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ORIGINAL ARTICLE

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Clutch size adjustment, information use and the evolution of gregarious development in parasitoid wasps

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Abstract As larvae, solitary species of parasitoid wasps eliminate rivals, including sibs, through contest competition. In gregarious species, larvae tolerate each other and several individuals can develop from a single host. We report experiments on two congeneric braconid species aimed at understanding how gregarious development evolved in one genus, and the consequences of larval interactions for parental behavior. In the transition from solitary to gregarious development, one possible intermediate stage is if solitary species frequently lay more than one egg per host. If the risk of conspecific superparasitism (another female of the same species oviposits into a single host) is high, optimal clutch size theory predicts the laying of multiple-egg clutches, because a female will increase the probability of the surviving larva being her own. Under the same conditions, theory predicts that gregarious species should reduce their clutch size. In our experiments, the solitary *Aphaereta genevensis* increased their clutch size under the risk of intraspecific competition for hosts whilst the gregarious *A. pallipes* reduced their clutch size, as predicted. In *A. genevensis*, the response to the risk of competition was dependent upon body size, being greatest amongst small females. In *A. pallipes*, the response did not depend on female body size, but clutch size increased with body size in all treatments, unlike in *A. genevensis*. Under the risk of competition from the other species, the response differed relative to the intraspecific treatments and differed between species. Thus, our data suggest that: (1) competition for hosts may have contributed to the evolution of gregarious development in this genus; (2) female wasps can combine information from both external and internal cues in complex ways during clutch size adjustment; and (3) the social environment may alter the adaptive response to both external and internal cues in adult females.

Keywords Siblicide · Parent–offspring conflict · Decision making · Hymenoptera · Adaptation

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Introduction

Sibling rivalry is a widespread phenomenon that occurs, to varying degrees, amongst the offspring of both animals and plants. Sibling rivalry and the associated concept of parent–offspring conflict, encompass two fundamental themes of continuing interest to evolutionary biologists. First, what selective pressures and constraints result in extreme sibling rivalry in some species, but not in others (see Cheplick 1992; Elgar and Crespi 1992; Mock and Parker 1997)? A second focus is upon what consequences sibling rivalry, or its absence, might have for the evolution of adult life-histories and parental behavior (see Smith 1991; Svensson and Sheldon 1998; Pexton and Mayhew 2002; Stockley and Parker 2002). Here, we address the evolutionary causes and consequences of sibling rivalry in a genus of parasitoid wasps.

Parasitoid wasps lay clutches of eggs in or on discrete resources of limited size (the host organism), usually the immature stage of another insect species. After a clutch has been laid hosts are, in most species, left and may be re-encountered by other females (for an exception see Stokkebo and Hardy 2000). Wasp larvae develop to adulthood by feeding on the body of the host, which is at some stage killed. Developing wasps are likely to compete against each other for host resources as they are trapped together in a ‘developmental nursery’ (Mock and Parker 1997). Parasitoid wasps are classified as solitary or gregarious, dependent upon the behavioral phenotypes of

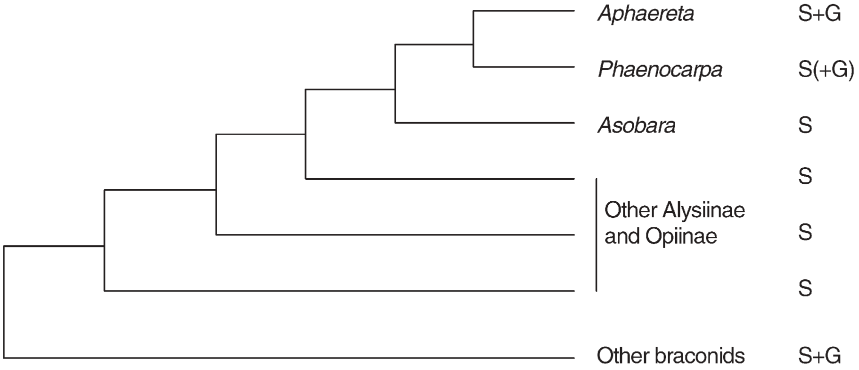

Fig. 1 The phylogenetic distribution of solitary and gregarious development in *Aphaereta* and related genera. Relationships are from Gimeno et al. (1997, their Fig. 4), branch lengths are arbitrary. See text for development sources. *S*: solitary, *G*: gregarious, (*G*): a single known gregarious species. Note that some authors treat *Asobara* as a subgenus of *Phaenocarpa* and that *Phaenocarpa* is a heterogeneous genus that may not be monophyletic (Wharton 1980)

the larvae when placed together in or on a host. Solitary species are distinguished by having ultra-aggressive larvae with a strong form of contest competition (rivals are usually killed in a series of physical fights but perhaps also by physiological suppression), so that only one individual is left to successfully develop. The relentless nature of this proactive, overt and consistently aggressive behavior directed towards sibs has led to it being referred to as 'ultra-siblicide' (Mock and Parker 1997). Gregarious species are generally regarded as being non-siblicidal, thus allowing several individuals to tolerate each other and successfully develop together (Godfray 1994; Quicke 1997), so that the mode of interaction between competitors resembles that of a scramble competition for resources.

The evolution of gregarious development presents a challenge within social evolution because solitary development is predicted to be evolutionarily stable to invasion by a rare tolerant (non-fighting) allele except under stringent conditions; the direct fitness of individuals developing together must rise with clutch size (Godfray 1987). However, solitary development has been replaced by gregarious development on numerous occasions (Rosenheim 1993; Mayhew 1998a) and is probably the derived state within the parasitic Hymenoptera (Mayhew 1998a, 1998b). Recent theoretical work has suggested several routes by which gregarious development may arise (Rosenheim 1993, Ode and Rosenheim 1998; Pexton et al. 2003). One intermediate step in the transition from solitary to gregarious development could involve solitary species that lay clutches of more than one egg. This scenario, under appropriate conditions, could facilitate the invasion of a gregarious phenotype, and allow a tolerant (non-siblicidal) allele to spread in an intolerant (siblicidal) population (Rosenheim and Hongkham 1996; Ode and Rosenheim 1998).

