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Sequences of sex allocation and mortality in clutches of *Metaphycus* parasitoids of soft scale insects and the prevalence of all-female broods

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Abstract. 1. In many gregarious or quasi-gregarious parasitoids that experience local mate competition, precise sex ratios with low variance are observed. Precise sex ratios can be achieved by laying male and female eggs in non-random sequences.

2. Developmental mortality can also alter sex ratios of emerging offspring, and subsequently influence sex ratio optima.

3. The present study investigates sex allocation by *Metaphycus flavus* Howard, *M. luteolus* Timberlake, and *M. angustifrons* Compere (Hymenoptera: Encyrtidae), endoparasitoids of soft scale insects, in the laboratory.

4. All three *Metaphycus* species had precise secondary sex ratios when parasitising brown soft scale, *Coccus hesperidum*, L. in the laboratory. Moreover, we documented that all three species lay fertilised (= female) eggs first followed by unfertilised (= male) eggs at the end of the oviposition bout. However, there were significant differences in sex allocation sequences among species.

5. Mortality rates of eggs allocated within an oviposition bout also varied considerably, indicating that there is a significant interspecific variation in sequence position-specific mortality.

6. Using a stochastic Monte Carlo simulation approach, we provide evidence that the incidence of all-female broods in these parasitoid wasps appears mainly due to developmental mortality and not due to decisions by the ovipositing female. In two species the prevalence of all-female broods was independent of clutch size, contrary to what is expected from theory. The influence of mortality on optimal sex allocation in these parasitoids is discussed.

Key words. All-female broods, developmental mortality, encapsulation, *Metaphycus*, precise sex ratios, sex allocation sequence pattern, soft scale insects.

Introduction

In many parasitic Hymenoptera, mating is confined mostly among siblings, and is often restricted to a local area or natal patch instead of being panmictic (Godfray & Cook, 1997). Under this scenario of spatially-structured populations, males compete for mates. This is known as local mate competition and production of female-biased sex ratios is usually the optimal strategy for ovipositing females (Hamilton, 1967). Hamilton's Theory of Local Mate Competition predicts that the optimum sex ratio for mothers depends on the number of colonists (foundresses) allocating offspring to a patch (Hamilton, 1967, 1979). When there are only a few foundresses in the patch, the optimal sex ratios are highly female-biased, thus reducing competition among males for mating opportunities. In extreme situations where only one female is allocating offspring in a patch, allocating more sons than is necessary to inseminate their sisters is a waste of host resources better provisioned for the production of daughters. In such a situation, the allocation of 'precise sex ratios' with

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low binomial variance is favored (Green *et al*., 1982; Hardy, 1992). One way to achieve 'precise sex ratios' is to lay sexes in non-random sequences. Due to their haplo-diploid sex determination system, parasitic Hymenoptera can control sex allocation by selectively fertilising eggs which become females (diploid) or withholding fertilisation (haploid males) (Godfray, 1994). Non-random sequences of sex allocation are widespread in many parasitic Hymenoptera that have been studied (Hardy, 1992).

Another important factor that influences sex ratio optima, is developmental mortality among offspring that can change the sex ratios of broods between oviposition (primary sex ratio) and adult emergence (secondary sex ratio). If females arise from broods or patches of parasitised hosts without males, and if mating is strictly local, then these females remain unmated and are thus constrained to produce only sons (Green *et al*., 1982; Godfray & Hardy, 1993). Hence, if mortality rates are similar between sexes, then the optimal number of male eggs is predicted to increase with increasing clutch size and with mortality (Green *et al*., 1982). Furthermore, only the mortality of males and its distribution among broods affect the primary sex ratio adjustment under local mate competition (Nagelkerke & Hardy, 1994). Even when optimal primary sex ratio is predicted to be affected by mortality, production of all-female broods is not precluded, but is mostly restricted to small broods according to the predictions of Heimpel (1994). Indeed, given that each host represents a limited resource for developing offspring, parental females face a trade-off between maximising the number of their daughters' production while minimising the probability that they remain unmated (Hartl, 1971; Green *et al*., 1982; Nagelkerke & Hardy, 1994; Hardy & Cook, 1995). Thus, the fitness penalty of producing all-female broods is higher in larger clutches than to smaller clutches, and extra sons should be allocated to larger clutches when mortality risks are high (Heimpel, 1994).

Metaphycus is a genus of encyrtid parasitoids that commonly parasitise soft scale insects (Guerrieri & Noyes, 2000). They are idiobiont parasitoids of soft scales (Lampson *et al*., 1996; Bernal *et al*., 1998, 1999b). In California, a number of *Metaphycus* species attack brown soft scale, *Coccus hesperidum*, L. (Hemiptera: Coccidae) in the field (Kapranas *et al*., 2007). Moreover, these encyrtid parasitoids manifest female-biased precise sex ratios when they develop gregariously in brown soft scales (Kapranas *et al*., 2008). However, it was noted that a great percentage of the broods were all-female, whereas only few all-male broods were observed, and the importance of this finding for optimal sex allocation and the mating structure of these wasps was raised. Hence, the main objective of the current study was to conduct careful laboratory experiments and to assess whether developmental mortality explained the preponderance of the all-female broods found in our field collections. Developmental mortality is very frequent in the encyrtid parasitoid–soft scale associations (Kapranas, 2006). The most common cause of mortality is egg encapsulation, which is a cellular immune response mounted by the scale insect hosts to the evading parasitoid (Blumberg, 1997; Kapranas *et al*., 2009). In our experiments we used three *Metaphycus* species: *M. flavus* (Howard), *M. luteolus* (Timberlake), and *M. angustifrons* (Compere). The latter two species are the most dominant parasitoids of brown soft scale infesting citrus in California (Kapranas *et al*., 2007), whereas the former had been evaluated for biological control of other soft scale pests in California (Bernal *et al*., 1999b, 2001; Schweizer *et al*., 2002, 2003). In laboratory experiments: (1) we first assess whether secondary sex ratios of these parasitoids are precise, i.e. have low variance, (2) we test whether the prevalence of all-female broods depends on the clutch size, and (3) we test whether sex allocation and mortality follow particular sequence patterns within a clutch. Then we use a simulation approach to better understand whether all-female broods in these species are caused by developmental mortality, and we discuss the consequences that mortality can have on sex ratios of these three congeneric species of parasitoid wasps.

