# Factors influencing egg parasitism in sub-social insects: insights from the treehopper *Alchisme grossa* (Hemiptera, Auchenorrhyncha, Membracidae)

L U I S   C A M A C H O,[1]   C L I F F O R D   K E I L[1]   and   O L I V I E R
D A N G L E S[1,2,3,4]   [1]Facultad de Ciencias Exactas y Naturales, Museo de Invertebrados, Pontificia Universidad Católica del Ecuador, Quito, Ecuador, [2]LEGS-CNRS, Institut de Recherche pour le Développement (IRD), Gif-sur-Yvette, France, [3]Université Paris-Sud 11, Orsay, France and [4]Instituto de Ecología, Universidad Mayor San Andrés, La Paz, Bolivia

**Abstract.** 1. Subsocial insects rely on maternal care in order to enhance offspring survival. Subsocial Hoplophorionini treehoppers defend their egg masses from predatory and parasitoid attacks. Egg parasitic wasps are important enemies of the Membracidae.

2. We examined parasitism patterns and female defence mechanisms in the Hoplophorionini treehopper *Alchisme grossa* (Fairmaire). We examined the effect of aggregations of egg-guarding females, spatial aggregations of host plants and female *A. grossa*, and host plant identity on the incidence of egg mass parasitism. Additionally, we measured the effect of female maternal care strategies on reducing the amount of parasitism on their egg masses.

3. Aggregation of egg-guarding females on a single host plant was the most important factor affecting egg mass parasitism. Increased numbers of aggregated females lowered both parasitism risk and parasitism levels on their egg masses. When spatial clumping of host plants and egg-guarding females occurred simultaneously, parasitism risk was also reduced. Maternal care strategies such as coverage of the egg mass by the females' pronota and intensity of female defensive behaviour also seemed to lower parasitism on egg masses.

4. A reduction of parasitism by female aggregation on host plants can be explained by a selfish herd dilution effect. This dilution effect was potentiated by a possible cooperation between aggregated egg-guarding females on the host plant. The present study provides a new dimension to sub-sociality in Hoplophorionini treehoppers.

**Key words.** *Alchisme grossa*, female aggregation, maternal care, Membracidae, parasitism, subsociality.

## Introduction

The Membracidae (Hemiptera, Auchenorrhyncha) is a mainly tropical group (Wood, 1993) of exclusively phytophagous insects that also depend entirely on their host plant for reproduction (Godoy *et al.*, 2006). The most striking attribute of the group is the exaggerated and elaborate shape of the pronotum, which extends posteriorly and usually dorsally over the body.

Correspondence: Clifford Keil, Museo de Invertebrados, Escuela de Ciencias Biológicas, Pontificia Universidad Católica del Ecuador, 12 de Octubre 1076 y Roca, Apartado Postal 17-01-2184, Quito, Ecuador. E-mail: Keil617@yahoo.com

Many of the Membracidae are subsocial (Godoy *et al.*, 2006). Subsocial species are defined by the presence of maternal care of eggs and occasionally immatures (Godoy *et al.*, 2006). Numerous treehoppers form associations with hymenopterans, mainly ants. These mutualistic associations are based on the protection offered by the ants in exchange for honeydew produced by both adults and immatures (Godoy *et al.*, 2006).

Many treehopper species rely on ant associations to enhance offspring survival from predator and parasitoid attacks (Wood, 1993). Other species, however, do not form associations with ants. This is the case in the highly subsocial Hoplophorionini (Membracinae) treehoppers. All species of this tribe exhibit a highly developed maternal care strategy to protect offspring

(Wood, 1974; McKamey & Deitz, 1996). Females from this group actively defend their offspring from predator and parasitoid attack during all development stages (Wood, 1974; McKamey & Deitz, 1996). Among all treehoppers, this tribe shows the highest degree of maternal care investment (McKamey & Deitz, 1996). Additionally, this tribe shows morphological adaptations related to the development of maternal care. For instance, their pronota are dorsally expanded forming a shield-like structure under which females can shelter their egg mass (McKamey & Deitz, 1996). Metathoracic tibiae have a clavate morphology and the metathoracic tarsi are reduced. The mesothoracic coxae have an acute triangular process. These characteristics may be related to the lateral kicking behaviour involved in female defence of offspring that is unique to this tribe (McKamey & Deitz, 1996).

Parasitoids are important enemies of the Membracidae (Godoy et al., 2006). Wasps in the families Mymaridae and Trichogrammatidae parasitise treehopper eggs (Godoy et al., 2006). The interaction between parasitoids and their hosts results in the death of the host (Turlings et al., 1992). Potential host insects are under constant pressure to avoid parasitoid detection and parasitoids to develop ever more efficient ways to find them (Turlings et al., 1992). Chemical cues are among the most essential means parasitoids use to find their host prey (Fatouros et al., 2008). These cues include odours released by the host or the insect's host plant (Fatouros et al., 2008). Typically, chemical cues are released by the host plant after stimulation by feeding activity (De Moraes et al., 1998; Kessler & Baldwin, 2001).