In solitary species, multiple-egg clutches can be adaptive when there is a high risk of superparasitism (Ives 1989; Visser and Rosenheim 1998). Superparasitism is when more than one conspecific female lays a clutch in a single host. Under contest competition between larvae, only one offspring will complete development in each host. By laying more than one egg per host, a solitary female wasp raises the probability that the surviving offspring will be hers and not that of a subsequent female. Alternatively, the larvae of gregarious species experience scramble competition, where resources are more equally shared. In such cases, a female should decrease her clutch size when there is a risk of superparasitism, so that larvae will obtain sufficient resources to develop successfully once they have been shared between all brood members. These divergent expectations suggest a possible consequence of differences in larval behavior and developmental mode for parental behavior.

Prior work has shown that clutch sizes can be modified in a predicted way in two solitary parasitoids (Michaud and Mackauer 1995; Rosenheim and Hongkham 1996), and one gregarious species (Visser 1996). However, the gregarious species studied is only distantly related to the solitary species, neither of which is known to represent the sister group of a gregarious species. Other important differences are that the species studied attack very different host species; hence, the experiments necessarily involved divergent conditions and differences in experimental protocols. Thus, the direct comparability of these studies is restricted. Comparing close relatives is an important feature of comparative analyses because it can control for many confounding features of biology (see Harvey and Pagel 1991) and allows us to infer changes connected with a single evolutionary event.

In this study we exposed two congeneric species, caught sympatrically and attacking the same host species, to identical laboratory treatments and observe changes in their oviposition behavior. We used the experiments to address the causes and consequences of the divergence in developmental mode within the braconid genus *Aphaereta* (Hymenoptera: Braconidae: Alysiinae). With the exception of a single species of *Phaenocarpa* (van Achterberg 1998), *Aphaereta* is the only genus in its subfamily currently known to contain gregarious species, and it also contains solitary species (Wharton 1977, 1980, 1984; Shaw and Huddleston 1991) (Fig. 1). This makes it likely that gregarious development is the derived state, perhaps only evolving once within this genus. The species we used here, *Aphaereta genevensis* (solitary) and *Aphaereta pallipes* (gregarious), have been the subject of previous research that showed differences in larval competition characteristic of solitary and gregarious species (Mayhew and van Alphen 1999). We exposed the species to the risk of competition for hosts and observe their subsequent oviposition behavior. We asked if this implies a mechanism by which gregarious development may have arisen and whether gregarious development, once arisen, has subsequently altered how females respond to the risk of competition.

Methods

Insects

A pair of species from the braconid genus *Aphaereta* was used in this study. The genus *Aphaereta* belongs to the subfamily Alysiinae (containing over 1,000 described species in around 70 genera; Shaw and Huddleston 1991), occupying a derived position with the genera *Asobara* and *Phaenocarpa* (Wharton 1980; Gimeno et al. 1997; Fig. 1). The Alysiinae itself forms a monophyletic group with the subfamily Opiinae (itself containing over 1,300 species; Quicke and van Achterberg 1990; Quicke 1993; Belshaw et al. 1998). All members of this clade are koinobiont endoparasitoids of cyclorrhaphous Diptera. The hosts of these types of wasps tend to live in fluctuating environments consisting of temporary and ephemeral patches of substrates on which they aggregate. Wasps of this type tend towards pro-ovigeny (so females emerge with a large complement of matured eggs, see Quicke 1997). Previous experiments on these species have provided essential data on differences in both larval behavior (Mayhew and van Alphen 1999) and also internal state variables that are related to adult female body size such as fat reserves, life expectancy and egg load (Pexton and Mayhew 2002). Mayhew and van Alphen (1999) demonstrated that in *A. genevensis* if more than one larva was present in a host the sharp sclerotized mandibles of first instar larvae were used in lethal physical combat until a single larva remained. In contrast, despite a very similar first instar larval morphology, including the presence of similar sharp mandibles, there was no evidence for *A. pallipes* larvae engaging in combat, as no fighting was observed amongst larvae. Morphologically the species are almost indistinguishable as adults (Wharton 1977). Adult females can only be distinguished by small (overlapping) differences in the relative length of the ovipositor; males are indistinguishable at present (see Wharton 1977).

Cultures

Cultures of both wasp species were started from adults collected in New York State (U.S.A.), during 1995 and 1996, (see Mayhew and van Alphen 1999). Since 1997, both species have been reared on *Drosophila virilis*. The wasp species were identified by Dr C. van Achterberg (National Museum of Natural History, Leiden, the Netherlands) based on a large sample of specimens from culture. Voucher specimens were also deposited at the museum. The hosts were reared in glass bottles on standard yeast-based medium. Wasps were reared in 5-cm diameter glass jars with foam stoppers (see Pexton and Mayhew 2002 for details). The cultures were maintained at 20°C, constant light and ambient humidity.

The risk of intraspecific competition

In Ives' (1989) model, which describes how clutch size should vary with the density of conspecifics, predictions relate to the behavior of the first female to arrive at a host, but it is not specified how a female can assess the likelihood of superparasitism from former experience. There are two major possibilities; they might do this from directly sensing that some hosts have been previously parasitized, or they might use the presence of other adult females. Both could potentially be used as treatments to signify the risk of superparasitism. We chose to use the latter option because (1) the *Drosophila* hosts bury through the medium and are a less accessible source of information for the wasps, (2) exposure to hosts prior to the measurement of oviposition behavior would lead to confounding influences such as variable egg loads, and (3) previous work on other species has shown that the density of conspecific females can alter oviposition behavior as predicted.

Pupae containing developing wasps were gently washed out from culture jars with lukewarm water and placed into specimen tubes (75 mm×25 mm). Five pupae were placed per tube in *A. pallipes* and 10 per tube in *A. genevensis*. Since brood size in the former species is approximately double that of the latter in normal culture (see Mayhew and van Alphen 1999), this controlled for each wasps experience of conspecifics as far as possible prior to the treatments. Rearing tubes were checked every 24 h for newly emerged females. Upon emergence, mated females of both species were randomly assigned to one of two treatments: with four other newly emerged randomly chosen females (intraspecific competition treatment) or without any other females (controls). Females were placed into 50 mm×25 mm specimen tubes. All the tubes (in both treatments) contained a cotton wool ball moistened with a 35% sucrose solution covered by a circle of filter paper. The sucrose solution was refreshed, as needed, to ensure a constant supply of food.