Materials and methods

Host and parasitoid cultures

Brown soft scale was cultured on excised *Yucca recurvifolia* Salisbury (Agavaceae) leaves maintained hydroponically in the University of California (UCR) insectary at 27–28 °C, 60% RH and a LD 21:3 h photoperiod. The excised leaves were obtained from yucca plants grown at Agricultural Operations (University of California, Riverside, U.S.A.). Initial culture was established from crawlers collected from a guava tree (*Psidium guajava* L.: Myrtaceae) located on the UCR campus.

Parasitoid cultures were initiated after the following collections. *Metaphycus flavus* was collected from citricola scale in 1996, in Turkey, during an exploration for natural enemies of this scale (Bernal *et al*., 1999b). *Metaphycus luteolus* was obtained from brown soft scale collected in California, U.S.A., and is considered to be a North American species (Timberlake, 1913). *Metaphycus angustifrons* was collected during a recent survey of brown soft scale parasitoids in southern California citrus groves (Kapranas *et al*., 2007) and was introduced into California from Taiwan (Compere, 1957).

Parasitoid cultures were maintained by introducing mated females (*ca* 1 per 10 scales) into a 7.5 cm diameter × 50 cm long plastic tube containing one or two scale-infested yucca leaves with *ca* 300 brown soft scales per leaf. The tube was capped with a plastic lid at both ends. One of the caps had holes covered with a fine nylon mesh to allow air circulation, while preventing adult parasitoids escape or entrance. Honey was streaked on the inside wall of the tube as a carbohydrate source for the introduced or emerged parasitoids, and the tube was maintained at 26 ± 1 °C and 50–70% RH under continuous light. To obtain adult parasitoids for our experiments, we isolated groups of parasitised scales in the 'mummy stage' in glass vials (2.5 cm diameter × 9.5 cm long) sealed with tightly fitting plastic lids, bearing a central hole covered with fine nylon mesh for ventilation. Parasitoids emerged from the mummified scale as mixed-sex groups, and thus they were likely to have mated by the time we used them in our experiments (in the Results section most of the wasps produced female offspring, indicating that they were indeed mated). Each daily cohort was collected in a separate glass vial (2.5 cm diameter × 9.5 cm long). In our experiments, we used 3–4 day-old wasps to allow sufficient time for egg maturation (Kapranas & Luck, 2008). One day before the experiments, each female wasp was isolated in a 0.8 × 3.0 cm vial with a streak of honey on its inside wall and plugged with a piece of Kimwipe® (Roswell, Georgia).

Behavioural observations

In each replicate, an individual wasp was allowed to forage on a scale-infested yucca leaf area (arena) with two scales of an approximate similar size. In our experiments yucca leaf arenas that had a range of host sizes were used, but we used host scales that prompted the parasitoids to lay gregarious clutches (scale length ranging from 2.00 to 3.00 mm). Their behaviour was observed continuously using a dissecting microscope at 10× to 50× magnification and a cool fibre light. Wasps were allowed to oviposit in hosts and observations were terminated when (1) they lost interest in ovipositing in hosts as indicated by a prolonged period of grooming, (2) they began rejecting each host they encountered and run over the yucca leaf (i.e. loss of host searching–examination behaviour) for more than 10 min, or (3) initiated host feeding. All behavioural assays were conducted at room temperature (24–28 °C). We performed assays with 174, 200, and 65 observations with *M. flavus*, *M. luteolus*, and *M. angustifrons* wasps respectively. Successful ovipositions of wasps in hosts were evident, because *Metaphycus* have typical encyrtiform eggs with a stalked part that protrudes from the host dorsum (Maple, 1947). Oviposited eggs were recorded and mapped on a soft scale insect drawing. Parasitised scales along with their host plants were split into two groups. One group was incubated at 27 ± 1 °C, 50–70% RH and LD 14:10 h photoperiod, whereas the second group was incubated at a fluctuating temperature regime of ∼27 °C (day) to 18 °C (night), 55–60% RH and LD 14:10 h photoperiod. The incubation of parasitised scales at different temperature regimes is justified by the fact that temperature affects encapsulation rates and thus, may have a stochastic effect on sex primary sex ratios (see Discussion).

