1=5.21$, $P<0.025$; Table 1). Of 24 hosts from which some *A. genevensis* emerged successfully, 21 produced one wasp and three produced two wasps. Sex

Table 1. Comparison of life history variables between species and treatments (mean for each host rearing tube \pm SE, N)

Variable	<i>A. genevensis</i>		<i>A. pallipes</i>	
	Single parasitism	Superparasitism	Single parasitism	Superparasitism
Proportion dissected hosts containing eggs	0.340 \pm 0.063 (25)	0.967 \pm 0.023 (15)	0.751 \pm 0.080 (17)	1.000 \pm 0.000 (14)
Eggs per host when hosts contained at least one egg	1.115 \pm 0.069 (18)	12.734 \pm 2.907 (15)	2.210 \pm 0.228 (15)	8.423 \pm 1.259 (14)
Proportion pupating hosts from which adult wasps emerged	0.136 \pm 0.050 (24)	0.508 \pm 0.083 (15)	0.331 \pm 0.077 (17)	0.753 \pm 0.077 (14)
Wasps emerging per host when at least one emerged	1.058 \pm 0.044 (12)	1.115 \pm 0.079 (13)	2.242 \pm 0.055 (12)	4.768 \pm 0.483 (13)
Proportion males at wasp emergence	0.569 \pm 0.124 (12)	0.207 \pm 0.076 (13)	0.872 \pm 0.054 (12)	0.497 \pm 0.059 (13)
Wasp dry weight at emergence (mg)	0.132 \pm 0.009 (12)	0.147 \pm 0.006 (13)	0.082 \pm 0.007 (12)	0.059 \pm 0.006 (13)
Total dry weight of emerging brood (mg)	0.142 \pm 0.012 (12)	0.160 \pm 0.007 (13)	0.172 \pm 0.007 (12)	0.250 \pm 0.014 (13)
Time from oviposition to emergence (days)	28.45 \pm 1.69 (10)	28.48 \pm 1.00 (13)	25.73 \pm 1.16 (11)	23.31 \pm 0.31 (13)

ratio at emergence was male biased in both species, and the difference in sex ratio between species was marginally nonsignificant ($\chi^2_1=3.741$, $P<0.1$; Table 1). Sex ratio variance was nonsignificantly overdispersed in both *A. genevensis* ($R=1.490$, $U=1.414$, NS) and in *A. pallipes* ($R=1.429$, $U=1.720$, NS).

Mean time from oviposition to emergence did not differ significantly between species ($F_{1,20}=1.787$, NS; Table 1). None of the other variables investigated (brood sex ratio, the dry weight of adults emerging and the number of adults emerging) had significant effects on development time. The estimated dry weight of the wasp individuals emerging declined significantly with the total number emerging per host when fitted alone ($F_{1,23}=13.84$, $P<0.01$; Fig. 1), and this relationship did not depend on either the wasp species or sex ratio of the wasp at emergence. However, because more wasps per host emerged in *A. pallipes*, average wasp size at emergence was lower in *A. pallipes* ($F_{1,23}=10.27$, $P<0.01$; Table 1). The

estimated total dry weight of wasps emerging per host increased with brood size fitted alone ($F_{1,23}=4.409$, $P<0.05$), and was higher in *A. pallipes* when fitted alone ($F_{1,23}=4.285$, $P<0.05$; Table 1), but because these variables are almost completely confounded we cannot at present distinguish their effects.

Superparasitism

The proportion of dissected hosts that contained parasitoid eggs did not differ between species ($\chi^2_1=3.471$, NS; Table 1), and nearly every host contained eggs. In hosts that contained at least one egg, the number per host did not differ significantly between species ($\chi^2_1=1.735$, NS), and much larger clutches were found relative to the single parasitism treatment (Table 1).