In this study, we examined parasitism patterns and defence mechanisms in a hoplophorionine subsocial treehopper. As the Membracidae are phytophagous insects, parasitoids should react to either treehopper or its host plant odours to search for and find their prey. We hypothesised that host plant and treehopper spatial aggregation should affect the risk of parasitism among egg-guarding females' egg masses. Clumped treehoppers and host plants might attract parasitoids, increasing the risk of parasitism. Moreover, given the number of both behavioural and morphological defences against parasitism exhibited by subsocial treehopper females, we hypothesised that maternal care strategies might affect the level of parasitism on their egg masses. While we specifically focus on treehoppers in this study, our approach to understand parasitism patterns is discussed in the broader context of subsocial insects.

### Natural history of the study model

We studied the treehopper *Alchisme grossa* (Fairmaire) (Membracidae, Hoplophorionini), a species with complex maternal care of the offspring. Unlike many treehoppers, this behaviour is carried out without the interaction with ants, even in areas where ants are ubiquitous. *Alchisme grossa*'s maternal care involves protection of the offspring until they reach adulthood. In the majority of *Alchisme* females, the female makes a shallow cut in the stem or leaf midrib and deposits the eggs so that less than 15–25% of the egg is exposed. A minority of females will leave a large part of the egg mass
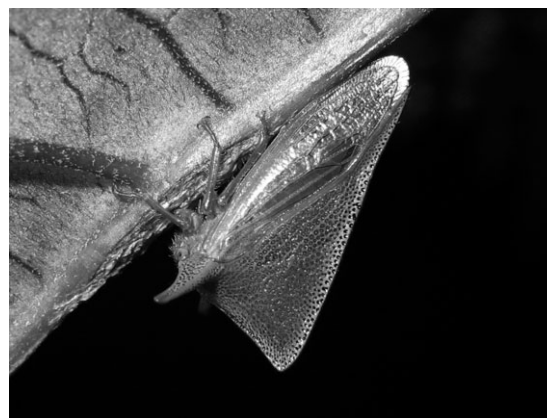


**Fig. 1.** *Alchisme grossa* female guarding an egg mass on the underside of the host plant leaf. Note that the large pronotum completely covers the female's body dorsally and extends laterally with prominent protonal horns. The egg mass has been placed in a slit cut by the female in the mid-rib of the leaf and is nearly completely covered by the leaf tissue and secretions of the female.

exposed. The female will also cover the egg mass with a frothy secretion. During the incubation period, the mother actively defends her eggs by covering the egg mass with her body (Fig. 1) and by repelling predators and parasitoids through wing fanning, lateral body movements, and powerful lateral kicks. During the nymphal stages, the nymphs form a column over the plant stem and their mother positions herself near the group. In the event of a predatory attack, typically wasps or reduviid bugs in this study, the attacked nymphs send vibratory alarm signals that are passed along the nymph column until the signal reaches the mother. This triggers a defensive response in the mother; she immediately walks towards the source of the alarm and confronts the attacker with the movements described above. This behaviour is similar to that reported for *Umbonia crassicornis* (Amyot & Serville) (Hemiptera: Membracidae) (Ramaswamy & Cocroft, 2009). As in all subsocial membracids, *A. grossa* males are not involved in the protection of the offspring. Additionally, *A. grossa* has the complete morphological defensive profile of a typical subsocial Hoplophorionini treehopper. The pronotum completely covers the insect dorsally, forming a shield-like structure. The humeral and dorsal processes of the pronotum are developed into spine-like structures. The metathoracic tibiae are clavate and the mesothoracic coxae have a triangular process that presumably aids in preparing the kick by keeping the mesothoracic leg out of the way. Females may be solitary, loosely grouped on adjacent host plants or aggregated on the same plant. The treehopper genus *Alchisme* has a strong preference for host plants in the Solanaceae (Godoy et al., 2006).

## Materials and methods

### Study area

The study was carried out in Mindo in Pichincha Province, located on the western slope of the northern Andes of

Ecuador (0°2′57.72″S, 78°36′30.74″W). The location was at an elevation of approximately 1300 m, and mainly consisted of evergreen low montane forest of the western Andes (Sierra *et al.*, 1999). All individuals were observed and collected in vegetation growing along the edge of secondary gravel roads. Sampling was carried out during June and July 2010, and March and April 2011.

*Data collection*

Once an egg-guarding female was found in the field, all the egg-guarding females on the plant were counted. In addition, both distances to the closest host plant and to the closest egg-guarding female were measured. Because the average straight-line distance between females was difficult to measure accurately, coordinates for each egg-guarding female in its plant were obtained using a Garmin 60 GPS unit (Olathe, Kansas). These coordinates were used to calculate the distance to the closest egg-guarding female using ArcGIS 9.3 software (Redlands, California). Samples of the host plants were collected for identification and archiving in the Herbarium (QCA) at the Pontificia Universidad Católica del Ecuador (PUCE) (Quito, Ecuador). The length and width of each egg mass was measured in the field using a Vernier caliper. The measurement of an egg mass typically triggered the mother's defensive response. Based on preliminary field observations, we used a six-level scale to evaluate the intensity of female response towards simulated attack: (1) the female absconded at the first sign of harassment; (2) the female walked away in response to the harassment, but then came back and resumed her initial position; (3) the female did not move away from the egg mass and held to it more strongly with her legs; (4) the female fanned her wings, held on more strongly to the egg mass, and kicked occasionally; (5) the female showed the full defensive repertoire which included wing fanning, kicking and sideway body movements; and (6) female behaved as in level 5, but more intensely, e.g. with constant and intense kicking, body shaking, and an aggressive attitude.