Determination of sex allocation and mortality sequences

Assignment of mortality of each egg and the sex determination of each egg that developed into an adult was made possible by using daily and detailed observations of immature development and adult emergence. The host's integument is transparent and thus, encapsulated eggs and dead larvae could be easily observed. Under our laboratory conditions, *Metaphycus* eggs hatched within 2–4 days. Encapsulated parasitoid eggs could be detected at around the time normal eggs hatch. They and the surrounding tissue become melanised. The young larvae were easily recognised within the scale by their light yellowish colour and peristaltic body movements. Observations were made possible by using fibre optics light. *Metaphycus* larvae are attached to the hatched egg for most of their development, and do not move much from the original oviposition site (as denoted by the stalk of the hatched egg) (Bartlett & Ball, 1964; Saakyan-Baranova, 1966; Kapranas, 2006; Tena *et al*., 2009). Occasionally, *Metaphycus* larvae engaged in lethal combat and the outcome of these aggressive interactions was the consumption of the losers (Kapranas, 2006; Tena *et al*., 2008). Such competitive interaction was rarely observed in *M. flavus* and *M. luteolus*, and was completely absent in *M. angustifrons*. During the late larval instars, septal walls are formed between the larvae within the same host (= a brood) and these walls persist through parasitoid pupation. At this stage, hosts appear compartmentalised with each cell containing an individual developing parasitoid. After pupation, adults emerge from the host dorsum via separate emergence holes. By comparing the size of the emerged wasps with that of the emergence holes, we were able to determine the sex of each non-encapsulated egg, because female wasps are larger than male wasps, and thus form larger emergence holes. Thus, continuous observations from oviposition to hatching of adult wasps allowed us to score the sex of each deposited egg that later developed into an adult.

Statistical analysis

To test whether brood sex ratios (secondary sex ratios) of the three *Metaphycus* species had a lower-than-binomial variance, we computed the variance ratio R of the observed variance of the number of males per brood divided by the variance expected from a binomial distribution. The variance of the binomial distribution was calculated as $p \times q \times N$, where p and $q = (1 - p)$ are the expected proportions of males and females, respectively, in a brood of size N. $R < 1$ thus indicates under-dispersion ('precision'), whereas $R > 1$ indicates over-dispersion. The statistical significance of deviations from binomial expectation was further tested by using the Meelis test (U) for equal clutch sizes. If the U statistic is significant and negative, it indicates under-dispersion, whereas if it is significant and positive, it indicates over-dispersion (Krackow *et al*., 2002). We also tested whether the prevalence of all-female broods was independent of clutch size using a logistic regression separately for each species, which is appropriate for proportional data (Wilson & Hardy, 2002). Computations were done with the statistical package JMP 7.0.

The analysis of sequences of sex allocation was performed by computing the non-parametric statistics that describe different sequential features of the oviposition sequences. This was developed by Wajnberg (1991, 1993) and further used by Colazza and Wajnberg (1998) and Bayram *et al*. (2004). For each oviposition sequence the following statistics were computed: (1) the sum of males' rank position (SMR), which indicates whether males are laid in the beginning of the sequence or not; (2) the variance of the males' rank position (VMR), which shows whether males are laid in the middle of the sequence or not; (3) the centre group of males (CGM), which shows whether there is any pooling of males within the sequence; (4) the centre group of females (CGF), which shows whether there is any pooling of females within the sequence; and (5) the number of runs of males and females (NR), which shows whether there is any autocorrelation of males or females within the sequence. To describe sequences differing in length or those composed of different sex ratios, these five parameters were first transformed into the exact probability corresponding to the first level risk of the associated null hypothesis (for details see Wajnberg, 1993). Thus, the resulting parameters then become uncorrelated with sequence length or sex ratio, and only describe the sequential organisation of males and females within each sequence described. Differences in these parameters for each species were compared with a one-way ANOVA. Each non-parametric test allowed comparison based on only different sequence attributes individually. To assess overall differences in sequential sex allocation among species, we used a canonical discriminant analysis that took into account the multivariate feature of the data.

This method allows the description of each oviposition sequence on successive canonical axes with decreasing importance (i.e. variance). These axes have the property of maximising the inter-/intra-variance ratio. To understand the meaning of the axes obtained, their correlation with the original parameters were computed and graphically represented in the standard format of a correlation circle. Such a correlation circle provides a graphical method for understanding how the computed axes are oriented in the space defined by the five original descriptive parameters used. All analyses were performed using SAS (PROC CANDISC) (SAS Institute, 2000). The same methodology (non-parametric statistics, one-way ANOVA, canonical discriminant analyses) was also employed for the analysis and comparison of sequence-specific mortality among species. In this last case, we calculated (1) the sum of dead offspring rank position (SdR), which indicates whether offspring laid in the beginning of the sequence died or not; (2) the variance of the dead offspring rank position (VdR), which shows whether dead offspring laid in the middle of the sequence died or not; (3) the centre group of dead offspring (CGd), which shows whether there is any pooling of dead offspring within the sequence; (4) the centre group of alive offspring (CGa), which shows whether there is any pooling of alive offspring within the sequence; and (5) the number of runs of dead and alive offspring (NR), which shows whether there is any autocorrelation of dead and alive offspring within the sequence.

Lastly, to test whether for each species the propensity of all-female broods is caused by developmental mortality and not simply by a female's sex allocation decision, we examined the resulting effect of a 'sequence of sex allocation by mortality pattern' product by means of a stochastic, Monte Carlo simulation approach. For this, both a potential sex and a mortality sequence was drawn randomly using the observed average sequences for each species, and the product between the two sequences obtained was computed to see whether an all-female brood is obtained. Such simulation was repeated 500 times using the distribution of clutch size observed in each species as a weighting factor. The simulation set-up was then repeated 30 times and was followed by a one-way ANOVA to compare the resulting probability of obtaining all-female broods among the three species.

