

The effect of multi-species host density on superparasitism and sex ratio in a gregarious parasitoid

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Abstract. 1. The ecological factors that influence key life-history traits such as brood size and sex ratio are enormously important to the survival and population dynamics of insect species.

2. The effects of host density on life-history characteristics of a parasitoid, *Pteromalus apum*, were examined in a field experiment conducted in the Åland Islands, Finland. This gregarious parasitoid preys on two co-occurring butterfly species, *Melitaea cinxia* and *Melitaea athalia*. The abundances of both butterfly species contribute to host density for the parasitoid.

3. The goals of the study were to: (i) test sex allocation theory in a field setting by evaluating how host density and species affect parasitoid brood size and sex ratio; and (ii) understand how parasitoid foraging behaviour and co-occurrence of multiple host species relate to life-history traits of the parasitoid.

4. Local host density and species were experimentally manipulated and natural parasitism was then allowed to occur. Larger brood sizes were found at low host density due to higher rates of superparasitism. Further, parasitoid brood size and sex ratio (proportion male progeny) were positively related, with a much stronger effect at low host density.

5. These results illustrate that host density affects life-history and population-level traits of parasitoids. The fact that low host density was associated with high superparasitism (larger brood size) supports predictions based on the ‘apparent commensal’ indirect interaction between the host species. This work highlights the value of research that integrates interactions between species with the study of foraging behaviour and life-history traits.

Key words. Apparent competition, brood size, life history, *Melitaea*, *Pteromalus apum*.

Introduction

Parasitoids have long been the focus of empirical and theoretical efforts to understand reproductive allocation, such as how females allocate progeny among broods (Godfray, 1987; Hardy & Mayhew, 1998; Traynor & Mayhew, 2005) and between the sexes (Suzuki & Iwasa, 1980; Mayhew & Godfray, 1997; West, 2009). Parasitoid Hymenoptera have

been particularly useful for studying sex allocation and sex ratio theory, because the females have facultative control over the sex of their offspring. Mate competition theory predicts that in organisms that mate locally, such as gregarious parasitoids (in which multiple offspring develop as a brood using a single host), an ovipositing female should produce just enough males to fertilise her own daughters as long as the host is parasitised by a single or low number of females (foundresses) (Hamilton, 1967). Thus, an extreme female-biased sex ratio is expected, with no resources expended in the overproduction of males.

A series of studies examining sex ratio theory using gregarious parasitoids has demonstrated that other factors, such as superparasitism (more than one foundress parasitising

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a host), increase competition between unrelated males and/or decrease host quality, thereby leading to the expectation of fewer female-biased sex ratios (Hamilton, 1967; Charnov *et al.*, 1981; Suzuki *et al.*, 1984; Shuker *et al.*, 2005). The shift in progeny sex ratio occurs due to adjustment by the superparasitising female(s) (Shuker & West, 2004; Dorn & Beckage, 2007). The predicted positive correlation between number of foundresses (degree of superparasitism) and sex ratio in parasitoid Hymenoptera has been supported by several empirical laboratory (Takagi, 1986; van Welzen & Waage, 1987; Santolamazza-Carbone & Rivera, 2003; Shuker & West, 2004; Shuker *et al.*, 2005) and field studies (Werren, 1983; Takagi, 1987; Burton-Chellew *et al.*, 2008). As expected, superparasitism has also been found to correlate with larger brood size (Taylor, 1988). Among species that superparasitise, the rate of superparasitism should decrease as the density of hosts increases. For parasitoids with a broad host range, this host density will depend on the combined densities of available host species, and their relative availability and suitability. Thus, the composition of the host community can influence the sex ratio and brood size of the parasitoids involved.