After 4 complete days in a tube (including the emergence day) either the single female (controls) or one of the females (intraspecific replicates) was transferred onto a patch of hosts (i.e. the experimental females were into their fifth day of adult life). Patches consisted of 12 late second instar/early third instar *D. virilis* larvae in Petri dishes (35 mm diameter×10 mm depth), with a 5-mm layer of nutrient agar and a spot of liquid yeast on the agar. Once a female had been introduced onto the patch, it was left for 2 h, then removed and killed by freezing at –20°C. The other females (in the intraspecific treatments) were also killed in the same way. The hind tibia length (mm) was recorded for all females (e.g. Visser 1994, Ellers et al. 1998, Mayhew and van Alphen 1999, Pexton and Mayhew 2002). The hosts in each replicate were dissected 4/5 days after exposure to the female wasp. The number of eggs found in each host was recorded.

Rosenheim and Hongkham (1996) have emphasized the distinction between eggs laid in one host encounter and eggs laid in two or more encounters (self-superparasitism). This distinction is not obvious in our study species. As they oviposit into concealed and aggregated hosts, the female must direct her ovipositor through the host medium when she is not in direct contact with them. Female wasps may repeatedly stab the same host with her ovipositor, or several different hosts, without repositioning her body (see Mayhew and van Alphen 1999). It is therefore not possible for the observer to distinguish between multiple-egg clutches and self-superparasitism as recommended by Rosenheim and Hongkham (1996).

The risk of interspecific competition

The interspecific competition treatments were the same as the intraspecific competition treatments except that one female of a given species was initially transferred to a specimen tube, then four females of the other species were also introduced into the tube. A behavioral assay was used to distinguish species after they had been mixed for an interspecific treatment. All females were first exposed to hosts to measure oviposition activity as in the intraspecific

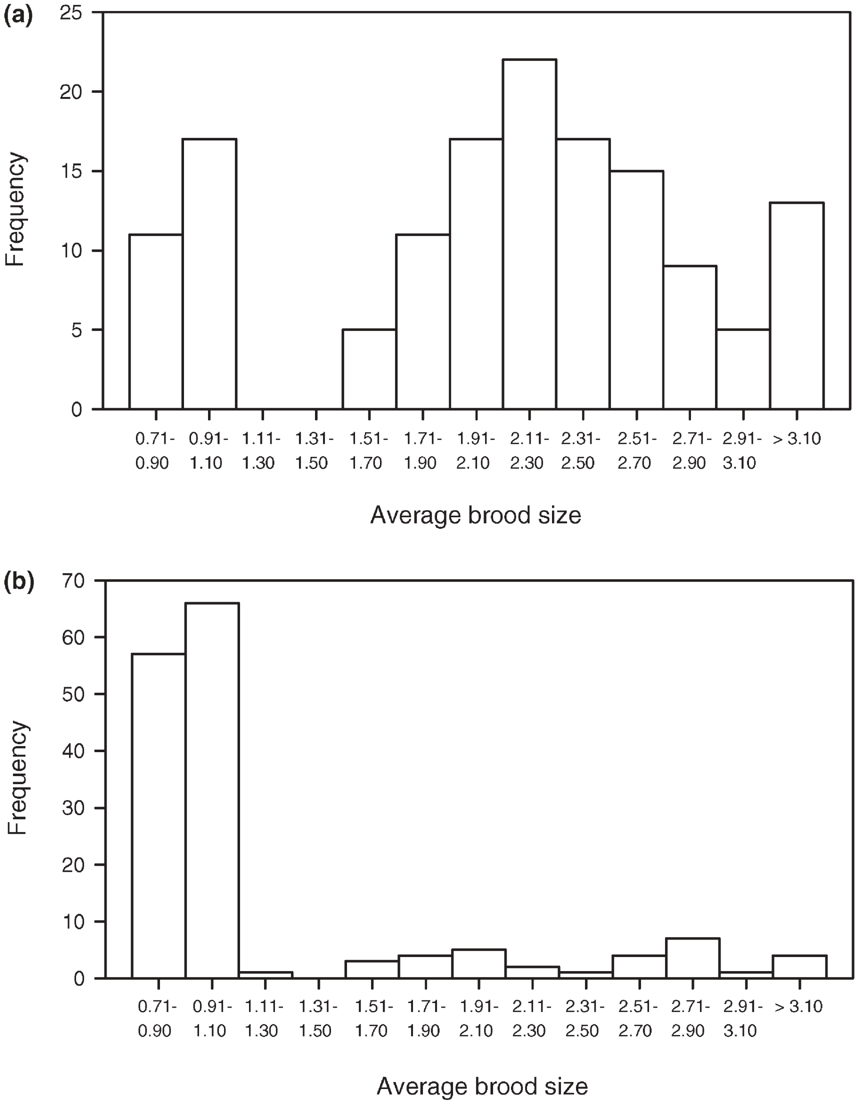

Fig. 2 The frequency distribution of the average brood size from experimental females in the post hoc identification assays from the interspecific experiments: **a** when a single *Aphaereta genevensis* female was kept with four *A. pallipes* females, and **b** when a single *A. pallipes* female was kept with four *A. genevensis* females. In both cases there is a bimodal distribution reflecting the difference in emerging brood size in the two species

treatment. Then, all females, instead of being immediately killed, were transferred into separate tubes (75 mm×50 mm) that had a 2-cm layer of nutrient agar and a group of approximately 25–50 second instar and third instar *D. virilis* larvae with some *Drosophila* medium. The females were left for 4 days (or until death whichever was sooner) in these tubes. Over this interval female wasps attack a very large proportion of hosts and also self-superparasitize hosts. Once the females had been removed, their hind tibia length (mm) was measured. The *D. virilis* larvae were allowed to complete development.