Once egg masses were measured and maternal care intensity was recorded, females and egg masses were collected in 70% ethanol. These specimens have been deposited in the Museum of Zoology, Invertebrate Section (QCAZ) at PUCE. In the laboratory, egg mass size was calculated using the formula for the area of an ellipse ($A$) with width and length of the egg mass as the ellipse's radii $r1$ and $r2$ ($A = \pi \times r1 \times r2$). To calculate the proportion of the area of an egg mass covered by its mother's body, the egg mass surface area was divided by the dorsal surface that the mother's body projects over it. The dorsal surface of each female's body was measured on dorsal photographs of each specimen. Photographs were taken with a PAXcam 2+ (Villa Park, Illinois) attached to a Wild Makroskop M420 (Heerbrugg, Switzerland) set at 6.3× of magnification. Then the dorsal area was obtained by measuring the surface of a drawn polygon that described the dorsal view of the female's body. This measurement was made with PAX-it 7.0 software (Villa Park).

The proportion of parasitised eggs in each egg mass was calculated by dividing the number of parasitised eggs by the total number of eggs. The parasitised eggs were identified by the drastic change in their appearance when they contain a pupa of a parasitic wasp. Healthy eggs were white compared with parasitised eggs that were brown. The parasitoid was identified as a member of the family Mymaridae (Hymenoptera) (Pitkin, 2004).

*Statistical analysis*

Data analyses were conducted to identify the factors that influenced the probability of a female having her egg mass parasitised or not, and the degree of parasitism for females with a parasitised egg mass. A generalised linear model with a binomial logit fit was used to examine the effect on presence and absence of parasitism using four variables: (1) the number of egg-guarding females on the host plant (more than one female nesting on the plant was considered an aggregation); (2) the distance to the closest neighbour host plant; (3) the distance to the closest egg-guarding female; and (4) host plant identity. Ecologically relevant interactions between some of these variables were also included in our model (see Results). Additionally, as a support model, a generalised linear model with a linear fit was run to examine the relationship between the number of aggregated females on a plant and (1) the distance to the closest neighbour host plant; (2) the distance to the closest egg-guarding female; and (3) the additive effect of these two variables. For these analyses, the most parsimonious model was identified using the Akaike information criterion (AIC) (Akaike, 1974). The likelihood ratio test (LRT) was used to find the difference between the initial models and the reduced models, dropping an effect term at each iteration.

Second, because of the limited amount of data on parasitised egg masses (11 observations), linear regressions and Lowess (Locally Weighted Scatter-plot Smoothing) analysis (Cleveland, 1979) were used to assess the relationship between the proportion of parasitised eggs and our three variables of interest: (1) the number of females aggregated on the plant; (2) the coefficient of maternal care aggressiveness; and (3) the proportion of egg mass covered. For these analyses the proportion of parasitised eggs was Arcsine-transformed and was calculated either as a mean per female or as an accumulated value for the whole plant. This allowed us to test the hypothesis of aggregated females as a parasitism dilution factor. If aggregated females on a plant represent a numerical factor that divides among all of their egg masses the maximum number of eggs a parasitoid can parasitise, the number of parasitised eggs on a plant would remain constant, regardless of how many females are guarding their eggs on it. This assumes that an individual parasitoid can lay fewer eggs than the number of available treehopper eggs in her vicinity. In addition, linear regressions were used to determine the effect of egg mass size and female pronotal surface area on the proportion of coverage. Pearson's correlations were used between the number of aggregated females, their coefficient of maternal care aggressiveness and egg mass coverage to find if female aggregation affects overall within-plant female maternal care strategies.

The generalised linear model, logistic regression, and linear regressions were carried out using SPSS Statistics 17.0 software (Atmonk, New York) whereas the Lowess curve and correlations were run with R 2.14.1 software (R Core Team, 2012).

## Results

### Female aggregation behaviour

In total, 50 *A. grossa* egg-guarding females were analysed during this study and 78% (39 females) of these were found in aggregations (i.e. sharing the host plant with at least one other egg-guarding female) in 11 different aggregations. Forty-four per cent (22) of these females were found on *Solanum* sp., 50% (25) on *Cestrum* sp., and 6% (3) on *Brugmansia arborea* (L.) Lagerh., all members of the Solanaceae. Sixty-eight per cent (15) of the egg-guarding females found in *Solanum* sp. were aggregated, 76% (19) in *Cestrum* sp., and 66% (2) in *B. arborea*. The spatial distribution of egg-guarding females was not evaluated within the aggregation on the host plant, but each female tended to use peripheral leaves and shoots to lay their eggs. The number of female aggregations significantly increased when both host plants and egg-guarding females were spatially clustered (GLM, $P = 0.01$, LRT $= 6.70$).

### Risk of parasitism

Of the 50 egg masses analysed, 11 (22%) contained parasitised eggs, in spite of being guarded by females. The number of egg-guarding females on a single plant significantly reduced the probability of an egg mass being parasitised (GLM, $p = 0.006$, LRT $= 7.53$, Table 1). The probability of a female's eggs being parasitised decreased consistently as the number of females with eggs on a same plant increased (logit regression, $P = 0.01$, Fig. 2). Neither the species of the host plant, nor the distance to the closest host plant (DH) or to the nearest egg-guarding female (DF) significantly affected the risk of a female's eggs being parasitised (Table 1). However, there was a potential effect of the interaction of distance to the nearest host plant (DH) and the distance to the nearest female guarding eggs (DF) on the risk of being parasitised (GLM, $P = 0.08$, LRT $= 3.10$, Table 1). This suggests that egg-guarding females may be at a higher risk when they are isolated (i.e. far from another host plant and from another egg-guarding female).