Overall, the relationship between host density and parasitoid progeny sex ratio in the field has been little studied (Heimpel & Casas, 2008), particularly in situations where more than one host species is involved. For the species in our study, the generalist parasitoid *Pteromalus apum* Retzius (Hymenoptera: Pteromalidae), host density (number of hosts available for parasitism in a locale) in the summer is to a large extent determined by the abundance of two Lepidoptera species, *Melitaea athalia* Rottemburg and *Melitaea cinxia* L. (Lepidoptera: Nymphalidae). In this situation, two scenarios are plausible, depending on parasitoid foraging behaviour and mobility. First, if parasitoids forage and mate locally, an increase in local host density could decrease the occurrence of superparasitism as foundresses become more likely to encounter unparasitised hosts. Sex ratio theory predicts that this would lead to a decrease in the proportion of male progeny laid by ovipositing females, because the males from different broods would not have to compete for access to mates (Suzuki & Iwasa, 1980). This scenario also predicts a negative correlation between brood size and host density. Secondly, for a more mobile parasitoid species, an increase in host density could lead to behavioural aggregation by individuals from outside of the patch (Bezemer *et al.*, 2010; Corley *et al.*, 2010), increasing the number of wasps in a given patch. This type of behavioural response may increase the proportion of male progeny due to increased superparasitism and competition for hosts. This response might additionally lead to short-term apparent competition between host species due to increased parasitism in one or both species (Holt, 1977; van Nouhuys & Hanski, 2000; Morris *et al.*, 2005). Under the mobile parasitoid scenario, higher superparasitism rates at greater host density would produce a positive correlation between brood size and host density. Thus, the behaviour of parasitoids should determine the relationship among host density, brood size, and sex ratio.

Empirical studies on this subject are few. King *et al.* (1995) used a laboratory experiment to show that host density can

indirectly influence sex ratio. However, the experimental setup was restricted to the biologically constrained scenarios of only one and two host individuals, and competition was limited to two parasitoids that were simultaneously presented with hosts. Shuker *et al.* (2007) went further by manipulating host to foundress ratios and testing the effect on sex ratio in the lab. They report increased sex ratios from focal females at low host to foundress ratios, but only when multiple foundresses were released on a patch simultaneously. This suggests that, at least in a laboratory setting, foundresses make sex allocation decisions based on both host density and expected or perceived superparasitism rates.

In this study, we examined how host density and indirect interactions between host species influence parasitoid sex ratios and superparasitism in the Glanville fritillary butterfly research system in Åland, Finland. The host butterfly is a model system for studies of population (Ehrlich & Hanski, 2004), community (van Nouhuys & Hanski, 2005), and evolutionary (Hanski & Saccheri, 2006) ecology in a natural spatial context. We studied the naturally occurring assemblage of two butterfly host species, *M. cinxia* and *M. athalia*, and a shared pupal parasitoid, *P. apum* (van Nouhuys & Hanski, 2005; Reudler Talsma *et al.*, 2008; van Nouhuys & Kraft, 2012). The results are interpreted in terms of parasitoid sex ratio and brood size to address: (i) how host density and species affect parasitoid life-history traits (sex ratio and brood size); and (ii) the effect of parasitoid foraging behaviour on the relationship between host density and parasitoid life-history traits. We analysed the effect of host density and species on parasitoid life-history traits before integrating this information with knowledge of parasitoid foraging behaviour.

Materials and methods

Study organisms

This study focuses on the parasitoid wasp *P. apum*, which is known to parasitise several Nymphalid butterfly species, as well as megachilid bees (Askew & Shaw, 1997; Stefanescu *et al.*, 2009). In the study area *P. apum* parasitises the butterflies *M. cinxia* and *M. athalia*. Its local use of other hosts is unknown, though rearing of other common Nymphalidae species suggests that species other than *Melitaea* are primarily parasitised by the ubiquitous congener, *Pteromalus puparum* L., rather than *P. apum* (S. van Nouhuys and N. Hirai, unpublished). *Pteromalus apum* is gregarious, producing broods of about 1–60 offspring in a single host. They also readily superparasitise, producing mixed broods of up to 110 offspring (van Nouhuys & Kraft, 2012). Under laboratory conditions *P. apum* is observed to mate immediately upon egression to adulthood. This type of local mating is common in gregarious Pteromalidae (Werren, 1980; Hardy, 1994) and we consider *P. apum* to mate locally, although both sexes are winged and so may also disperse.

The co-occurring host species in this study, *M. cinxia* and *M. athalia*, have a Eurasian distribution and are of conservation interest due to the negative effects of habitat fragmentation leading to the extinction of *M. cinxia* and

decline of *M. athalia* from many parts of Europe (Warren, 1991; Hanski & Kuussaari, 1995; Wahlberg, 1997). *Melitaea cinxia* inhabits open meadows, feeding on *Veronica spicata* L. and *Plantago lanceolata* L. (Plantaginaceae) (van Nouhuys & Hanski, 2005). *Melitaea athalia* has a wider host and habitat range (Warren, 1987; Schwarzwälder *et al.*, 1997), but in Åland it overlaps strongly with *M. cinxia*, feeding on *Veronica chamaedrys* L., *V. spicata*, and *P. lanceolata* in open meadows (Reudler Talsma *et al.*, 2008). The host plants are abundant, so the larvae of the two species do not generally compete for food. Both butterfly species lay eggs on food plants in June. The larvae feed through the summer, diapause over the winter, and feed again in the spring until pupation in mid-May. They pupate inconspicuously in the litter near host plants, often hanging under dead leaves.