Results

Overall, we obtained 137, 68, and 41 hosts parasitised by *M. flavus*, *M. luteolus*, and *M. angustifrons* respectively. These hosts were considered to be parasitised by mated females. Few females produced only male offspring in the two hosts they were offered, so these broods were discarded from the data set, because we assumed that females were unmated in these cases.

Secondary sex ratio

Overall secondary brood sex ratios (SR = proportion males) of all three *Metaphycus* species were female biased: *Metaphycus flavus* (SR = 0.38), *M. luteolus* (SR = 0.24), and *M. angustifrons* (SR = 0.26). Furthermore, they had low R values, suggesting that sex ratio was under-dispersed (i.e. low variance). *Metaphycus flavus* ($U = -5.09$, $P < 0.001$), *M. luteolus* ($U = -3.77$, $P < 0.001$), and *M. angustifrons* ($U = -2.91$, $P < 0.01$) manifested precise sex ratios when parasitising brown soft scale in the laboratory (Table 1).

Prevalence of all-female broods

From 137 broods of *M. flavus*, 21.9% were all-female, resulting in 22.5% virgin daughters (i.e. females emerging from gregarious broods without brothers to mate). There was no association between clutch size and production of all female broods ($\chi^2 = 0.261$, d.f. = 1, $P = 0.609$) (Fig. 1). From 68 broods of *M. luteolus*, 38.23% were all-female, resulting in 28.87% virgin daughters. In this case, however, there was a significant negative relationship between clutch size and production of all-female broods ($\chi^2 = 5.938$, d.f. = 1, $P < 0.05$). However, clutch size explained little of the variation in the prevalence of all-female broods (per cent of the deviance explained 6.5%) (Fig. 1). Lastly, from 41 broods of *M. angustifrons*, 36.6% were all-female, resulting in 36.00% of females being unmated (virgin), and the association between clutch size and production of all female broods was not significant ($\chi^2 = 2.467$, d.f. = 1, $P = 0.116$) (Fig. 1).

Sequences of sex allocation

Females of the three *Metaphycus* species allocated female (fertilised) eggs in the beginning, and one or few male (unfertilised) eggs at the end of the oviposition of a clutch of eggs respectively (Fig. 2). Whenever we had the complete sequences of sex allocation, in almost all cases female wasps of all three species laid at least one male egg at the end of the oviposition bout in a host (*M. flavus*: 94% from 66 sequences; *M. luteolus*: 95% from 19 sequences; *M. angustifrons*: 100% from 20 sequences). However, there were differences between species (Figs 2 and 3). Although all species tended to lay male eggs at the end of the sequence and female eggs at the beginning (high SMR values overall), *M. luteolus* regularly added females towards the end of the sequence, hence the lower SMR value for this species (one-way ANOVA on SMR:

Table 1. Brood sex ratio composition of three *Metaphycus* species parasitising brown soft scale with variance ratio *R* and Meelis test statistic *U* calculated for each brood size/species and overall for each species.

			Number of males						
Species	Brood size	Frequency	0	1	2	3	>3	*R*	*U*
Metaphycus flavus	1								
	2	59	11	45	3	–	–	0.454	72.795
	3	37	2	28	7	0	–	0.328	73.309
	4	8	0	5	3	0	0	0.297	21.645
	5	4	0	4	0	0	0	0.000	6.526
	6	2	0	0	2	0	0	0.000	9.455
	Totals	110	13	82	15	0	0		
	Overall							0.350	−5.09
Metaphycus luteolus	1								
	2	20	11	9	–	–	–	0.747	10.846
	3	13	2	11	0	–	–	0.232	16.789
	4	12	0	11	1	0	0	0.105	22.957
	5	4	0	4	0	0	0	0.000	6.526
	6	4	0	3	1	0	0	0.253	9.348
	Totals	53	13	38	2	0	0		
	Overall							0.287	−3.77
Metaphycus angustifrons	1								
	2	14	6	8	–	–	–	0.646	10.074
	3	9	1	8	0	–	–	0.178	12.308
	4	5	0	5	0	0	0	0.000	8.158
	5	2	1	1	0	0	0	1.111	1.000
	6	1	0	1	0	0	0	0.646	10.074
	Totals	31	8	23	0	0	0		
	Overall							0.338	−2.91

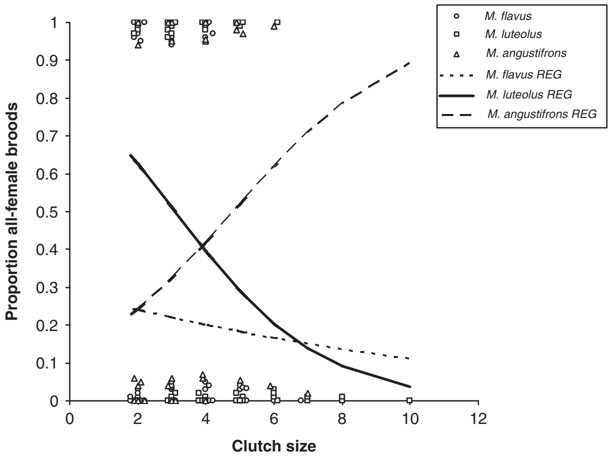

Fig. 1. Relationship between clutch size and the prevalence of all-female broods for three *Metaphycus* species. Only in the case of *M. luteolus* is the relationship significant (see text). Data points are slightly displaced to better indicate sample sizes. The lines indicate the logistic regression model (REG) fitted separately for each of the three species.