### Determinants of the level of egg mass parasitism

Further analysis of the 11 parasitised egg masses identified additional local factors that might affect the level of parasitism in each egg mass. As the number of egg-guarding females on the same plant increased, both the average proportion of parasitised eggs ($R^2 = 0.59$, $P = 0.001$, Fig. 3) and the accumulated number of parasitised eggs from all egg masses present on the plant decreased ($R^2 = 0.44$, $P < 0.01$, Fig. 3).

The proportion of the egg mass covered by the mother's pronotum depended on the egg mass size ($R^2 = 0.82$,

**Table 1.** Generalised linear model with a binomial logit fit of the incidence of egg mass parasitism depending on female aggregation on the same plant, host plant species, and spatial distribution of host plants and egg-guarding females.

| Factor | AIC[†] | $\Delta_i$[‡] | Likelihood ratio test | P |
|---|---|---|---|---|
| All (A) | 55.40 | 1.99 | – | – |
| Number of egg-guarding females on the same plant (NF) | 60.93 | 7.53 | 7.53 | 0.006* |
| Host plant species (HP) | 54.59 | 1.18 | 1.19 | 0.27 |
| Distance to the closest host plant (DH) | 53.46 | 0.06 | 0.07 | 0.80 |
| Distance to the closest egg-guarding female (DF) | 53.40 | 0.00 | 0.005 | 0.94 |
| HP × NF | 53.79 | 0.38 | 0.40 | 0.53 |
| HP × DH | 53.9 | 0.51 | 0.51 | 0.51 |
| DH × DF | 56.50 | 3.09 | 3.10 | 0.08 |

*Significant at $P < 0.01$.
†Akaike's information criterion (Akaike, 1974).
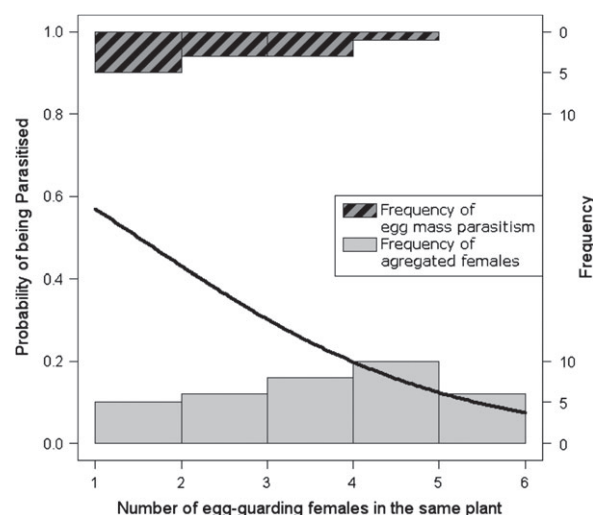‡$\Delta_i = AIC_{(DF)} - AIC_{(each\ model)}$..



**Fig. 2.** Probability of egg mass parasitism depending on female aggregation. The logistic curve shows a significant decrease in the probability of an egg mass to be parasitised as the number of egg-guarding females on a plant increased. Nagelkerke pseudo-*R*-squared is shown as $R^2 = 0.24$, $P < 0.01$.

$P < 0.001$) rather than the dorsal area of her pronotum ($R^2 = 0.03$, $P = 0.94$). There was a weak correlation between the proportion of egg mass coverage and the level of parasitism on egg masses ($r = -0.59$, $P = 0.07$). The correlation between females' coefficient of maternal care aggression and the level of parasitism was not significant ($r = -0.41$, $P = 0.21$). However, Lowess analysis showed a tendency towards a reduction in egg mass parasitism with an increase of egg mass coverage by the mother (Fig. 4a) and their readiness to defend it (Fig. 4b). There was a significant positive correlation between
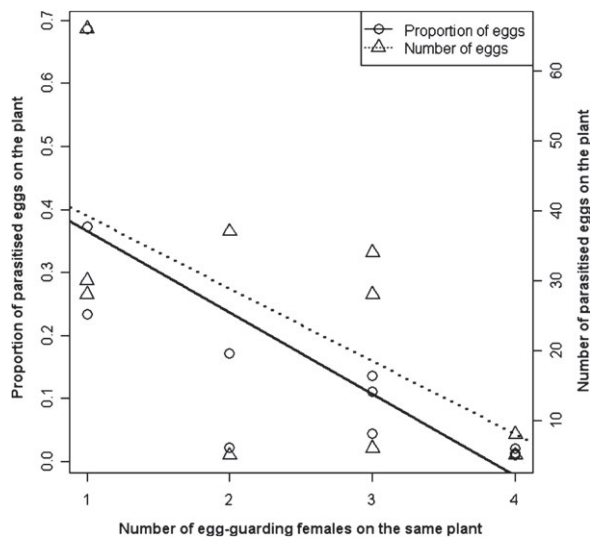
**Fig. 3.** Female aggregation: dilution effect on parasitism. The proportion of parasitised eggs on a plant as well as the overall number of parasitised eggs on the plant significantly decreased as the number of egg-guarding females on a plant increases. For the number of parasitized eggs, $R^2 = 0.44$, $P < 0.001$, $y = -10.40x + 49.64$ and for the proportion of parasitized eggs, $R^2 = 0.59$, $P < 0.007$, $y = -12.94 + 49.51$.

female aggregation on the plant, and the proportion of egg mass coverage ($r = 0.79$, $P < 0.01$) and the coefficient of maternal care aggression ($r = 0.64$, $P = 0.03$) (Fig. 5a, b).