Study area

The study took place in the Åland Islands, which are located 60 km off the west coast of mainland Finland. This Baltic archipelago provides a suitable but fragmented habitat for *M. cinxia*, *M. athalia*, and their parasitoids (Ehrlich & Hanski, 2004; van Nouhuys & Hanski, 2005). Of the 4000 habitat patches suitable for *M. cinxia* in the Åland islands, 400–500 are occupied by localised populations (Fig. 1; Nieminen *et al.*, 2004). The butterfly *M. athalia* is present where *M. cinxia* is found in Åland as well as in the surrounding forest edge areas (Reudler Talsma *et al.*, 2008). The Åland Island landscape provides a large number of similar, somewhat isolated, habitat

patches that can be used as replicates within an experimental framework. Eleven field sites were used in 2008, and 10 in 2009, with an overlap of six sites that were used in both years (Fig. 1). The sites were selected by geographical separation, knowledge of local density of naturally occurring host butterflies, uniform topography, and sufficient size to accommodate the experimental setup.

Materials and experimental design

In this experiment, host larvae of both species were placed in the field together (high density) and alone (low density) in replicated plots over two field seasons. Upon pupation, the hosts were parasitised by naturally occurring *P. apum*, after which they were brought into the laboratory where the rate of parasitism, parasitoid brood sizes and individual sexes were scored. Further details of the experimental set up are presented in van Nouhuys and Kraft (2012), which addresses the effect of multi-species host density on the rate of parasitism of the butterflies.

Three treatment plots were placed at least 30 m apart in 11 (2008) and 10 (2009) (Fig. 1) replicate field sites. Each treatment plot consisted of a 5 × 6 rectangular grid of 30 points, 50 cm apart (overall grid dimension 2 m × 2.5 m). The treatment plots were:

- 1 High-density multi-species, in which each spot in the 5 × 6 grid 'checkerboard' included alternating *M. cinxia* and *M. athalia* pupae (30 pupae);

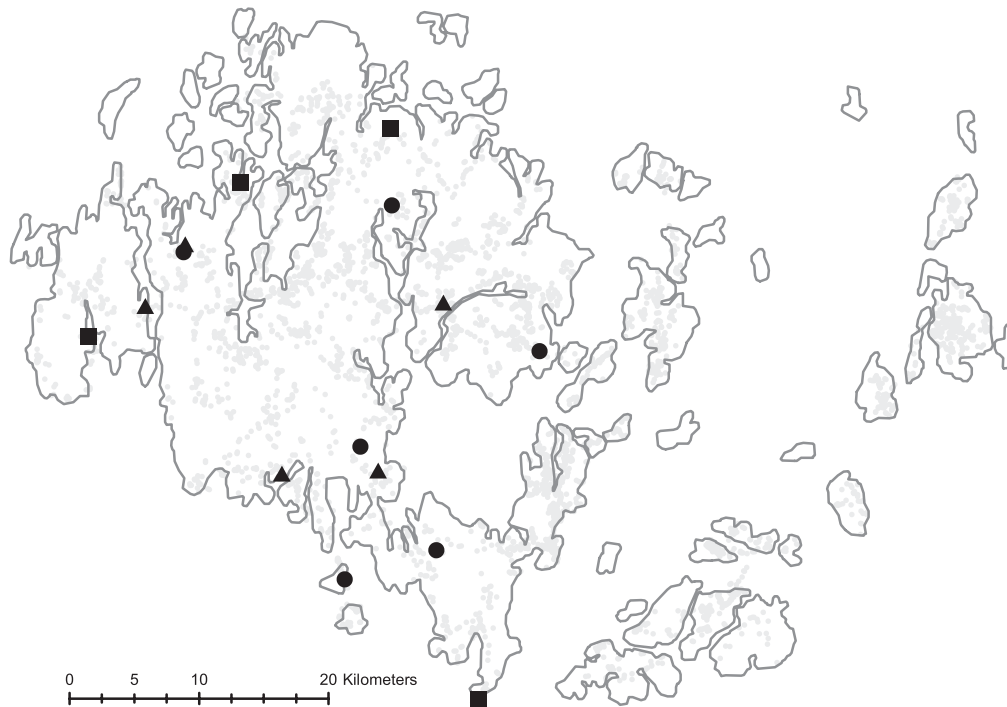


Fig. 1. Map of suitable habitat patches for *Melitaea cinxia* in the Åland Islands, Finland. Light grey spots represent habitat patches suitable for *M. cinxia*. Bold circles are patches used in the experiment in 2008. Bold squares are patches used in the experiment in 2009. Bold triangles are patches that were used for the experiment in both years.