The proportion of pupating hosts that successfully produced emerged adult wasps did not differ significantly between species, although the difference approached significance ($\chi^2_1=3.638$, $P<0.1$; Table 1). From those hosts where at least one wasp emerged successfully, significantly more per host emerged in *A. pallipes* than *A. genevensis* ($\chi^2_1=31.74$, $P<0.001$). Of 33 hosts that produced at least one wasp in *A. genevensis*, 30 produced one wasp and three produced two wasps. Sex ratio at emergence was female biased in both species, and a significantly greater proportion of males emerged in *A. pallipes* relative to *A. genevensis* ($\chi^2_1=5.594$, $P<0.025$; Table 1). Mean time from oviposition to emergence, assuming that eggs were laid on average half-way through the 24-h exposure to parasitism, was significantly greater in *A. genevensis* than *A. pallipes* ($F_{1,25}=12.61$, $P<0.01$; Table 1), but development time also increased significantly with the estimated dry weight of the emerging wasp individuals ($F_{1,25}=13.45$, $P<0.01$), with which species is confounded (see below). The estimated dry weight of the wasp individuals emerging was significantly higher in *A. genevensis* compared with *A. pallipes* ($F_{1,24}=10.07$, $P<0.01$; Table 1) and declined significantly with the total number emerging per host ($F_{1,24}=10.68$, $P<0.01$; Fig. 1) with which species is confounded. Overall the total dry weight emerging per host was significantly higher in *A. pallipes* than *A. genevensis* ($F_{1,25}=14.51$, $P<0.01$; Table 1) and

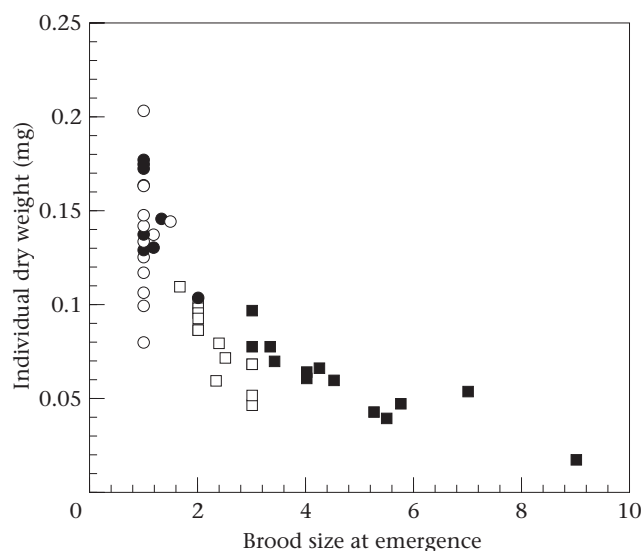


Figure 1. The estimated size of individual wasps emerging from broods of a given size. Points are means for a given rearing tube. ○, ●: *A. genevensis*; □, ■: *A. pallipes*. ○, □: Single parasitism; ●, ■: superparasitism.

increased significantly with the total number emerging per host ($F_{1,24}=12.26$, $P<0.01$) with which species is confounded.

Comparisons between Treatments

In an analysis of deviance, there was a significant interaction between treatment and species on the number of eggs per host ($\chi^2_1=6.084$, $P<0.05$). Species on its own was not significant ($\chi^2_1=1.779$, $P>0.1$), although treatment was ($\chi^2_1=75.56$, $P<0.001$). Thus, on average, more eggs per host were laid in the superparasitism treatment than in the single parasitism treatment, and the strength of this relationship varied with species (it was stronger in *A. genevensis*; Table 1). The square root transformation of the number of adults emerging was analysed, using normal errors, because the number of adults emerging showed heavy underdispersion under Poisson errors. The treatment \times species interaction had a significant effect on the (square root) number of wasps emerging ($F_{1,47}=16.55$, $P<0.01$), and both species ($F_{1,48}=32.62$, $P<0.01$) and treatment ($F_{1,48}=13.82$, $P<0.01$) were significant on their own. Thus, on average more wasps emerged from the superparasitism treatment than from the single parasitism treatment, more wasps emerged per host in *A. pallipes* than *A. genevensis*, and the effect of treatment was different in the two species, with almost no treatment effect in *A. genevensis* (Table 1).

The sex ratio of emerging wasps was significantly affected by both species ($\chi^2_1=9.33$, $P<0.005$) and treatment ($\chi^2_1=14.27$, $P<0.001$), but the interaction between species and treatment was not significant ($\chi^2_1=0.001$, NS). Thus, *A. pallipes* had a consistently more male-biased sex ratio than *A. genevensis*, and both species responded in the same way to treatment, with a lower proportion of males in the superparasitism treatment (Table 1).