## Discussion

### *Female aggregation behaviour*

More than half of the analysed females were aggregated. The increase in aggregation number with increasing spatial clustering of egg-guarding female and host plants suggests that *A. grossa* females aggregate by choosing the same host plant instead of dispersing to nearby unoccupied plants. Female spatial distribution within aggregations suggests egg-guarding females did not preferentially select one single spot on the plant.

### *Risk of parasitism*

A reduction of predation has been reported as an advantage of gregarious life styles in several animal taxa (Turchin & Kareiva, 1989; Uetz *et al.*, 2001; Cresswell, 2002; Shiojiri & Takabayashi, 2003; Spieler, 2003). One proposed benefit of aggregations against predators is the avoidance effect (Hamilton, 1971; Turner & Pitcher, 1986). These authors suggest that a predator is less likely to find a particular aggregation rather than all the member individuals if they were scattered in space (Hamilton, 1971; Turner & Pitcher, 1986). Another proposed benefit of aggregations is the dilution effect or selfish herd, which suggests that the risk of predation for a single individual in an aggregation is lower than for

an individual in solitude (Hamilton, 1971; Turner & Pitcher, 1986). Our results showed that *A. grossa* females experienced a reduction in the probability of egg mass parasitism when aggregated. Hence, egg-guarding females may benefit from both the avoidance effect and selfish herd dilution effect against parasitoids when aggregated. Female aggregations imply that females concentrate on one plant and do not put their eggs at risk by scattering them individually among nearby host plants. In addition, aggregated females may also benefit from the group if an attacking parasitoid can only parasitise a certain number of egg masses and then departs leaving other egg masses untouched. For instance, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) larvae benefit from a gregarious lifestyle provided that, regardless of how many larvae are present on the plant, its parasitoids (*Cotesia plutellae* Kurdjumov, Hymenoptera: Braconidae) parasitise only one or two individuals before leaving (Shiojiri & Takabayashi, 2003).

Parasitoids search for potential hosts using several signals, of which chemical cues seem to be the most important (Vinson, 1976; Fatouros *et al.*, 2008; Wickremasinghe & Van Emden, 2008). These chemical signals have been reported to be those of the host's host plant, host plant emissions induced by feeding activity of the host, and host insect itself (Vinson, 1976; Fatouros *et al.*, 2008). The present study shows that *A. grossa* egg-guarding females densely distributed over groups of host plants decreased the risk of parasitism on their egg masses. This contradicts what might be expected given that aggregations of females on clumped host plants may represent an increase in emission of chemical signals, and consequently, potentially increase parasitoid attraction. This apparent contradiction could be as a result of the added effect we found of egg-guarding female and host plant spatial aggregation on increasing the number of egg-guarding females on one plant. As mentioned above, female aggregation was the principal variable reducing the risk of parasitism. As a result, the effect of aggregation of egg-guarding females on reducing parasitism risk might be overpowering the effect of host plant and female spatial grouping on the attraction of parasitoids by concentrated chemical cues.

### *Determinants of the level of egg-parasitism*

Subsocial maternal care strategies in treehoppers have been reported to be effective against predatory attacks but not against parasitoids (Eberhard, 1986; Perotto *et al.*, 2002). In the present study with *A. grossa*, both maternal defensive behaviour and egg mass coverage decreased the level of parasitism. *Alchisme grossa* is a member of the tribe Hoplophorionini which has the highest degree of maternal care investment among the treehoppers (McKamey & Deitz, 1996). This highly developed maternal care may have evolved when treehoppers moved into higher altitudes where there was not enough ant diversity to support ant-membracid mutualism interactions for offspring defence (Wood, 1984). However, it should be noted that some hoplophorionine species occurring at low elevations, where ants are ubiquitous, also lack mutualistic relationships with ants (Wood, 1984). Thus,
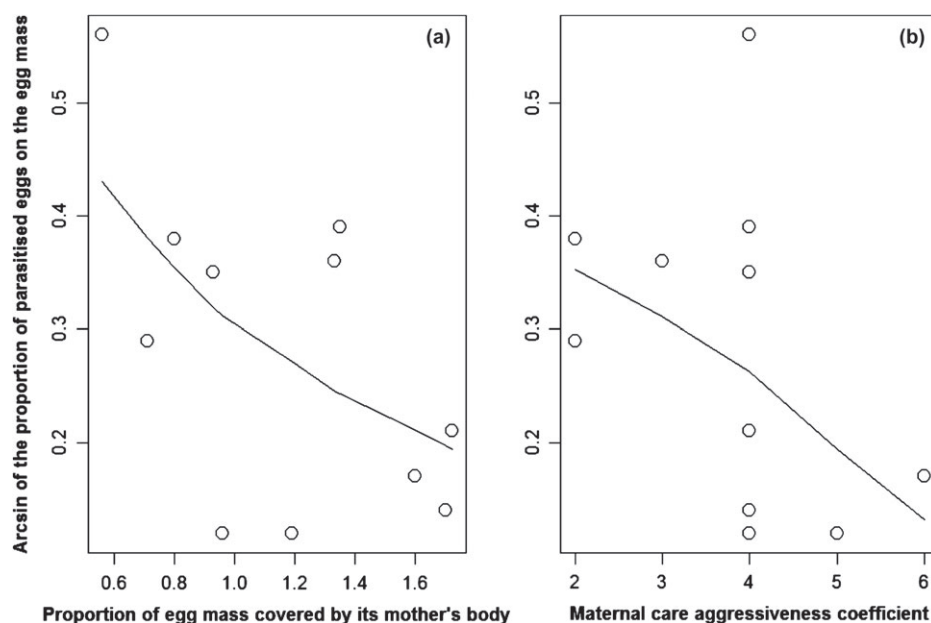
**Fig. 4.** Egg mass parasitism levels in relation to the (a) proportion of the egg mass covered by the female and (b) maternal care aggression coefficient. The proportion of parasitised eggs in the egg mass decreased with increasing egg mass coverage by the mother and maternal care aggression. The proportion of parasitised eggs on the egg mass was arcsine transformed (Lowess analysis, f = 1, t = 2, W = tri-cube weight function, d = 1).