- 2 Low-density *M. cinxia*, in which alternating spots on the 5 × 6 grid ‘checkerboard’ included *M. cinxia* pupae (15 pupae, remaining spaces empty);
- 3 Low-density *M. athalia*, in which alternating spots on the 5 × 6 grid ‘checkerboard’ included *M. athalia* pupae (15 pupae, remaining spaces empty).

Individual last-instar host larvae were placed on potted plants at the initiation of the experiment. Final-instar larvae were used rather than pupae to allow natural foraging by parasitoids that may forage for hosts by following odours associated with pupation, larval-produced silk, or larval feeding. Each pot was covered by a coarse mesh cloth to prevent escape. *Pteromalus apum* is very small (2–3 mm) and tests in the previous year indicated that the mesh cloth did not hinder parasitism by *P. apum*. The plants were placed at each field site for 14–18 days, which was long enough to ensure that sufficient time was available for host pupation and parasitism to occur. The pupae are vulnerable to parasitism by *P. apum* for the first 5 days of their 14–21 day development, after which *P. apum* attempt to parasitise but are unsuccessful (van Nouhuys & Kraft, 2012).

The potted plants used in the experiment (*V. spicata*, *P. lanceolata*) were transplanted from wild populations the previous year and maintained in a greenhouse until use. The *M. cinxia* and *M. athalia* larvae came from our laboratory colonies. These colonies were derived from local Åland butterfly populations, and new individuals are added each year. For each field site we used whichever plant species (*V. spicata* or *P. lanceolata*) was locally most abundant.

In 2008, the mesh cloth was left on the pot for the duration of the experiment. In 2009, the mesh cloth covering the plant was removed after the larvae pupated to allow potential access to pupal parasitoid species that were larger than *P. apum*. Upon completion of exposure to parasitism in the field, the pupae were collected and reared individually in plastic cups in the laboratory until a butterfly or parasitoid emerged. Specimens from which nothing emerged were dissected to determine whether an individual was parasitised or not. Parasitoid species identification was based on a taxonomic key (Askew & Shaw, 1997). To verify the species identity, samples were examined by two Hymenoptera taxonomists (R. Askew and R. Hoebcke). The number of offspring in each brood was counted and the sex of each individual was determined under a light microscope. Female *P. apum* are two to three times larger than males and have visible ovipositors.

Statistical analysis

All data analysis was performed using the statistical package, R (R Core Development Team, 2011). We examined whether brood size varied with host density and species (*M. athalia* and *M. cinxia*) using generalised linear mixed-effects models (GLMMs) constructed using the ‘lme4’ package (Bates *et al.*, 2011). We used a Poisson error distribution and a log link function to best model count (brood size) data. Eleven candidate models that tested the relevance of five independent

Table 1. A list of variables included in the presented models and relevant descriptions.

| Variable | Description |
|--------------------------|---|
| Parasitised | Yes or no variable describing whether a host pupa was parasitised or not |
| Host species | <i>Melitaea athalia</i> or <i>Melitaea cinxia</i> |
| Host density | High- or low-host-density experimental treatment |
| Patch | Field site where the host was placed |
| Sex ratio | The proportion of a brood male |
| Brood size | Number of parasitoids emerging from or found within a parasitised host pupa |
| Development time | Number of days from the placement of a host larva in the field to when parasitoids emerged from the pupa |
| Local rate of parasitism | Proportion of pupae of a given species that were parasitised in a given experimental treatment plot (excluding pupa that were designated ‘unknown’) |
| Year | Year: 2008 or 2009 |
| ID | Individual ID assigned to host pupae |

fixed-effect variables (see Table 1) were generated by removing one or two factors from the full model at a time. ‘Patch’ (site) was included as a random effect in all models. In order to account for overdispersion, an observation level random effect (‘ID’) was also included (Bolker *et al.*, 2009). Sex ratio was not included as a factor because brood size is not dependent on sex ratio. Although the two variables are related, a given sex ratio is unlikely to cause an ovipositing female to alter clutch brood size. Both study years were in the model, with ‘year’ included to account for variation between 2008 and 2009. We implemented AICc model comparison (Hurvich & Tsai, 1989) using the ‘AICcTab’ function in the ‘bbmle’ R package (Bolker & R Development Core Team, 2011), which computes Δ AICc and Akaike weights for all candidate models.