The estimated dry weight of emerging wasp individuals was significantly affected by the interaction between species and treatment ($F_{1,47}=6.48$, $P<0.05$), and although species was significant on its own ($F_{1,48}=30.73$, $P<0.01$), treatment was not ($F_{1,48}=0.336$, NS). Thus, *A. genevensis* had consistently larger offspring than *A. pallipes*, and the effect of treatment was different in the two species: in *A. genevensis* superparasitism had little effect (if anything a slight increase in body size) whereas in *A. pallipes* superparasitism led to a decrease in body size (Table 1). Figure 1 suggests that for a given species of wasp, even after brood size is controlled for, wasp size is greater if wasps developed under superparasitism than under single parasitism. However, once wasp species and brood size are controlled for, treatment is not significant ($F_{1,46}=0.702$, NS) but sex ratio is ($F_{1,47}=6.13$, $P<0.05$). This suggests that females, which have greater dry weights for a given tibia length than males, are able to extract more resources from a given host than males. Together with the fact that the total dry weight of offspring was much greater under superparasitism than single parasitism in *A. pallipes* (Table 1), this suggests that hosts may not be a fixed quantity of resource, but that the wasps are able to keep their host alive and growing to some extent if they require more resources for development.

Assuming that emerging wasps from the superparasitism treatment started to develop on average half-way through the 24-h oviposition period, treatment had no significant effect on time from oviposition to emergence ($F_{1,45}=1.27$, NS), but development time was significantly longer in *A. genevensis* than *A. pallipes* ($F_{1,46}=11.19$, $P<0.01$; Table 1). The interaction between species and treatment was not significant ($F_{1,44}=1.30$, NS).

Development under Superparasitism

Dissected hosts contained either large eggs or first-instar larvae 5–6 days after oviposition (Figs 2, 3a, b). The first-instar larvae of both species were similar in general morphology. They were hymenopteriform, with a blind gut and large head capsules (Figs 3b, 4). The ridges surrounding the mouthparts were strengthened and sclerotized, as were the mandibles which were sharply pointed (Fig. 4d, e). In both species the first instar was initially surrounded by an extraembryonic 'serosal' membrane, which in *A. pallipes* consisted of very large cells covered with villi (Fig. 3b). In *A. genevensis* the first-instar larvae used their mandibles to attack other eggs and larvae (Fig. 4a, b), and the serosal membrane was sometimes very damaged and dissociated (Fig. 4c). Of 30 larvae dissected on days 5–7 and found to contain at least two *A. genevensis* offspring, seven instances (in six hosts) were observed of one first-instar larva with its mandibles embedded in another first-instar larva (six instances, e.g. Fig. 4a) or egg about to hatch (one instance; Fig. 4b). Of 29 *A. pallipes* larvae dissected on the same days with more than one wasp inside, no such fights were observed. The difference in the frequency of fights observed is highly significant ($\chi^2_1=10.82$, $P<0.005$), and coincides with the presence of unhealthy first-instar larvae in *A. genevensis* but not *A. pallipes* (Fig. 4c).

In both species the serosal membrane was shed at ecdysis into the second instar or shortly afterwards (if not already lost in *A. genevensis*). The second instar was different morphologically from the first: the head was completely unsclerotized (unsclerotized sharp mandibles were present) and the body of the larva rapidly became swollen into a sausage shape (Fig. 3c). The pharynx could be seen to make strong suction movements, indicating that the diet at this stage may be largely host haemolymph. The second instar was seen from days 6–7 after parasitism with a peak occurrence on days 7–9 in *A. genevensis* and days 6–7 in *A. pallipes* (Fig. 2).

The third and final instar larva was again different morphologically (Fig. 3d), with sclerotized mouthparts in both species (see Capek 1970) between which we noticed no gross differences. Third-larval instars appeared from days 7–8 in both species (Fig. 2), and whilst the number per host peaked at over four in *A. pallipes*, it only reached just over one in *A. genevensis*, whilst numerous unhealthy first-instar larvae littered the host (Figs 2, 4c). Pupae were of the typical exarate form (i.e. with appendages free of the body, Quicke 1997; Fig. 3e). The total number of wasp offspring per host dropped from nearly seven in both species on days 5–6 to between four and five per host on days 14–15 in *A. pallipes*, and just over one per host in