The number of wasps emerging was counted along with the numbers of flies as well as the total number of pupae. The average emerging brood sizes (no. wasps/no. pupae minus no. flies) was used to identify the species of the female in the 'identification' tube. Under such circumstances, female *A. genevensis* only produce one offspring per host whilst female *A. pallipes* produce an average emerging brood size of two or three (Mayhew and van Alphen 1999). These observations allowed each female to be identified post hoc (Fig. 2). The assay appeared to be completely reliable; identifying in each case either one solitary and four gregarious individuals per replicate or one gregarious and four solitary individuals per replicate. Subsequently the correct replicate could be identified (i.e. that of the single female exposed to a group of females from the other species) and used in the data analysis.

Statistical analyses

The theoretical predictions of Ives (1989) refer to clutch size, and are our primary interest. However, only recording clutches that contain eggs is a potential source of bias: solitary species laying only one egg per clutch by definition cannot reduce their clutch size, and yet might display a reduced motivation to oviposit by laying fewer clutches overall, something that might go undetected using the above measure. Therefore we also recorded the total number of eggs laid per patch to gain an overall measure of oviposition motivation.

Data analysis was performed by general linear modeling in the GLIM statistical package (see Crawley 1993). Significance was assessed by the change in deviance by stepwise subtraction from a full model of all potential explanatory variables. Significance was assessed either under normal errors by an *F*-test or under Poisson errors by a chi-squared test. The appropriateness of the error structure 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).

Table 1 General linear models of clutch size in both species (comparing control treatments against intraspecific treatments)

Species	*Aphaereta genevensis*		*Aphaereta pallipes*	
Explanatory variable	*F* value	*P*	*F*-value	*P*
Treatment	$F_{1,65}$=24.32	<0.001	$F_{1,76}$=40.75	<0.001
Hind tibia	$F_{1,64}$=12.78	<0.001	$F_{1,75}$=16.19	<0.001
Interaction	$F_{1,63}$=12.85	<0.001	$F_{1,74}$=1.49	NS 0.226

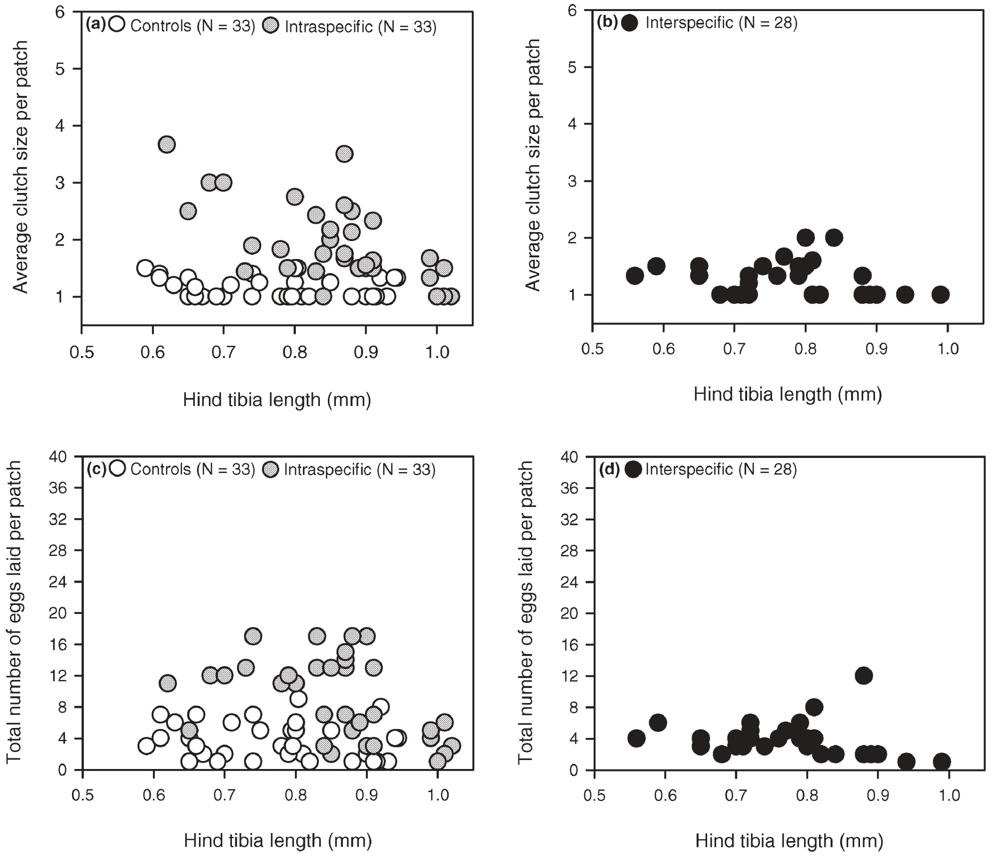

Fig. 3 The oviposition behavior of *A. genevensis*. The average clutch size of *A. genevensis* experimental females from **a** the control and intraspecific treatments and **b** the interspecific treatment. In addition, the total number of eggs laid by *A. genevensis* experimental females from **c** the control and intraspecific treatments and **d** the interspecific treatment. For clarity, standard errors are not shown

Results

The risk of intraspecific competition

The average clutch size for females did significantly differ in the control and intraspecific competition treatments for both species (see Table 1). *A. genevensis* laid larger clutches on average in the intraspecific treatment than in the control treatment (Fig. 3a). However, *A. pallipes* laid smaller clutches in the intraspecific compared to the control treatment (Fig 4a). The total number of eggs laid by females (see Fig. 3c and 4c) also differed with experimental treatment in both species, in the same direction as for clutch size (see Table 2). The size of females (as measured by hind tibia length) was also a significant explanatory variable for both the average clutch size and the total number of eggs laid by females of both species. In *A. genevensis* female size had a negative effect on oviposition activity (Fig. 3a), whilst in *A. pallipes* it had a positive effect (Fig. 4a, c). For *A. genevensis* there was a significant interaction between female size and treatment with respect to average clutch size. As female size declined the average clutch size in the intraspecific treatments rapidly increased compared with females of similar sizes from the control treatment. In *A. genevensis*, there was no significant interaction between size and treatment with respect to the total number of eggs laid. For *A. pallipes*, there was no significant interaction between size and treatment for average clutch size or the total number of eggs laid.