for *A. grossa*, subsocial maternal care is an adaptive response to increase offspring survival (Wood, 1984). The decreasing parasitism levels with increasing defensive behaviour observed in this study with *A. grossa* are congruent with selection pressure from predation and the evolutionary trends within the tribe. Moreover, as in all members in the tribe, *A. grossa*'s maternal care also has associated morphological adaptations. The pronotum completely covers the insect dorsally, forming a shield-like structure. This is of great importance as it provides the female with a wide, solid dorsal surface under which she can hide and protect her egg mass (Wood, 1993). This is consistent with the trend of a decrease in parasitism with an increasing proportion of egg mass coverage. Studies in different Hemiptera support this point. Some Pentatomidae and Acanthosomatidae (Heteroptera) use their broad shield-like body to cover their egg masses (Eberhard, 1975; Mappes & Kaitala, 1994). In *Elasmucha grisea* (L.) (Acanthosomatidae), females adjust their egg masses to a size they can cover with their bodies, and eggs that a female fails to cover are readily parasitised (Mappes & Kaitala, 1994).

Aggregated *A. grossa* egg-guarding females experienced a dilution effect on the parasitism levels in their egg masses. This has also been reported in the wasp *Crabro cribrellifer* (Packard) (Hymenoptera: Sphecidae), which experiences a reduction in parasitism levels with increasing nest aggregation (Wcislo, 1984). Wcislo (1984) also suggests that these wasps choose to aggregate their nests because it is more likely that a neighbouring nest will be parasitised, in a classic selfish herd model. If, aggregated *A. grossa* females on a plant represent a numerical factor that divides among their egg masses the number of eggs a parasitoid can parasitise, the overall number of parasitised eggs on a plant would remain constant, regardless

of how many females are guarding their eggs on it. In this study, however, the number of parasitised eggs on a single plant as well as the proportion of parasitised eggs decreased with an increased number of egg-guarding females on the plant. This suggests that *A. grossa* female aggregations were not only diluting parasitoid pressure among them, but discouraging the parasitoids from oviposition.

This discouraging effect on parasitoids might be explained by the positive effect female aggregation has on egg mass coverage combined with aggressive maternal care. Aggregated females increased their proportion of egg mass coverage by laying smaller egg masses, and benefitted from this behaviour through reduced parasitism. Additionally, *A. grossa* egg-guarding females sent substrate-borne alarm signals by drumming with their abdomen on the branch they were located on when threatened (L. Camacho & C. Keil, unpublished). This signalling has also been reported in the hoplophorionine treehopper *U. crassicornis* (Cocroft, 1996, 1999), and other treehoppers (Cocroft, 2003, 2005; Cocroft *et al.*, 2008). Hence, if females attacked by parasitoids sent a distress signal, this may have put other aggregated egg-guarding females on alert and increase their irritability. This could explain why aggregated females intensified their maternal defensive behaviour. This phenomenon has been reported for *Metepeira incrassate* F.O. Pickard-Cambridge, a social spider that uses substrate vibrations on the web to warn other spiders about predatory attacks (Uetz *et al.*, 2001). In that case, a reduction in the predator's success was found with an increase of the number of spiders on the web (Uetz *et al.*, 2001). Accordingly, both female coverage of the egg mass and maternal care aggressiveness had a negative relation with parasitism levels in *A. grossa*. Therefore, aggregating *A. grossa* females enhanced
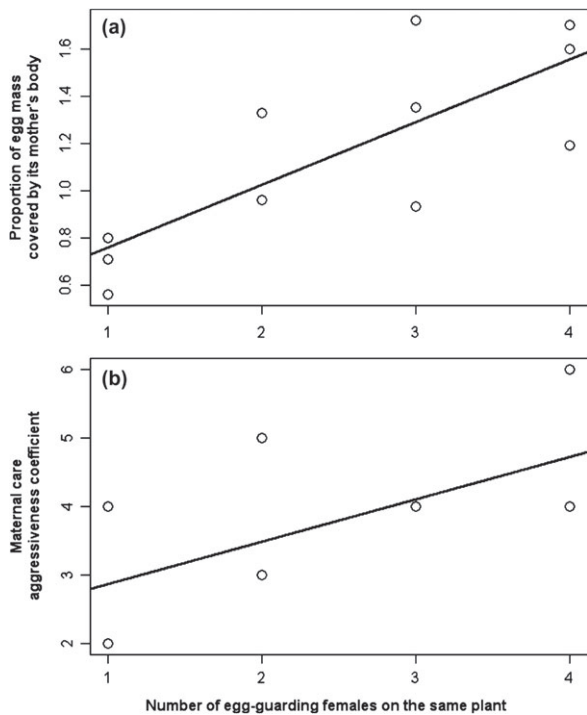
**Fig. 5.** The effect of female aggregation on the (a) proportion of egg mass coverage and (b) coefficient of maternal care aggression. An increase in both egg mass coverage by the female as well as maternal care aggression was significantly correlated with increasing numbers of egg-guarding females on the host plant. (a) $r = 0.79$, $P < 0.01$; (b) $r = 0.64$, $P < 0.03$.