$F_{2,243} = 4.05$, $P < 0.05$). Both *M. angustifrons* and *M. flavus* apparently laid males more intensively on the fourth and third position respectively. This which was not the case for *M. luteolus* for which males were laid progressively more and more along the sequence, leading to a lower VMR value (one-way ANOVA on VMR: $F_{2,243} = 4.63$, $P < 0.05$). Furthermore, *M. luteolus* males were laid later in the sequence leading to males being more 'pooled' at the end, and thus resulting in a lower value of CGM for this species (one-way ANOVA on CGM: $F_{2,243} = 3.1$, $P < 0.05$). For *M. luteolus*, males had started to be laid later in the sequence, and females were more 'pooled' toward the beginning of the sequence, leading to lower values of CGF relative to the other two species (one-way ANOVA on CGF: $F_{2,243} = 11.08$, $P < 0.05$). Lastly, NR values did not differ significantly among species (one-way ANOVA on NR: $F_{2,243} = 2.97$, $P = 0.053$). Significant differences among species regarding the structure of their sex allocation sequences are shown using a factorial discriminant analysis (Fig. 4). Specifically, *M. luteolus* appears to be different from the other two species in the way that it structures sequences of sex allocation.

Patterns of mortality within sequences of egg allocation

In all species, egg mortality rates tended to be higher either at the end or at the beginning of the sequences (Fig. 5). However, for some species this effect is stronger; in *M. angustifrons* eggs laid at the end of the sequence had a high mortality risk, leading to higher SdR values (one-way ANOVA on SdR: $F_{2,243} = 3.36$, $P < 0.05$), whereas in *M. flavus*, eggs laid at the beginning of the sequence had a higher mortality risk

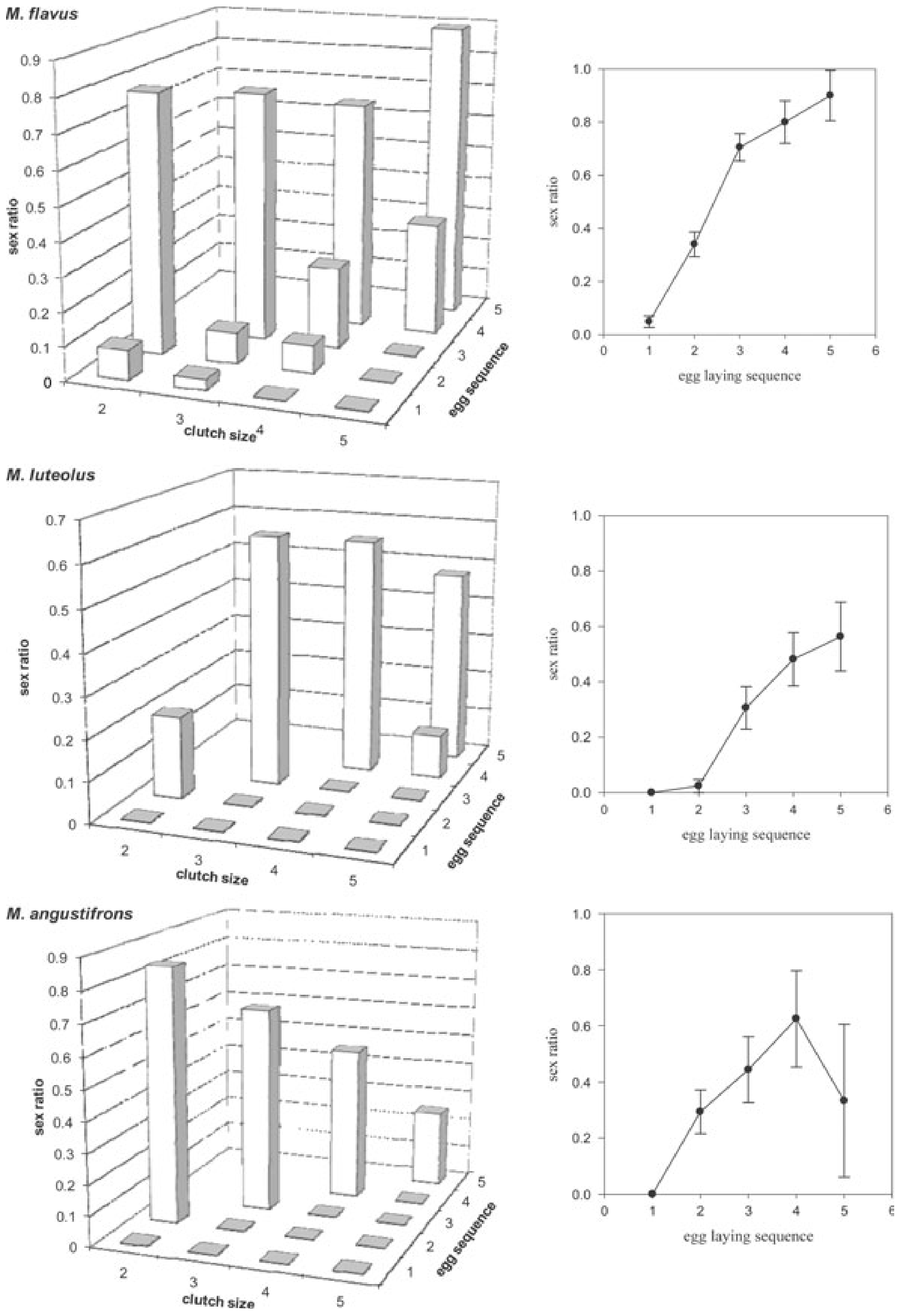

Fig. 2. Sex ratios (proportion males; left-hand graphs) and cumulative sex ratios (right-hand graphs; ±SE) produced by *Metaphycus flavus*, *M. luteolus*, and *M. angustifrons* females as a function of the sequence of oviposition in brown soft scales. The graphs show sequences of sex allocation for clutches up to five eggs only.