The possibility that, not only the presence of but also the size of, other females in the intraspecific replicates influenced oviposition behavior was assessed. However, in the intraspecific treatments, three size-related variables representative of the other females present in the experimental tubes were not significant in accounting for average clutch size of the experimental females: mean hind tibia length of other females (*A. genevensis*) $F_{1,32}$=0.02, *P*=0.888; (*A. pallipes*) $F_{1,35}$=0.02, *P*=0.888; variance in hind tibia length of other females (*A. genevensis*) $F_{1,32}$=3.14, *P*=0.086; (*A. pallipes*) $F_{1,35}$=1.81, *P*=0.187; maximum hind tibia length of the other females (*A.

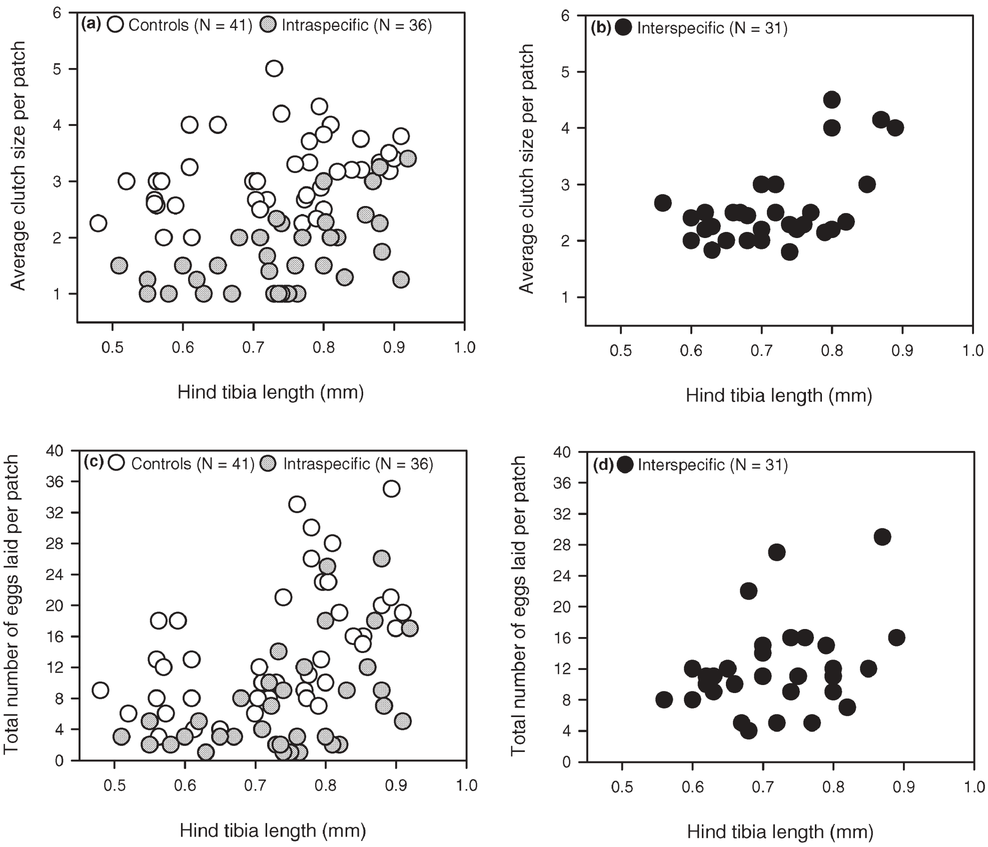

Fig. 4 The oviposition behavior of *A. pallipes*. The average clutch size of *A. pallipes* experimental females from **a** the control and intraspecific treatments and **b** the interspecific treatment. In addition, the total number of eggs laid by *A. pallipes* experimental females from **c** the control and intraspecific treatments and **d** the interspecific treatment. For clarity, standard errors are not shown

Table 2 General linear models of the total number of eggs laid in both species (comparing control treatments against intraspecific treatments)

Species	*A. genevensis*		*A. pallipes*	
Explanatory variable	X^2	*P*	X^2	*P*
Treatment	X^2_1= 37.10	<0.001	X^2_1= 27.09	<0.001
Hind tibia	X^2_1= 8.10	<0.005	X^2_1= 28.37	<0.001
Interaction	X^2_1= 0.88	NS	X^2_1= 2. 25	0.134

genevensis) $F_{1,32}$=0.32, *P*=0.576; (*A. pallipes*) $F_{1,35}$=0.40, *P*=0.531.

The same trio of size-related variables (representative of other females present in the experimental tubes) were not significant in accounting for the total number of eggs laid by the experimental females: mean hind tibia length of other females (*A. genevensis*) $F_{1,32}$=0.01, *P*=0.921; (*A. pallipes*) $F_{1,35}$<0.01, *P* >0.921; variance in hind tibia length of other females (*A. genevensis*) $F_{1,32}$=0.03, *P*=0.864; (*A. pallipes*) $F_{1,35}$=0.01, *P*=0.921; maximum hind tibia length of the other females (*A. genevensis*) $F_{1,32}$=0.01, *P*=0.921; (*A. pallipes*) $F_{1,35}$<0.01, *P*>0.921.

The risk of interspecific competition

For both species the mean clutch size laid (Fig. 3b and 4b) and the total number of eggs (Fig. 3d and 4d) laid by experimental females were separately compared against both the control data and the intraspecific data, as either set of replicates could represent an appropriate experimental control (Table 3 for *A. genevensis* and Table 4 for *A. pallipes*). The general response of *A. genevensis* females in the interspecific treatments (to *A. pallipes* females) differed from that observed in the intraspecific treatments, but did not differ from the control treatments. The response of *A. pallipes* to *A. genevensis* females was intermediate between the controls and the intraspecific treatment.