A GLMM was also constructed to evaluate how the sex ratio of broods (from individual parasitised pupae) varied according to brood size, host density, and host species. We used a binomial error distribution and a logit link function to best model proportion (sex ratio) data. Seven candidate models were created in order to test the importance of the interaction between brood size and host density, in addition to the factors development time, year, and local rate of parasitism. ‘Patch’ and ‘ID’ were included as random effects. The same model selection procedure was then performed as previously described.

Results

Over the course of both years in this experiment, 333 of 758 (approximately 44%) host pupae were parasitised by *P. apum*. This number excludes larvae that died before pupation or were eaten by predators, and the small number (22) that were parasitised by other species of parasitoids. The relatively large proportion of parasitised pupae suggests that our methods did not discourage parasitism.

Table 2. Generalised linear mixed effects model (using log link function) of factors affecting brood size.

| Response variable: brood size | | | | |
|-------------------------------|-------------|-------|---------|---------|
| Fixed effect | Coefficient | S.E. | z | P-value |
| Intercept | 7.168 | 0.423 | 16.957 | <0.001 |
| Species | -0.105 | 0.080 | -1.320 | 0.187 |
| Host density | 0.223 | 0.079 | 2.807 | 0.005 |
| Development time | -0.157 | 0.015 | -10.520 | <0.001 |
| Year | 0.958 | 0.106 | 9.067 | <0.001 |

Random effects: Patch + ID.

'Patch' and 'ID' were included as random effects.

Brood size

Brood sizes ranged from one to 110, with an average of 27.5 ± 19.3 (SD) parasitoids per brood. This average reflects the majority of broods, given that median brood size was 24.0. The species of host pupae had no effect on brood size ($z = -1.320$, $P > 0.05$), and brood size varied significantly with host density ($z = 2.807$, $P = 0.005$) (Table 2). Large brood size was strongly associated with low host density, with an average difference in brood size of approximately six between pupae at low and high host density (Fig. 2).

Brood size was also found to correlate with several other factors. Namely, large broods developed faster than small broods ($z = -10.520$, $P < 0.001$; Table 2) and year exhibited a strong effect due to significantly larger broods in 2009 than in 2008 ($z = 9.067$, $P < 0.001$).

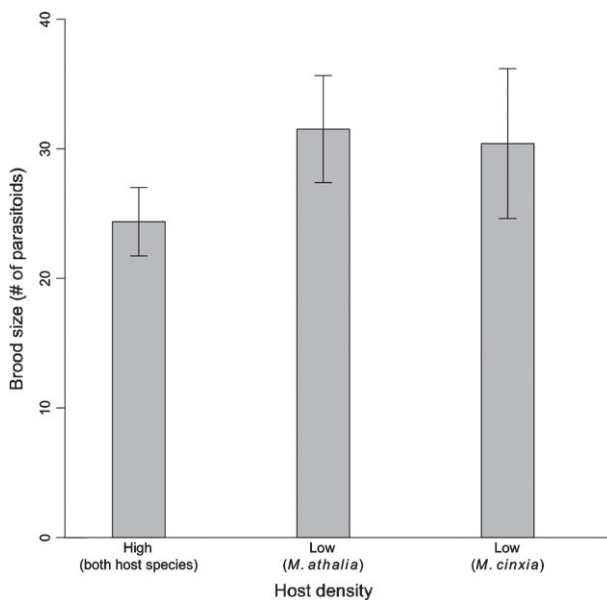


Fig. 2. Brood size versus host density. Mean brood size was 24.4 in high-density pupae, 31.2 in low-density pupae (*Melitaea athalia*), and 30.4 in low-density (*Melitaea cinxia*) pupae. The height of the bars corresponds to mean values, and error bars represent 95% confidence intervals. High, $n = 164$; low (*M. athalia*), $n = 83$; low (*M. cinxia*), $n = 62$.

Table 3. Generalised linear mixed effects model (using a logit link function) of factors affecting brood sex ratio.

| Response variable: sex ratio (proportion males) | | | | |
|---|-------------|-------|--------|---------|
| Fixed effect | Coefficient | S.E. | z | P-value |
| Intercept | 0.533 | 1.136 | 0.469 | 0.639 |
| Brood size | 0.004 | 0.006 | 0.648 | 0.517 |
| Species | -0.218 | 0.161 | -1.347 | 0.178 |
| Host density | -0.694 | 0.305 | -2.278 | 0.023 |
| Development time | -0.101 | 0.036 | -2.777 | 0.005 |
| Local rate of parasitism | 0.731 | 0.362 | 2.023 | 0.043 |
| Brood size \times host density | 0.021 | 0.008 | 2.564 | 0.010 |
| Year | 0.600 | 0.236 | 2.540 | 0.011 |

Random effects: Patch + ID.