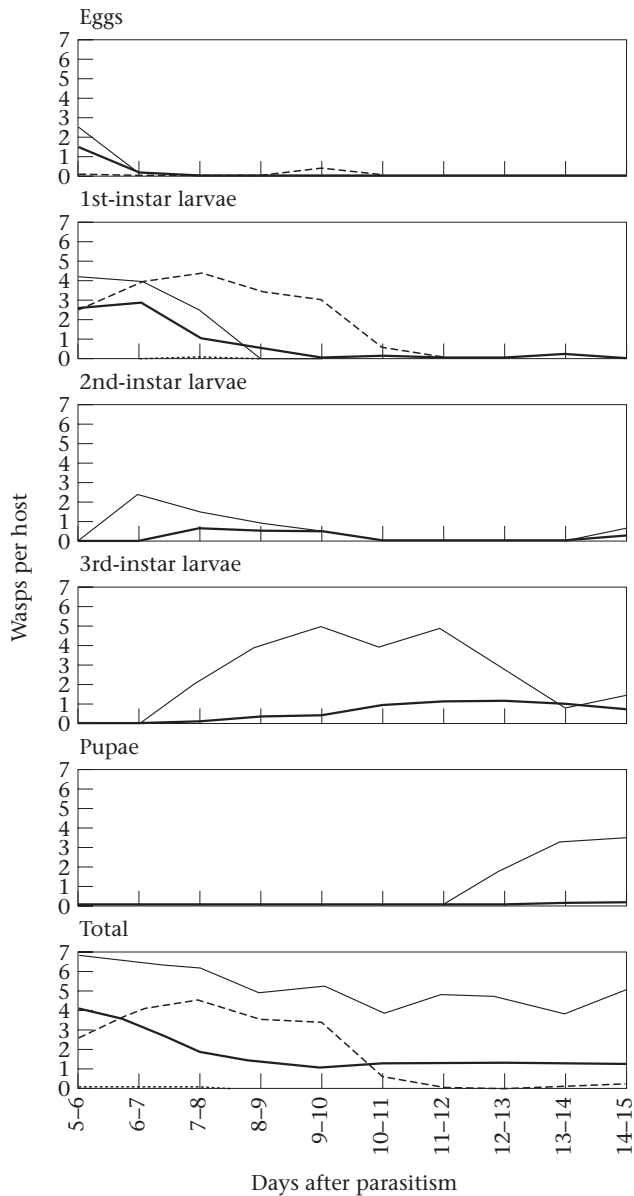


Figure 2. The number of wasps dissected from each host, of each developmental stage (\bar{X} per rearing tube) under superparasitism at different times after the host was parasitized. —: Healthy *A. genevensis*; —: healthy *A. pallipes*; ---: unhealthy *A. genevensis*; -.-: unhealthy *A. pallipes* (of which there were very few). To improve clarity errors are not shown, but the largest standard error was 1.42 individuals.

A. genevensis, a level that it had reached by days 7–8 (Fig. 2). This suggests that brood reduction occurs early in development in *A. genevensis* over about a 2-day period.

DISCUSSION

We have demonstrated differences in oviposition and brood reduction behaviour between two closely related *Aphaereta* species developing in the same host. *Aphaereta genevensis* laid only a few eggs per *D. virilis* host (usually one) and only one (occasionally two) offspring survived