Discussion

The main findings of our study are: (1) that the solitary *A. genevensis* lays more eggs per host in response to competition for hosts, whilst the gregarious *A. pallipes* lays fewer; (2) that clutch size adjustment in *Aphaereta* females depends on both endogenous and exogenous information that female wasps are able to integrate, so that oviposition behavior can exhibit complex interactive responses to both extrinsic and intrinsic variables; and (3) that the behavioral responses to both internal and external factors have changed in *Aphaereta* in conjunction with, and likely as a result of, the evolution of gregarious development. Below we put these findings in the context of previous work and discuss their significance.

Table 3 General linear models of oviposition behaviour in *A. genevensis*, comparing pairwise combinations of treatments

Dependent variable	Average clutch size			
Comparison	Interspecific versus intraspecific		Interspecific versus controls	
Explanatory variable	*F*-value	*P*	*F*-value	*P*
Treatment	$F_{1,60}$=15.82	<0.001	$F_{1,60}$=3.69	0.059
Hind tibia	$F_{1,59}$=12.79	<0.001	$F_{1,59}$=1.35	0.250
Interaction	$F_{1,58}$=8.84	<0.005	$F_{1,58}$=0.07	0.790
Dependent variable	Total eggs laid			
Comparison	Interspecific versus intraspecific		Interspecific versus controls	
Explanatory variable	X^2	*P*	X^2	*P*
Treatment	X^2_1= 30.46	<0.001	X^2_1= 0.00	1.00
Hind tibia	X^2_1= 8.27	<0.005	X^2_1= 1.54	0.215
Interaction	X^2_1= 0.63	0.427	X^2_1= 0.10	0.752

Table 4 General linear models of oviposition behaviour in *A. pallipes*, comparing pairwise combinations of treatments

Dependent variable	Average clutch size			
Comparison	Interspecific versus intraspecific		Interspecific versus controls	
Explanatory variable	*F*-value	*P*	*F*-value	*P*
Treatment	$F_{1,66}$=18.26	<0.001	$F_{1,71}$=11.35	<0.005
Hind tibia	$F_{1,65}$=21.44	<0.001	$F_{1,70}$=14.85	<0.001
Interaction	$F_{1,64}$=0.21	0.648	$F_{1,69}$=2.40	0.126
Dependent variable	Total eggs laid			
Comparison	Interspecific versus intraspecific		Interspecific versus controls	
Explanatory variable	X^2	*P*	X^2	*P*
Treatment	X^2_1= 12.35	<0.001	X^2_1= 3.06	0.080
Hind tibia	X^2_1= 15.06	<0.001	X^2_1= 20.48	<0.001
Interaction	X^2_1= 4.60	<0.05	X^2_1= 1.04	0.380

Multiple-egg clutches in solitary species

The laying of more than one egg per host in solitary species is important for the evolution of gregarious development for two reasons: (1) gregarious development requires an increase in clutch size in and of itself and (2) more importantly, such clutches also provide the context in which a rare non-siblicidal allele can be exposed to selection against a resident siblicidal allele and possibly spread. If tolerant larvae are competitively inferior (because they cannot fight), such larvae will not survive when they share a host with a larva that has the intolerant (fighting) phenotype. This is the reason why it has been suggested that solitary development may frequently represent an example of irreversible/directionally biased evolution, with siblicidal fighting being a locally absorbing state (Godfray 1987; Harvey and Partridge 1987; le Masurier 1987; Williams 1992).

However, if the frequency with which phenotypically homogenous broods are laid (resulting in more all-tolerant broods) increases, the evolutionary transition from a siblicidal to a non-siblicidal phenotype is theoretically possible. In haplo-diploid organisms such an increase in the average relatedness within multiple-egg clutches can be generated if ovipositing wasps have a female-biased sex ratio or lay an increased proportion of single-sexed broods. Female-biased sex ratios can result from adaptive maternal control, or from infection by maternally transmitted genetic entities, such as the symbiotic bacteria *Wolbachia* (Godfray 1994). It has been argued that laying single-sexed broods is adaptive, under the risk of superparasitism, by allowing a female wasp greater control over the sex of her surviving offspring (Rosenheim and Hongkham 1996). A number of gregarious wasps are distinguished by only laying single-sexed broods, or laying a very high proportion of single-sexed broods (Rosenheim 1993; West et al. 1996; Mayhew 1998b). Such a bias in the tendency to lay a high proportion of single-sexed broods has also been observed in a solitary species, which also has a female-biased sex ratio (Ode and Rosenheim 1998).

Solitary species of wasps are known to lay more than one egg per host in response to several factors including: the presence of conspecifics (Michaud and Mackauer 1995; Rosenheim and Hongkham 1996), low encounter rates with hosts (Rosenheim and Hongkham 1996; Chau and Mackauer 1999; Darrouzet et al. 2002), high egg loads (Michaud and Mackauer 1995) and increasing host size (Chau and Mackauer 1999). Rosenheim and Hongkham (1996) list 23 solitary wasp species from a diverse array of taxa that are known to occasionally lay multiple-egg clutches.

The significance of our results is not only that we have explicitly distinguished a new factor (the body size of the ovipositing female—see below) that can encourage or discourage this behavior, but also that the genus investigated in this paper is known to contain many gregarious species. Hence, we can more realistically suggest the behavior is a possible transitional state towards gregarious development. In addition, as our experiments were conducted with two very closely related species under identical laboratory circumstances, we can be much more confident that the response to the risk of intra-specific competition reflects differences in the form of larval interactions rather than other differences in biology or experimental environment. In comparative terms our data represent a single independent contrast (Harvey and Pagel 1991); alternative interpretations of individual comparisons are always possible, but previous work on the biology of these species also suggests that the variation in larval behavior is a key factor modulating the divergent biology of these species (Mayhew and van Alphen 1999; Pexton and Mayhew 2002).