'Patch' and 'ID' were included as random effects.

Sex ratio of parasitoid broods

Observed brood sex ratios ranged from 0 to 100%, with an average of approximately 23.4% male. Thus, despite the fact that broods were female-biased overall, in many cases individual broods were highly (some completely) male-biased. The relationship between sex ratio, host density and brood size is understood by examining the effect of the interaction between brood size and host density on sex ratio; highly male-biased sex ratios were more strongly associated with large brood size in low-host-density treatments than in high-host-density treatments (Fig. 3; $z = 2.564$, $P = 0.010$; Table 3).

In the model, sex ratio was also significantly correlated with development time, with more male-biased broods tending to develop faster ($z = -2.777$, $P = 0.005$; Table 3). This relationship probably reflects the fact that male-biased broods tended to be large. Further, the model indicates that sex ratio was greater in the year 2009 ($z = 2.540$, $P = 0.011$), that sex ratios were slightly more male-biased when the local rate of parasitism was higher ($z = 2.023$, $P = 0.043$) and that the sex ratio of broods emerging from *M. athalia* pupae were not significantly different from those emerging from *M. cinxia* ($z = -1.347$, $P > 0.05$).

Total number of parasitoids

To estimate the effect of multi-species host density on the overall size of the next parasitoid generation, sum totals of parasitoid offspring were calculated at low and high host densities. Total numbers of *P. apum* offspring were 4637 (31.2% male) and 3945 (24.0% male) at low (combined *M. cinxia* and *M. athalia* treatments) and high host densities, respectively. The same number of host pupae therefore yielded an additional 692 parasitoids in low-host-density treatments, meaning that low-density plots contributed 17.5% more *P. apum* to the next generation than high density plots. This difference was mainly due to the production of males (1445 at low density vs. 947 at high density) rather than females (3192 at low density vs. 2998 at high density).

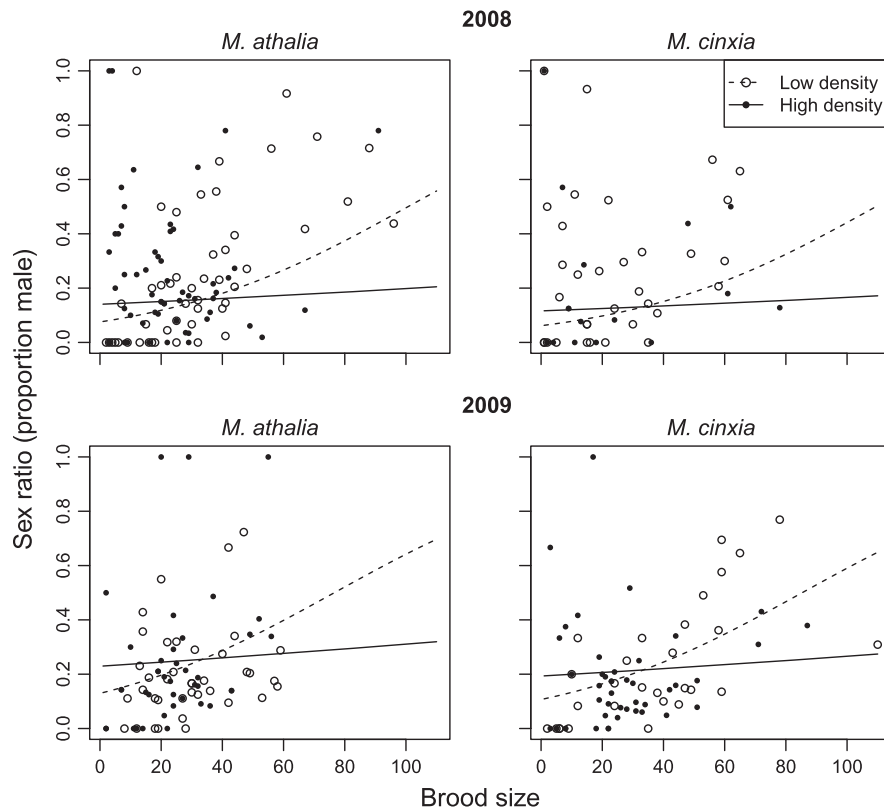


Fig. 3. Predicted sex ratio (proportion of brood male) plotted across the range of observed brood sizes (1–110) at high (solid lines) and low (dotted lines) host density. Predicted sex ratios were calculated using coefficients from the final GLMM presented in Table 3 followed by logit back transformation (the continuous variables “Development time” and “Local rate of parasitism” were both fixed at median values). Raw data is plotted for low (open circles) and high (closed circles) density treatments.