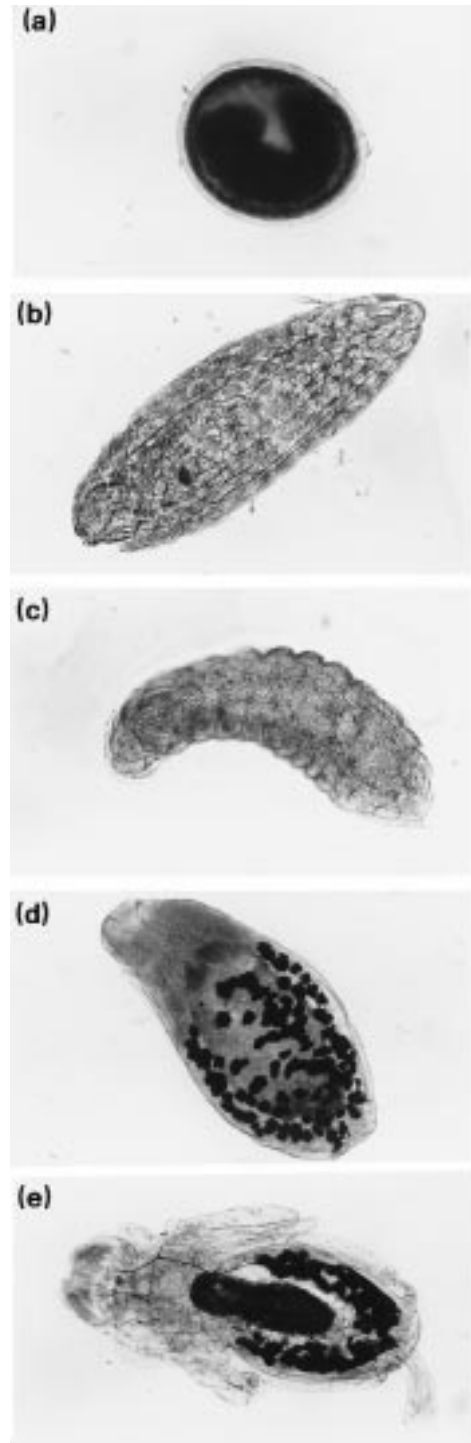


Figure 3. Developmental stages of *Aphaereta* spp. Photographs of specimens mounted whole in gelvatol, all with anterior towards the left of the picture. (a) *Aphaereta genevensis* egg just prior to eclosion (length 0.5 mm); (b) first-instar *A. pallipes* larva (length 1.5 mm) surrounded by serosal membrane; (c) second-instar *A. genevensis* larva (length 2 mm); (d) third-instar *A. genevensis* larva (length 2.5 mm); (e) *A. pallipes* pupa (length 2.5 mm).

to adult emergence, even under superparasitism. In *A. pallipes*, however, several eggs could be laid per *D. virilis* host, of which more than one usually survived,

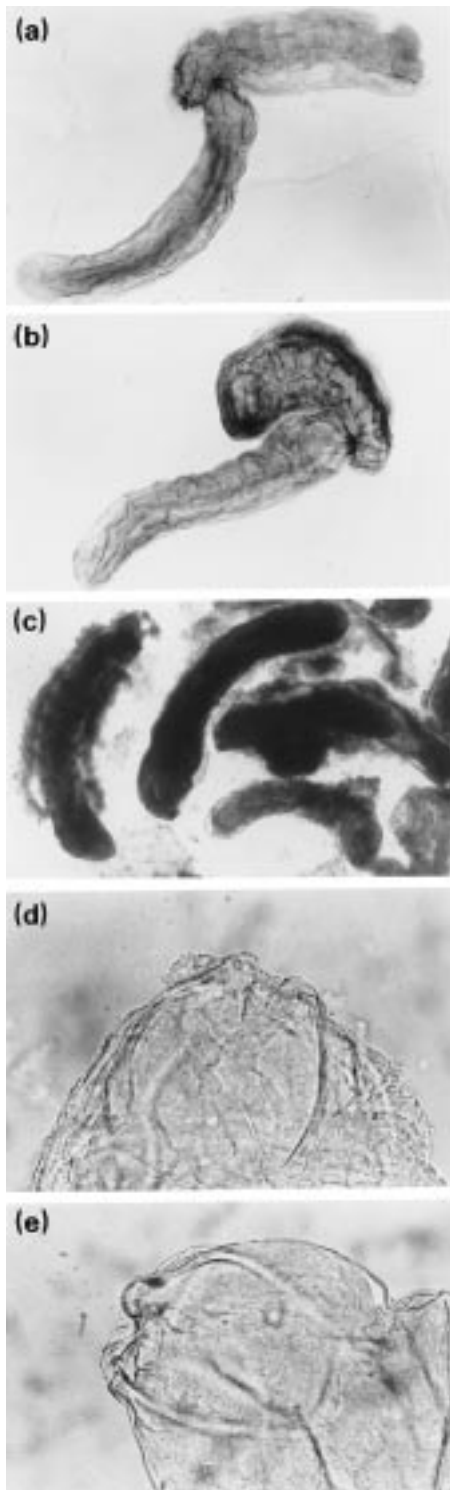


Figure 4. Combat between *A. genevensis* larvae. Photographs of specimens mounted whole in gelvatol. (a) First-instar larva (length 1 mm) biting head of first-instar larva; (b) first-instar larva (length 1 mm) biting abdomen of first-instar eclosing from egg; (c) dead first-instar larvae dissected from one superparasitized host, with the serosal membranes very dissociated and mixed with extruded body fluids and organs (cf. Fig. 3b); (d) head and mouthparts of first-instar *A. genevensis* larva showing sharp, sclerotized mandibles; (e) head and mouthparts of first-instar *A. pallipes* larva showing sharp, sclerotized mandibles.