An investigation of the primary sex ratio of clutches containing more than one egg in *A. genevensis* would now appear warranted, to examine if sex allocation behavior is such that it could also promote the spread of a tolerant phenotype. Although *Aphaereta* species have not, to our knowledge, been investigated for *Wolbachia* infection, numerous *Asobara* populations are infected (Dedeine et al. 2001). An investigation of the presence and effects of sex distorters in *Aphaereta* species would now appear warranted. Since *Aphaereta* is almost unique in its subfamily in having gregarious development, it would be advantageous to compare the response of more remotely related alysiines in this respect: (1) to assess if the risk of superparasitism is higher in *Aphaereta* species, or; (2) if divergences exist between females from different species, with respect to the possible constraints upon the use or processing of information, such that females from different species vary in their ability to assess the likely risk of superparasitism by other competitors. Although we are unaware of any similar studies on non-parasitoid taxa, the costs and benefits of siblicide in most taxa (see Mock and Parker 1997) may be altered by the clutch size adjustment of adults. Therefore, this phenomenon may represent a more general explanation for the loss or gain of siblicide.

Reduction in clutch size in gregarious species

Ives (1989) developed a theoretical framework for the effect on clutch size of variable numbers of competitor females ovipositing on a patch. Ives built upon models by Parker and Courtney (1984) and Skinner (1985) that predicted a general increase in clutch size under the risk of intraspecific competition. Parker and Courtney (1984) and Skinner (1985) only considered a limited number of competition functions. By describing a wider range, Ives (1989) was able to show that the direction of response to change could be completely reversed depending on whether competition was a contest or scramble process. This approach, which considered a wider number of parameters to more accurately predict adaptive responses, has been supported by subsequent empirical work (Visser 1996; Rosenheim and Hongkham 1996; see also Visser and Rosenheim 1998) as well as our data.

In contrast to the solitary *A. genevensis*, the gregarious *A. pallipes* reduces its clutch size in response to intraspecific competition for hosts. Rosenheim and Hongkham (1996) emphasized two possible selective forces underlying such clutch size adjustments. The first, and the one which motivated Ives' (1989) model and hence our study, is that it reflects the risk of superparasitism. Another possible selective force, however, is that the presence of other females indicates future changes in host density. For example, if it reflected a reduction in future host density, an increase in clutch size might be adaptive because of reduced opportunity costs (see Skinner 1985). This would however require that solitary and gregarious species take the presence of conspecifics to mean opposite things (i.e. an increase in future host density in gregarious species but a decrease in solitary species). This is possible, but perhaps a less parsimonious interpretation. Whatever the underlying selective force, our study has shown that the changes involved can evolve between species in the same genus, which may even be sister species. This change also adds to the number of known differences in life-history traits and parental behavior that can likely be attributed to the evolution of gregarious development in this species (see Mayhew and van Alphen 1999; Pexton and Mayhew 2002); on this occasion involving a change in the response to endogenously and exogenously derived information.

Effects of body size

In both *A. genevensis* and *A. pallipes*, the precise quantitative response of the females taken from the intraspecific replicates was strongly dependent upon a female's size. In *A. genevensis,* large females showed little or no response to the risk of competition, laying mostly solitary clutches. In contrast, small females dramatically increased their average clutch size. This discovery is to our knowledge entirely novel and may require the development of explicit theory to test its adaptive value. As adults, parasitoids seemingly cannot increase their fat reserves during life (no lipogenesis occurs, see Ellers 1996; Olsen et al. 2000; Rivero and West 2002). The continual presence of food can drastically reduce the rate at which fat reserves are used but not prevent their decline (Ellers 1996). Fat reserves upon emergence are positively associated with adult size (Ellers 1996; Pexton and Mayhew 2002). In turn, female longevity is likely to be strongly size-dependent both when food is available and particularly so in stressful conditions when food is not available (e.g. Pexton and Mayhew 2002). A possible adaptive explanation of the size-related patterns is therefore that small females are extremely unlikely to survive long enough to encounter many patches of hosts in their lives so would pay few costs from adjusting their clutch size upwards (see Roitberg et al. 1993; Fletcher et al. 1994). Hence they may maximize their reproductive success by increased allocation of eggs to existing hosts (see Ellers and Jervis 2003). Large females may be more likely to encounter subsequent patches of hosts so that the benefits derived from an upward adjustment of clutch size are significantly lowered.

In *A. genevensis*, the egg load of newly emergent females is relatively invariant with body size, yet females show a significant increase in fat levels with increasing size (Pexton and Mayhew 2002). Small females in *A.*

genevensis carry much less fat and have reduced life expectancies (Pexton and Mayhew 2002). If the risk of competition is absent, it may not pay for solitary individuals of whatever size to allocate to hosts extra eggs that would inevitably die (Skinner 1985; Waage and Godfray 1985; Mayhew and Glaizot 2001). Hence, we would expect clutch size to be independent of female size in the absence of intraspecific competition, as seen in our results.

In *A. pallipes*, clutch size increased with body size in all treatments. The number of eggs a female wasp has upon emergence rapidly increases with body size in *A. pallipes*: large females have hundreds of more eggs upon emergence than the smallest females, which have an initial egg load similar to *A. genevensis* (Pexton and Mayhew 2002). The positive effect of body size on oviposition in *A. pallipes* is consistent with the increase in egg load with body size found in this species (Pexton and Mayhew 2002), and would be predicted from state-dependent adaptive models (see Iwasa et al. 1984; Mangel 1987). Female wasps with a greater reproductive investment (i.e. carrying a large number of matured eggs) typically have a higher motivation to oviposit and/or to lay larger clutches (Collins and Dixon 1986; Rosenheim and Rosen 1991; Flanagan et al. 1998).

Under the risk of superparasitism the offspring fitness-enhancing strategy is to reduce clutch size in *A. pallipes*, rather than to increase it. This means that adults actually reduce their costs (time and eggs) by following the strategy that enhances offspring fitness. This is beneficial to all adults regardless of their size.Therefore the size dependence of the effect of competition was not expected to be so great. Formal modeling of the interaction between body size and competition on clutch size will be necessary to confirm this. Hence, our results suggest an immediate need for new adaptive models that can incorporate such interactions.