Discussion

Life-history traits in the parasitoid P. apum

For the parasitoid *P. apum*, brood size is a meaningful life history trait that is controlled by the foundress(es), varies with ecological context, and presumably has a strong association with fitness (Hardy *et al.*, 1992). We found that parasitoid brood size did not differ between the two host species. However, brood size decreased with increasing host density such that, on average, large *P. apum* brood sizes occurred in low-host-density treatments (Fig. 2). A subsequent laboratory experiment has demonstrated that when multiple *P. apum* foundresses parasitise a host pupa, the resulting brood size increases more than two-fold between one and three foundresses, presumably as a result of superparasitism (S. van Nouhuys and N. Hirai, unpublished). This same trend has been shown in other gregarious parasitoid species (Taylor, 1988), supporting the idea that the large brood sizes observed in this study resulted from superparasitism.

Superparasitism is avoided by many solitary parasitoids because it is a waste of resources if only a single offspring can develop in a host (Godfray, 1994). However, it is fairly common among gregarious parasitoids and has been shown to have adaptive benefits (van Alphen & Visser, 1990; Dorn &

Beckage, 2007). For example, superparasitism can be adaptive under conditions of low host availability (Takagi, 1987), as a mechanism for overwhelming the immune response of hosts (Hegazi & Khafagi, 2008), and under several other scenarios (Dorn & Beckage, 2007).

There was a strong positive correlation between brood size and sex ratio among broods at low host density, contrasting with a negligible correlation found among broods at high host density (Fig. 3). For gregarious species that mate locally, such as *P. apum*, superparasitism should increase competition for mates between male progeny of different foundresses. It would therefore be beneficial for a superparasitising foundress to produce a high proportion of sons, in order to increase the proportion of female progeny in a mixed brood mating with her male offspring (Hamilton, 1967; Suzuki & Iwasa, 1980; Werren, 1980; van Welzen & Waage, 1987; Shuker *et al.*, 2005).

In addition, theory predicts that host quality and differential fitness gains according to the size of male and female progeny will influence sex ratio (Charnov *et al.*, 1981; Henri & van Veen, 2011). Given the relative size difference between male and female *P. apum* (males are less than half the size of females), superparasitised hosts that are reduced in quality may be more suitable for the production of small males than small females. Indeed, development time was found to be a significant predictor of both brood size and sex ratio (Tables 2

and 3). Larger broods with greater (more male-biased) sex ratios tended to develop faster. This relationship is likely a result of the limited resources available from a given host pupa with large broods depleting those resources faster than small broods (Dorn & Beckage, 2007), supporting the idea that brood size affects host quality.

Thus, at low host density there was a strong correlation between brood size and sex ratio because superparasitism is high, both increasing competition between males (Werren, 1980) and lowering the quality of hosts below the threshold necessary to produce high fitness female progeny (Charnov *et al.*, 1981; Henri & van Veen, 2011). By contrast, it is likely that low rates of superparasitism occurred in high-host-density treatments, resulting in a negligible correlation between brood size and sex ratio in these treatments, due to decreased expected competition among unrelated parasitoid offspring and greater overall parasitoid offspring fitness/survival among a host population of uniform quality.

Indirect interactions and the relationship between host density and parasitoid aggregation

In a companion publication (van Nouhuys & Kraft, 2012), we show that parasitism by *P. apum* leads to short-term 'apparent commensalism' [+ , 0] between the two host species. Specifically, we found that the addition of *M. athalia* reduced the rate of parasitism in *M. cinxia*, whereas the presence of *M. cinxia* had no effect on the rate of parasitism in *M. athalia*. Our experimental design made the realistic assumption that the presence of both host species results in relatively high host density, compared with lower host density in areas with only a single local host species. The short-term 'apparent commensal' interaction between host species reveals that *P. apum* do not aggregate in response to increased host density.