their maternal care capabilities generating a better defence against parasitoids. Cooperative defence among individuals in insect aggregations has been reported in social and a few non-social insects (Evans, 1990). As a result, the dilution of risk of parasitism by aggregated *A. grossa* females by a selfish herd effect may also include a kin selection effect. These results might give a new dimension to the term 'sub-sociality' in Hoplophorionini treehoppers.

Further evidence of female aggregation as a defence mechanism against parasitism can be found in an *A. grossa* population in Tandayapa. Tandayapa is approximately 20 km east and 900 m higher in elevation than Mindo. Studies of this population of *A. grossa* found no egg mass parasitism. Only 38% of the egg-guarding *A. grossa* females in Tandayapa were aggregated (L. Camacho & C. Keil, unpublished) in contrast to 78% of the egg-guarding females from Mindo in this study. Moreover, females from Tandayapa were not as aggressive as those in Mindo when defending their egg masses. The individual egg masses were about twice as large as those from Mindo and consequently the proportion of the egg mass covered by the female was reduced (L. Camacho & C. Keil, unpublished). Therefore, it appears that in the absence of egg mass parasitism, *A. grossa* females in Tandayapa did not have such a strong propensity to aggregate and invest in maternal care strategies such as aggressive behaviour or minimising egg mass size.

Future studies should aim towards studying the genetic relationship among aggregated *A. grossa* females. When *A. grossa* immatures reach adulthood and gain flight capabilities, they begin to disperse in a similar fashion to *U. crassicornis* (Wood & Dowell, 1985), although sisters may stay on the host plant and rear their own offspring there. Hence, cooperation between egg-guarding sibling females would be strongly justified by genetic relatedness. Because of the tendency to aggregate for oviposition, it is possible that adult females have limited dispersal and hence may show some degree of relatedness. If that is the case, *A. grossa*'s subsociality would include a kin selection component added to the selfish herd effect and simple cooperative behaviour proposed in this study. The existence of kin selection within the species' subsocial behaviour would put it, or even the whole tribe, a step closer towards the evolution of eusociality (Anderson, 1984).

## References

Akaike, H. (1974) A new look at the statistical model identification. *IEEE Transactions on Automatic Control*, **19**, 716–723.

Anderson, M. (1984) The evolution of eusociality. *Annual Review of Ecology and Systematics*, **15**, 165–189.

Cleveland, W.S. (1979) Robust locally weighted regression and smoothing scatterplots. *American Statistical Association*, **74**, 829–836.

Cocroft, R.B. (1996) Insect vibrational defence signals. *Nature*, **382**, 679–680.

Cocroft, R.B. (1999) Parent-offspring communication in response to predators in a subsocial treehopper (Hemiptera: Membracidae: *Umbonia crassicornis*). *Ethology*, **105**, 553–568.

Cocroft, R.B. (2003) The social environment of an aggregating, ant-attended treehopper (Hemiptera: Membracidae: *Vanduzea arquata*). *Journal of Insect Behavior*, **16**, 79–95.

Cocroft, R.B. (2005) Vibrational communication facilitates cooperative foraging in a phloem-feeding insect. *Proceedings of the Royal Society of London*, **272,** 1023–1029.

Cocroft, R.B., Rodriguez, R. & Hunt, R. (2008) Host shifts, the evolution of communication, and speciation in the *Enchenopa binotata* species complex of treehoppers. *Specialization, Speciation and Radiation: The Evolutionary Biology of Herbivorous Insects* (ed. by K. J. Tilmon), pp. 88–100. University of California Press, Berkeley, California.

Cresswell, W. (2002) Flocking is an effective anti-predator strategy in redshanks, *Tringa totanus*. *Animal Behaviour*, **47**, 433–442.

De Moraes, C.M., Lewis, W.J., Paré, P.W. & Tumlinson, J.H. (1998) Herbivore infested plants selectively attract parasitoids. *Nature*, **393**, 570–574.

Eberhard, W. (1975) The ecology and behaviour of a subsocial pentatomid bug and two scelionid wasps. Strategy and counterstrategy in a host and its parasitoids. *Smithsonian Contributions to Zoology*, **205**, 1–39.