leading to higher VdR values (one-way ANOVA on VdR: $F_{2,243} = 7.74$, $P < 0.001$) (Fig. 6). There were also significant differences in CGd values among species (one-way ANOVA on CGd: $F_{2,243} = 8.24$, $P < 0.001$). *Metaphycus angustifrons* had a high CGd value, because mortality rates of eggs were progressively increasing as more eggs were allocated, whereas *M. flavus* had a high CGd value, because mortality rates of eggs significantly decreased at the end of the sequence. *Metaphycus luteolus* has the lowest CGd value, because mortality rates of eggs are relatively constant within the allocation sequence

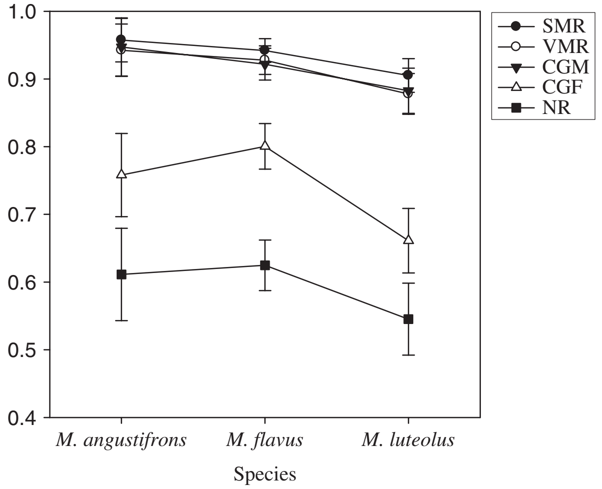

Fig. 3. Average (±SE) values of the descriptive parameters used to describe sequences of sex allocation of three *Metaphycus* species parasitising brown soft scale. SMR, sum of males' rank position; VMR, variance of the males' rank position; CGM, centre group of males; CGF, centre group of females; NR, number of runs of males and females.

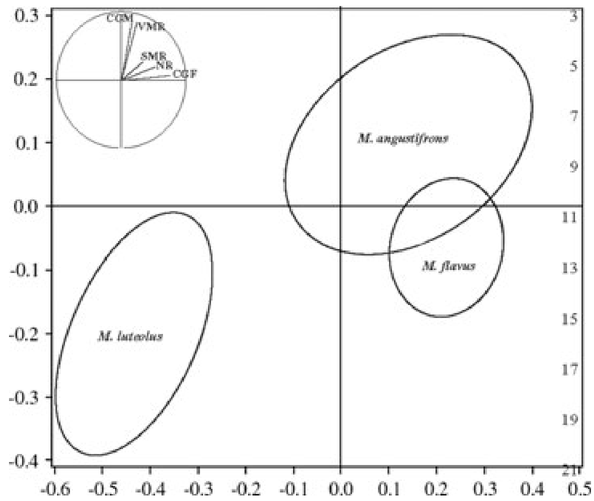

Fig. 4. First and second axis of a factorial discriminant analysis done on the statistical description of three *Metaphycus* species oviposition sequences on brown soft scales with five statistical descriptive parameters. Ellipses represent 95% confidence intervals around the means. The correlation circle of the corresponding plan is provided. CGM, centre group of males; VMR, variance of the males' rank position; SMR, sum of males' rank position; NR, number of runs of males and females; CGF, centre group of females.

(Fig. 6). There were no differences is the CGa values among species (one-way ANOVA on CGa: $F_{2,243} = 2.17$, $P = 0.12$), whereas NR values were only slightly different among species (one-way ANOVA on NR: $F_{2,243} = 3.22$, $P < 0.05$). Overall, patterns of mortality within sequences of egg allocation were different among species (Fig. 7).