In the absence of behavioural aggregation by the parasitoid, superparasitism should be greater in low-density plots where host choice is more restricted and theory predicts a general positive correlation between brood size (superparasitism) and sex ratio, which is what we observed. A different pattern of parasitism, superparasitism, and sex ratio would have been expected if the parasitoids had responded differently to either host species or density. For example, aggregation in response to increased host density could result in a reciprocal (apparent competition) or non-reciprocal (apparent amensalism) negative indirect interaction between host species (Holt, 1977; Chanton & Bonsall, 2000; DeCesare *et al.*, 2010). A negative indirect interaction due to behavioural aggregation would result in low-density treatments incurring relatively low rates of parasitism compared with high-density treatments. In turn, low-host-density treatments would experience less superparasitism and therefore yield smaller, female-biased broods, which is the opposite of the outcome we found.

Implications for parasitoid foraging ecology

The observed relationship between brood size (resulting from superparasitism) and host density provides insights

regarding the scale of foraging behaviour of *P. apum*, and presumably other parasitoids. Specifically, this study demonstrates conditional superparasitism behaviour. Experimental increases in host density were associated with smaller average brood size in this experiment, either because *P. apum* uses general knowledge of host density for host selection or due to stochastic effects of a low foundresses-to-host ratio in a patch. In the latter case, lower rates of superparasitism could have occurred by chance in high-host-density treatments because more hosts were available for the same number of parasitoids. This scenario is likely if individual foundresses are limited in the number of hosts they can parasitise (due to long handling time, egg limitation, or the inability to locate all hosts in a patch).

Alternatively, host selection rather than chance may account for the observed trend. Parasitism is associated with energy and time costs, therefore a foundress must balance the inherent trade-offs involved in choosing a host, depending on its value as a resource (Charnov & Skinner, 1984). Host quality can differ due to species-specific traits such as body size, or due to proximate effects such as parasitism by competing foundresses. Thus, differences in host quality can alter the optimal foraging strategies and sex allocation of parasitoids (Henri & van Veen, 2011). Although it is now generally accepted that superparasitism can be an adaptive strategy for gregarious parasitoids (Dorn & Beckage, 2007), avoiding superparasitism is probably beneficial when it reduces resource competition among unrelated progeny (van Alphen & Visser, 1990). Some species of Hymenoptera parasitoids are capable of distinguishing between unparasitised hosts, self-parasitised hosts, and hosts previously parasitised by a conspecific (Shuker *et al.*, 2007; Darrouzet *et al.*, 2008). Thus, *P. apum* foundresses in high-host-density treatments may have actively avoided previously parasitised hosts while foundresses in low-host-density treatments could not avoid them. If host discrimination by foundresses is occurring, our study results support the hypothesis that host selection is dependent on host density (van Alphen & Visser, 1990).

The difference in *P. apum* population size due to host density (692, or 17.5%, more *P. apum* offspring from low host density pupae compared to high density pupae) suggests that heterospecific host aggregation may limit the total reproductive output of a shared parasitoid. Even though the presence of multiple host species increases the total number of available hosts and probably *P. apum* density, host aggregation may serve to limit the number of parasitoid offspring generated by the same number of hosts. The difference in offspring number was largely due to the number of males, and thus host density also affected the sex ratio of the next generation.

Conclusions

Parasitoid–host assemblages are model systems for studies of life history, behaviour and population biology (Godfray & Shimada, 1999; Wajnberg *et al.*, 2008). In addition, parasitoid–host systems involving two or more shared host species offer the opportunity to examine how indirect interactions between host species might influence life-history traits (Holt, 1977).

In this study, a parasitoid, *P. apum*, uses two congeneric host species, *M. cinxia* and *M. athalia*. We found that the wasps forage locally rather than aggregate from the surroundings, and that local host density plays an important role in determining brood size (degree of superparasitism) and sex ratio of the parasitoid. Specifically, larger average brood sizes were found at low-host-density treatments, and the positive correlation between brood size and sex ratio was significantly stronger at low rather than high host density. We also found that differences in local parasitoid foraging behaviour, due to host density, drive an 'apparent commensal' relationship between host species (van Nouhuys & Kraft, 2012). These patterns involving host density, parasitoid foraging behaviour, and parasitoid population size demonstrate the complex nature of parasitoid–host interactions that govern the survival and persistence of interacting species in an ecological assemblage. Specifically, the behavioural response of a parasitoid to host co-occurrence has the potential to alter parasitoid population sex ratio and size in the short term, thereby eliciting changes in parasitoid density over time. Accordingly, this study demonstrates an important link among life-history variation, foraging behaviour and community complexity.

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