Eberhard, W. (1986) Possible mutualism between females of the subsocial membracid *Polyglypta dispar* (Homoptera). *Behavioral Ecology and Sociobiology*, **19**, 447–453.

Evans, D. (1990) *Insect Defenses: Adaptive Mechanisms and Strategies of Prey and Predators*. State University of New York Press, New York, New York.

Fatouros, N., Dicke, M., Mumm, R., Meiners, T. & Hilker, M. (2008) Foraging behaviour of egg parasitoids exploiting chemical information. *Behavioral Ecology*, **19**, 677–689.

Godoy, C., Miranda, X. & Nishida, K. (2006) *Treehoppers of Tropical America*. Instituto Nacional de Biodiversidad, San José, Costa Rica.

Hamilton, W.D. (1971) Geometry for the selfish herd. *Journal of Theoretical Biology*, **31**, 295–311.

Kessler, A. & Baldwin, I.T. (2001) Defensive functions of plant volatile emissions in nature. *Science*, **291**, 2141–2144.

Mappes, J. & Kaitala, A. (1994) Does a female parent bug lay as many eggs as she can defend; experiments with *Elasmucha grisea* L. (Heteroptera; Acanthosomatidae). *Behavioral Ecology and Sociobiology*, **35**, 314–317.

McKamey, S.H. & Deitz, L.L. (1996) Generic revision of the New World tribe Hoplophorionini (Hemiptera: Membracidae: Membracinae). *Systematic Entomology*, **21**, 295–342.

Perotto, M., Buffa, L. & Delfino, M. (2002) Efectos de la atención de *Camponotus rufipes* (Fabricius) (Hymenoptera, Formicidae) sobre agregaciones de *Enchenopa cericea* Walk. (Hemiptera: Membracidae). *Ecología Austral*, **12**, 91–97.

Pitkin, B. (2004) *Universal chalcidoidea database: a key to the families of chalcidoidea and mymaromatoidea* [WWW document]. URL http://www.nhm.ac.uk/research-curation/research/projects/chalcidoids/keys1_14.html [accessed on 4 February 2012].

R Core Team (2012) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria [WWW document]. URL http://www.R-project.org [accessed on 18 February 2012].

Ramaswamy, K. & Cocroft, R.B. (2009) Collective signals in treehopper broods provide predator localization cues to defending mothers. *Animal Behaviour*, **78**, 697–704.

Shiojiri, K. & Takabayashi, J. (2003) Effects of specialist parasitoids on oviposition preference of phytophagous insects: encounter–dilution effects in a tritrophic interaction. *Ecological Entomology*, **28**, 573–578.

Sierra, R., Cerón, C., Palacios, W. & Valencia, R. (1999) *Propuesta preliminar de un sistema de clasificación de vegetación para el Ecuador continental*. Proyecto INEFAN/GEF-BIRF y EcoCiencia, Quito, Ecuador.

Spieler, M. (2003) Risk of predation affects aggregation size: a study with tadpoles of *Phrynomantis microps* (Anura: Microhylidae). *Animal Behaviour*, **65**, 179–184.

Turchin, P. & Kareiva, P. (1989) Aggregation in *Aphis varians*: an effective strategy for reducing predation risk. *Ecology*, **70**, 1008–1016.

Turlings, T., Wackers, F., Vet, L., Lewis, J. & Tumlinson, J. (1992) Learning of host-finding cues by hymenopterous parasitoids. *Insect Learning: Ecological and Evolutionary Perspectives* (ed. by D. Papaj, A. Lewis), pp. 59–75. Springer, New York, New York.

Turner, G. & Pitcher, T. (1986) Attack abatement: a model for group protection by combined avoidance and dilution. *American Naturalist*, **128**, 228–240.

Uetz, G., Boyle, J., Hieber, C. & Wilcox, S. (2001) Antipredator benefits of group living in colonial web-building spiders: the 'early warning effect'. *Animal Behaviour*, **63**, 445–452.

Vinson, S.B. (1976) Host selection by insect parasitoids. *Annual Review of Entomology*, **21**, 109–133.

Wcislo, W. (1984) Gregarious nesting of a digger wasp as a "selfish herd" response to a parasitic fly (Hymenoptera: Sphecidae; Diptera: Sarcophagidae). *Behavioral Ecology and Sociobiology*, **15**, 157–160.

Wickremasinghe, M. & Van Emden, H. (2008) Reactions of adult female parasitoids, particularly *Aphidius rhopalosiphi*, to volatile chemical cues from the host plants of their aphid prey. *Physiological Entomology*, **17**, 297–304.

Wood, T.K. (1974) Aggregating behaviour of *Umbonia crassicornis* (Homoptera: Membracidae). *Canadian Entomologist*, **106**, 169–173.

Wood, T.K. (1984) Life history patterns of tropical membracids (Homoptera: Membracidae). *Sociobiology*, **8**, 299–344.

Wood, T.K. (1993) Diversity in the new world Membracidae. *Annual Review of Entomology*, **38**, 409–435.

Wood, T.K. & Dowell, R. (1985) Reproductive behaviour and dispersal in *Umbonia crassicornis* (Homoptera: Membracidae). *Florida Entomologist*, **68**, 151–158.