Another finding of this study was that, whilst experience of conspecific females did generally result in the modification of oviposition behavior, the size of the other females did not have a significant effect upon oviposition. This is in contrast to the findings of Flanagan et al. (1998) who reported that under intraspecific competition that some aspects of oviposition behavior changed with the body size of competitors. None of the measures used to characterize the sizes of the other females or the relative size of the subject female to the non-subject females had any significant explanatory power with respect to the aspects of oviposition behavior recorded in this study. It is possible that sex ratio decisions may have been changed with female body size but the primary sex ratio of clutches cannot be easily measured in these species. A difference in the sensitivity to the precise size of other females may also reflect basic biological differences; *Nasonia vitripennis* is an idiobiont, so its host does not grow after parasitization, whilst *A. genevensis* and *A. pallipes* are koinobionts. Their hosts (in this study *D. virilis*) do grow considerably after parasitization. Thus, there may be more tolerance to minor ‘errors’ in ovipositional decisions in koinobionts and the lack of such additional information about potential competitors may have little consequence for female fitness (with respect to clutch size per se). West and Sheldon (2002) have recently invoked the difference between koinobiont and idiobiont wasps to be fundamental to the expression of adaptive sex ratio adjustment, for similar reasons. A final difference between our study and Flanagan et al.’s (1998) is that females in our study were exposed to hosts after, not during, their experience of other females. It is possible that size assessment occurs only during oviposition, or that females forget their assessment over time.

Whilst our experiments were not designed to investigate mechanistic processes, our results do imply mechanisms behind the patterns we observed. Responses to the density of competitors are learned in the sense that they represent an adaptive modification of behavior based on experience. It is unknown how females perceived each other, although they had the opportunity to do so by several means, for example using tactile, chemical or visual cues. Nor is it known how this translated into changes in oviposition activity; possibilities include reduction in egg maturation rate, or increased tendency to retract the ovipositor from the host. The effects of body size also imply some, as yet unknown, mechanism by which behavior can be altered. These possibilities are intriguing avenues for further study.

Interspecific competition

The effects of interspecific competition, compared to intraspecific competition for hosts, have received little attention theoretically for parasitoid wasps. Multiparasitism studies, in which females of different species oviposit in the same host, have investigated the course of competition within hosts, but to our knowledge no empirical studies have examined how the risk of multiparasitism might alter clutch size decisions. It is common for several parasitoid species to be associated with a single host species (Hawkins 1994), so the risk of multiparasitism is another factor that might induce parasitoids to adjust their behavior (Weisser and Houston 1993). Oviposition behavior and average clutch size in *A. genevensis* females showed no response to this risk. Oviposition behavior in *A. pallipes* females did change, being intermediate between the control and intraspecific treatments. One possible reason for reduced responses to interspecific competitors is that interspecific competition may be rarer than intraspecific competition (see Atkinson and Shorrocks 1981; Hanski 1981; Shorrocks and Rosewell 1987; Ives 1988). Little is known about the range of host species that *A. genevensis* uses in nature although *A. pallipes* does use a wide range of host species, as well as having a wider known geographic range (Wharton 1984; Whistlecraft et al.1984; Shaw and Huddleston 1991). If the niches of *A. genevensis* and *A. pallipes* are substantially different then selection may be more intensive with respect to the phenomenon of intraspecific competition.

With regard to competitiveness superiority, it is generally assumed that contest competitors are superior to scramble competitors (see Pexton and Mayhew 2001). If true, this could explain the absence of response to interspecific competition in *A. genevensis*. Under the same paradigm, *A. pallipes* would be expected to reduce its clutch size relative to controls, which it does, but not as substantially as the reduction observed under the risk of intraspecific competition. The response of *A. pallipes* females could indicate some very limited recognition of *A. genevensis* resulting in an incomplete reduction in clutch sizes (compared with the risk of intraspecific competition). Alternatively, it may indicate complete recognition of *A. genevensis* and that the competitive interactions are more evenly balanced within the two species. Recent studies have demonstrated that some gregarious species can be competitively equal or even superior when faced with competition from solitary larvae (Boivin and van Barren 2000; Marklt et al. 2002).

The response of *A. pallipes* to *A. genevensis* females indicates at least some species-specific recognition ability, and thus that constrained recognition cannot be a complete explanation for our observations. Several closely related species show no interspecific discrimination ability, (Bakker et al. 1985; Turlings et al. 1985) although others do. *Asobara tabida*, another alysiine species, can recognize the mark left by its sympatric sister species *A. rufescens* but not those left by allopatric congeners (Vet et al. 1984). Martel and Boivin (2004) showed that *Trichogramma minutum* females will not adjust their offspring sex ratio to the presence of heterospecifics, but will adjust it to the presence of conspecifics. However, *T. pintoi* adjusts its offspring sex ratio to heterospecifics in the same way as to conspecifics. This suggests some species-specific recognition in the former species, but not the latter. Such results are reminiscent of ours. Martel and Boivin (2004) suggest that they may reflect differences in the likelihood of heterospecific encounters in the field. Tests of the frequency of heterospecific encounters in the field, and of existence of constraints (if any) upon the ability of our species to recognize each other's oviposition markers may shed some light on the adaptive value of the recorded responses.

Although our studies indicate some differences in the way females respond to heterospecifics as opposed to conspecifics, it may be that an alternative experimental treatment, such as on-patch encounters between females, would lead to greater differences since the time delay between experience and oviposition would be reduced.

Conclusions

Our experiments were designed to investigate whether competition for hosts is a potential selective pressure on the evolution of gregarious development in *Aphaereta*, and whether gregarious species differ from solitary species in their response, indicating a behavioral change in information use shaped by the underlying extent of sibling rivalry. Our results are supportive of both of these suggestions. In addition, the species differ in how body size affects and interacts with this response, a novel finding that may also be linked with a change in development mode (hence larval behavior), that indicates complex integration of both external and internal cues. The adaptive significance of these responses requires further work, as does the response to both species to interspecific competitors. Our results should stimulate equivalent studies in other non-parasitoid taxa: on whether competition between individuals and clutch size adjustment can facilitate the gain and loss of siblicide, on whether changes in the nature of sibling rivalry can affect parental decision making, and how both endogenous and exogenous information is utilized by organisms.

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