and under superparasitism final brood size increased relative to that under single parasitism. Thus, the species that displayed the most extreme brood reduction also had the lowest clutch size at oviposition, and the largest body size, as predicted by several optimization models (Skinner 1985; Waage & Godfray 1985; Parker & Mock 1987). Brood reduction in *A. genevensis* occurred in the first larval instar, at least partly by physical attack, with the sharp mandibles which both species possess. Thus, differences in brood reduction appear to be largely or purely behavioural differences. Together, these data suggest that the evolution of gregariousness in alysiines is directly linked to the invasion of nonsiblicidal larval behaviour into siblicidal populations. The data raise the questions of how this has occurred in alysiines, and why not all species have undergone this transition. Theory suggests that three groups of factors are likely to be important: the direct costs and benefits of brood reduction; the relatedness of broodmates; and underlying genetic, mechanistic and phylogenetic constraints.

One possible direct benefit of brood reduction is an increase in individual size at adult emergence. Individual body size decreased with the number of offspring emerging and was thus smaller overall in the gregarious species (Fig. 1, Table 1). We have no estimate of the relationship between adult size and fitness for either of the species in question, but field-based estimates exist for two other alysiines. In *Asobara tabida* (a solitary species) fitness increases exponentially with size, with only very large females ever expected to leave surviving offspring (Ellers et al. 1998). In *Aphaereta minuta* (a gregarious species), fitness is related asymptotically to size (Visser 1994; West et al. 1996), and even quite small females can leave surviving offspring. If the first estimate were appropriate to either species in our study, optimal clutch size would probably be a single egg for both parents and offspring because the loss in fitness resulting from sharing a host is very great. The second estimate is more likely to make multiple egg clutches optimal for parents, and whether it also makes them optimal for offspring depends on several other factors including relatedness between broodmates. Similar studies on *A. genevensis* and *A. pallipes* are needed to see if differences in the fitness consequences of size across species are in the predicted direction (i.e. a greater benefit of size in the solitary species).

Even when it occurred, siblicide was sometimes incomplete. In *A. genevensis*, more than one individual sometimes survived, in both single parasitisms (where females laid more than 1 egg per clutch) and under superparasitism. This suggests that siblicide may not always allow an individual parasitoid to monopolize host resources, as assumed by theoretical models (Godfray 1987; Rosenheim 1993; Ode & Rosenheim 1998). If it were possible for nonsiblicidal individuals to survive in the presence of siblicidal individuals, this might facilitate the spread of nonsiblicidal alleles, but whether this can occur is also unknown. Experiments involving oviposition of both species into the same host (multiparasitism) might reveal this, but they are not feasible at present for this species pair because we cannot distinguish individuals from the two species morphologically; molecular markers

or new diagnostic morphological characters would be required.

Adult size is but one possible fitness component that may be affected by brood reduction behaviour. Our data suggest that another possible component, development time, is longer in the siblicidal species, and that this might be a cost of brood reduction. Other studies have found that superparasitism in solitary species lengthens development (e.g. Gerling 1972; Vinson & Sroka 1978; Wylie 1983; Eller et al. 1990; Harvey et al. 1993). In *A. genevensis*, it is not clear if the lengthened development time results from time needed to grow larger or from time taken up in combat rather than feeding. The difference in development time was more significant under superparasitism suggesting the latter explanation, although even there species and body size were confounded, and treatment did not have a significant effect when data from both treatments were pooled. If development time were an important component of total offspring fitness, such as might occur in populations that undergo rapid increases in size at certain times of the year, this might select for loss of siblicide. More data on the field biology of these species are needed before its importance can be properly assessed.