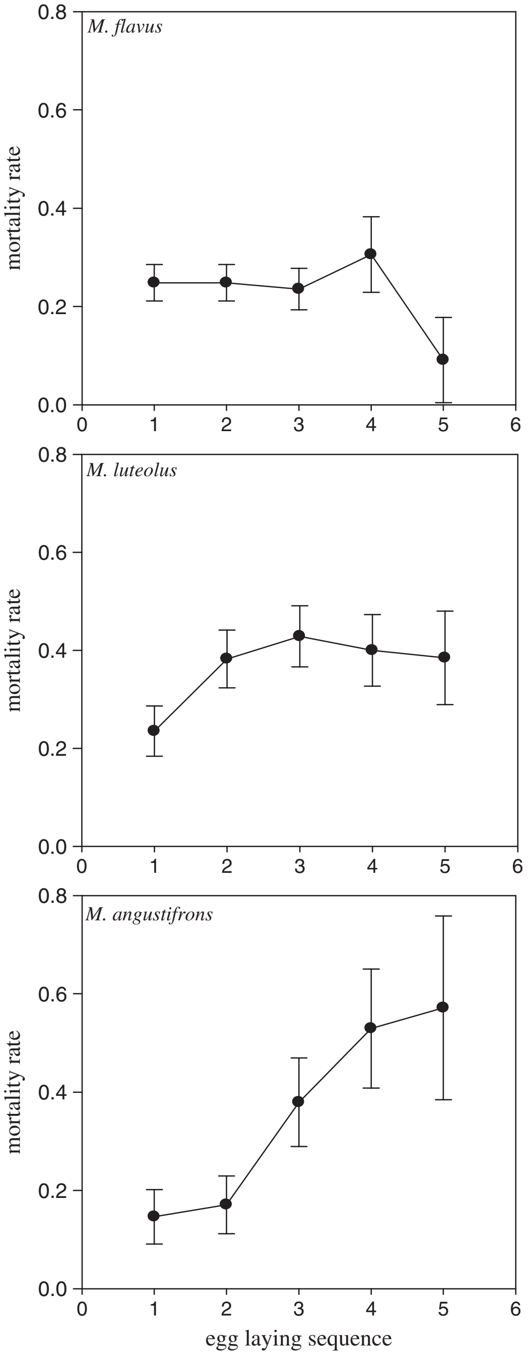

Fig. 5. Cumulative mortality rates (±SE) of eggs allocated by *Metaphycus flavus*, *M. luteolus*, and *M. angustifrons* females as a function of the sequence of oviposition in brown soft scales. The graphs show mortality rates for clutches up to five eggs only.

Effect of mortality on the incidence of all female broods

Generally, the incidence of all-female broods calculated from a Monte Carlo simulation model and using the product between sequences of sex allocation and patterns of mortality, was relatively high in all species. Developmental mortality causes

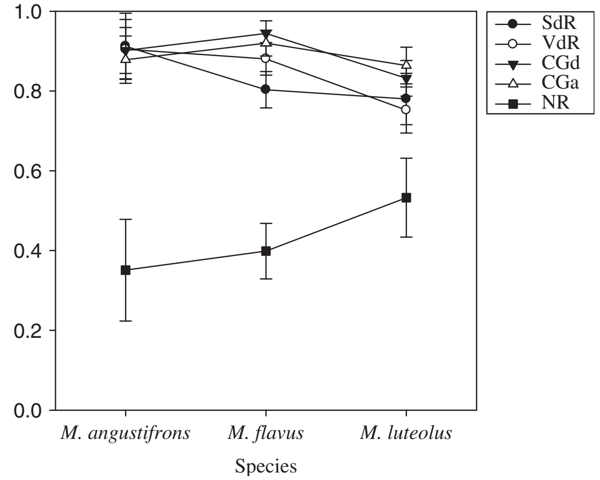

Fig. 6. Average (±SE) values of the descriptive parameters used to describe sequence-specific mortality of three *Metaphycus* species parasitising brown soft scale. SdR, sum of dead offspring rank position; VdR, variance of dead offspring rank position; CGd, centre group of dead offspring; CGa, centre group of alive offspring; NR, number of runs of dead and alive offspring.

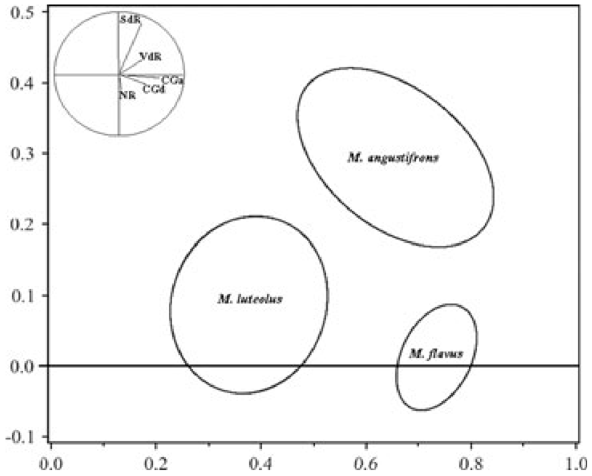

Fig. 7. First and second axis of a factorial discriminant analysis done on the statistical description of three *Metaphycus* species patterns of mortality on brown soft scales with five statistical descriptive parameters. Ellipses represent 95% confidence intervals around the means. The correlation circle of the corresponding plan is provided. SdR, sum of dead offspring rank position; VdR, variance of dead offspring rank position; CGa, centre group of alive offspring; CGd, centre group of dead offspring; NR, number of runs of dead and alive offspring.

37.03 ± 0.05, 55.33 ± 0.06, and $51.94 \pm 0.06\%$ of the broods of *M. flavus*, *M. luteolus*, and *M. angustifrons* respectively to be all-female. There was a strong difference of the incidence of all-female broods among species (ANOVA: $F_{2,87} = 875.5$, $P < 0.0001$).

Discussion

Our experiments show that *Metaphycus* wasps laid precise sex ratios while parasitising gregariously brown soft scale in the laboratory. *Metaphycus luteolus* and *M. angustifrons* have been reported to allocate female-biased, precise sex ratios in brown soft scale in the field (Kapranas *et al*., 2008). Generally, female-biased, precise sex ratios allocated by these wasps are attributed to extreme local mate competition where mating opportunities for females are limited within the natal patch (Green *et al*., 1982; Hardy, 1992, 1994). Our study also shows that production of such precise sex ratios is achieved by ordering of sex within an oviposition bout: fertilised (female) eggs are laid first in sequences, while one or few unfertilised (male) eggs are laid at the end of the sequences. This 'male-last' strategy can be explained by the type of hosts these wasps exploit. In gregarious parasitoids, such as *Metaphycus* species, females generally assess host quality/resources first and regulate their brood sizes accordingly. This pattern of sex allocation commonly occurs in gregarious parasitoids such as Pteromalids, Eulophids, Bethylids, and Encyrtids (Putters & van den Assem, 1985; Dijkstra, 1986; Rojas-Rousse *et al*., 1988; Strand, 1989; Hardy, 1992; Ode *et al*., 1996). On the other hand, male-first strategy or laying of males in intervals within an oviposition bout might be more adaptive in quasi-gregarious parasitoids, such as some egg parasitoids that do not have an exact estimation of the number of host eggs per egg mass in the beginning of the oviposition bout (Waage, 1982; Waage & Ng, 1984; Wajnberg, 1993, 1994; Colazza & Wajnberg, 1998; Bayram *et al*., 2004).