A final important individual fitness component that may be affected by brood reduction behaviour is developmental mortality. Our clutch size data are destructive, so estimating developmental mortality requires the use of untested assumptions. If we assume, in the first instance, that developmental mortality does not occur before eggs are counted, does not strongly vary with clutch size within treatments, and that the probabilities of a parasitized and an unparasitized host dying before completing development are approximately equal, then an estimate of relative developmental mortality can be calculated from the ratio of the number of adults emerging per host to the number of eggs dissected per host (Table 1). In the single parasitism case the relative probabilities are approximately the same for both species (0.620 for *A. genevensis* and 0.553 for *A. pallipes*). Under superparasitism, relative mortality is much higher for *A. genevensis* as expected (0.954), but for *A. pallipes* is approximately the same as under single parasitism (0.574). This suggests that the evolution of nonsiblicidal larval behaviour has not greatly affected the probability of larval survival when only one female oviposits in a host.

In our dissections of superparasitized hosts, we only rarely encountered a host that lacked at least one healthy *A. genevensis* larva. This suggests that direct costs such as the probability of all larvae dying in combat may be quite small. Similar results have been obtained in *Venturia canescens*, where the number of eggs laid in a host does not affect the chance that a host will produce a surviving wasp (Harvey et al. 1993), suggesting that combats usually have one clear winner, although in vitro observations suggest that fights in which both competitors are wounded may be reasonably common (Marris & Casperd 1996).

The relatedness and number of broodmates that must be killed by siblicidal larvae determine the inclusive fitness costs of siblicide. Sex ratios and sex ratio variances

affect relatedness between broodmates because full-sibling sisters are more related to each other than full-sibling brothers under haplodiploid inheritance. Overdispersed and female-biased sex ratios would promote the spread of nonsiblicide, whereas underdispersed (precise) and male-biased sex ratios would promote the spread of siblicide (Rosenheim 1993). In both species studied, sex ratio variances did not differ significantly from binomial. Both species produced more female-biased sex ratios under the superparasitism treatment, but *A. genevensis* had consistently more female-biased sex ratios than *A. pallipes*. This is the opposite from what one would expect if sex ratios were the major factor contributing to the stability of larval behaviour, and suggests that other influences are more important in this system. Other contributions to relatedness between broodmates that may be more important are the incidence of superparasitism or multiparasitism encountered by each species, and the mating system of the wasp species, both of which remain to be investigated.

With regard to the number of broodmates that must be killed by siblicidal wasps, our data have shown that even in the absence of superparasitism, some relatives may be killed by siblicidal larvae as a result of females laying multiple egg clutches. Factors that may contribute to this tendency have been discussed by Rosenheim (1993), Rosenheim & Hongkham (1996) and Ode & Rosenheim (1998). Since in *A. genevensis* two larvae may sometimes complete development successfully, such larvae may not necessarily be 'supernumerary', and may thus have contributed towards the tendency for *A. genevensis* to produce multiple egg clutches. We do not know at present what developmental mortality is suffered in such multiple egg clutches, but if there is at least some siblicidal mortality, as seems likely, then this adds an inclusive fitness cost to siblicide that is not present in single egg clutches, and may help select against siblicidal behaviour.

Finally, our data suggest that genetic or mechanistic constraints on the evolution of siblicide may be low in this genus: since both species possess what we infer to be the morphological apparatus for brood reduction, yet only one species practises it, behaviour and morphology appear to be separable. This suggests that highly constrained scenarios, in which gross and unlikely changes in morphology are required to affect behavioural changes, may be inappropriate; the larva in *Aphaereta* spp. is a rather generalized endoparasite and both siblicidal and nonsiblicidal behaviour occur within this morphological framework. Whilst conclusions must remain tentative in the absence of genetic data, we believe our results should lead researchers away from highly constrained scenarios for the evolution of siblicide/nonsiblicide in this genus.

The genus *Aphaereta* is clearly an interesting taxon with regard to the evolution of sibling interactions and their effects on parents. We see several opportunities for further investigation. First, we need more breadth of biological knowledge across the genus of the sort described here. Second, we need more depth of knowledge, preferably from field estimates, of parameters such as size-fitness relationships, relatedness between broodmates, and the genetics and morphology of brood

reduction behaviour. Such parameters are needed to test our current theoretical models. Finally, we need robust estimates of phylogenetic relationships within the genus, so we can begin to map the evolution of biological traits within the genus, and perhaps pinpoint the events associated with transitions in behaviour and development.

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