Our results also suggest that there are some differences in the sequence structure among species. Particularly, *M. luteolus* appears to allocate sex in clutches of eggs differently from the other species overall. These differences in the structure of sequences among species could be explained from a host utilisation perspective; different wasp species assess host resources in brown soft scales with a different degree of accuracy, because they utilise scales of different stages/sizes, as has been documented in field studies (Bernal *et al*., 2001; Kapranas *et al*., 2007). Although these species readily accept scales of different sizes for oviposition, as has been observed in our laboratory experiments, some species may indeed be better in assessing resources in larger hosts more efficiently than others. Moreover, some of these species might not be well adapted in parasitising brown soft scale, a scale species of probably African origin (Kozár & Ben-Dov, 1997). Alternatively, differences among species in terms of sex allocation pattern might be due to different degrees of non-local mating that these species experience. Although there is an advantage of precise sex ratio control in cases of intermediate non-local mating (Nagelkerke, 1996), slight permutations along the average pattern of sex allocation sequence could be allowed, assuming that there is no overall effect on optimum sex ratios. For *M. luteolus* and *M. angustifrons*, there is some field-based evidence that non-local mating is apparent (Kapranas *et al*., 2008).

Although these wasps order the sex of their eggs in particular sequences to achieve precise sex ratios with low variance (i.e. allocate the minimum number of males to mate their sisters), a great portion of the broods are all-female, resulting in the production of many virgin females. This is also commonly observed in brood sex ratios of *M. luteolus*, *M. angustifrons*, and other encyrtid parasitoids of brown soft scale in the field (Kapranas *et al*., 2008). Our laboratory experiments prompt the hypothesis that production of all-female broods is caused by developmental mortality and not solely because of a female's decision to allocate only female offspring in hosts. However, the use of sex allocation data from the complete broods (sequences) to estimate sex allocation in broods with developmental mortality, is likely not accurate enough. This is because if differential mortality exists between males and females, the sample of broods that escape mortality will be biased in favour of the sex with the greater survivorship (Fiala, 1980). This is the reason why we used stochastic, Monte Carlo simulations and the result obtained provides direct evidence that the combined effect of the observed sequences of sex allocation and patterns of mortality within oviposition sequences results in the production of all-female broods. Although our approach predicts a greater prevalence of all-female broods than what is actually observed in our experiments, simulation results are in qualitative agreement with our observations. In the case of *M. flavus*, the species wherein the prevalence of all-female broods is the lowest compared with the other two species, mortality rates of eggs are lower at the end of the oviposition bout, i.e. where males are typically allocated. Hence, overall all broods have at least one male at emergence.

Generally, developmental mortality can have implications for optimal sex allocation and as Nagelkerke and Hardy (1994) suggested it is the male mortality that affects sex allocation decisions by ovipositing females. In cases of high mortality, allocation of extra, insurance males should be favoured. In our study some species tend to allocate more males as the oviposition bout progresses, i.e. as clutch size increases (*M. flavus*), while others do that to a lesser extent or not at all (*M. luteolus* and *M. angustifrons*). Thus, slight differences in the sequences regarding positioning of male offspring can also represent an adaptive reproductive decision of parasitoid wasps to accommodate for male mortality. In our laboratory experiments, it appears that secondary sex ratios are overall precise, despite the prevalence of all-female broods caused by developmental mortality. However, the predictions of Heimpel (1994) that the prevalence of all-female broods is negatively correlated with clutch size are not met for *M. flavus* and *M. angustifrons*. In *M. luteolus*, there is a significant negative relationship between clutch size and prevalence of all-female broods as has been demonstrated in other host–parasitoid associations (Hardy & Cook, 1995; Hardy *et al*., 1998). This relationship between clutch size and brood sex ratios is mechanistically explained by differences in sex allocation sequence and sequence-specific patterns of mortality of this species. Furthermore, our study contrasts with field studies of *M. luteolus* parasitising brown soft scale in California's citrus groves, wherein no significant relationship was observed between clutch size and production of all-female broods (Kapranas, 2006). Perhaps abiotic factors such as temperature that influence developmental mortality rates are a confounding factor in testing this hypothesis. In the case of the encyrtid parasitoids, egg encapsulation rates by their hosts are higher in higher temperatures (Blumberg, 1997 and references cited therein). In our study, incubation of parasitised scales in different temperature regimes may represent some environmental stochasticity. Furthermore, egg encapsulation rates are known to be strongly influenced by the host plant (Blumberg, 1997 and references cited therein), and based on the fact that brown soft scale is a polyphagous insect. It is likely that parasitoid brood sex ratio composition might differ on different host plants. Future studies should seek to compare sex ratio variance, sequences of sex allocation, and sequence-specific mortality of individual parasitoid species attacking different hosts and/or feeding on different plants. Our study highlights that the influence of mortality on optimal sex allocation may be hindered by stochasticity imposed by exogenous factors. Thus, results from laboratory studies may not clearly represent the impact of mortality on optimal sex allocation in field populations of parasitoids.

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