Effect of *Calliphora erythrocephala* Meigen Puparial Size on the Sex Ratio of *Melittobia acasta* Walker (Hymenoptera: Eulophidae)

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Abstract

The most important biological control agents which are pro-ovigenic and synovigenic parasitoids usually assess their hosts critically before laying eggs in them. *Melittobia acasta* Walker, a synovigenic parasitoid of several dipteran species is potentially an agent for the biological control of such nuisance dipteran fly pests as *Calliphora* and *Musca* species. A cardinal requirement before a parasitoid could be deployed for the control of any pest is a thorough understanding of the parasitoid – host interactions. In line with this requirement the aim of this work was to determine the effect of *Calliphora erythrocephala* Meigen puparial size on the sex ratio of *M. acasta*. *M. acasta* of uniform size (head width = 0.36 – 0.37 mm) were given five puparia of similar age (48 – 72h) from three experimental host size grades for a period of 24h. After oviposition, the parasitized puparia were reared at 25°C for fourteen days and then dissected to count the number of male and female parasitoid pupae. The results show that *M. acasta* laid a similar proportion of male to female eggs on large as well as on small hosts. This pattern of sex allocation and its significance in the reproductive biology of the parasitoid is discussed.

Keywords: *Melittobia acasta*, *Calliphora erythrocephala*, Puparial size, Sex ratio

Introduction

A majority of the Eulophidae have been employed in classical biological control (Greathead 1986). The identifying characteristics of the family (Eulophidae) have been recorded by Richards and Davies (1977). *Melittobia acasta* belongs to this family. Its male and female morphology has been described by Waterston (1917) and Browne (1922) with additional contributions from Dahms (1984a) and Imandeh (1998).

Within genetic limits, the adult size of *Melittobia acasta* and indeed other parasitoids is largely determined by the amount and quality of food consumed by the larva. Decisions made by the ovipositing female regarding the choice of a host, clutch size, superparasitism and sex-ratio [proportion of unfertilized male eggs to fertilized female eggs] thus have major effects on the resulting progeny size (Godfray 1994). King (1987) reviewed evidence to show that female fitness increases with adult size. In both pro-ovigenic species (Iwata 1966) and synovigenic species (Rosenheim and Rosen 1991), more eggs and often more ovarioles as well as maximum egg number are positively correlated with body size, larger ones laying more eggs. To achieve the maximal body size for progeny therefore, the ovipositing females assess their hosts before accepting them, and will adjust the clutch size and / or sex-ratio to maximize fitness. Since arrhenotoky is generally the rule rather than the exception (Askew 1971), the decision to fertilise or not to fertilise an egg before laying it is made on the basis of the stimuli related to host quality among other factors (King 1993). Size of a host is of particular importance because it determines the eventual size reached by the son or daughter as an adult, which in turn affects its reproductive prospects. Clausen (1939), van den Assem (1971), King (1988), Sandlan (1979), Charnov et al. (1981), Rivers and Denlinger (1994) and others have provided evidence to show that a parasitoid assesses its host's size and acts on the information to decide what clutch size to lay. Assem (1971) and Charnov et al. (1981) have shown that in *Lariophagus distinguendus* Foerster, a small (1-3 mm) parasitic wasp (family Pteromalidae) which attacks larvae of the common granary weevil *Sitophilus granarius* L., the parasitoid lays more daughters in big hosts and sons in smaller hosts. A similar fertilised versus unfertilised egg pattern has been described in *Spalangia cameroni* Perkins by King (1994).

Charnov et al. (1981) suggested that host size may have greater effect on females than on male's reproductive success if host size is positively correlated to size and if size increases the reproductive potential of females more than the reproductive potential of males. This was based on the assumption that a small male may still be able to inseminate as many females as a large male, but a small female may not be able to produce as many offspring as a larger female. Alternatively, host size may have a differential effect on reproductive success of females versus males through other factors such as an effect on the development time. Faster development of males from smaller hosts will mean shorter generational times and a net gain in inseminations of females.

The bulk of experiments done on sex ratio manipulation in response to host size has been on solitary parasitoids (King 1989). In this group, only one offspring develops per host, thus the quantity of resources available to a developing wasp is closely correlated to the size of its host. In *M. acasta* and other gregarious parasitoids, the expected relationship between host size and resources available to a developing offspring is complicated by the ability of ovipositing females to adjust their clutch size. Since females often increase clutch in larger hosts (King 1987), the quantity of resources available to a developing individual will not necessarily be related to the host size. If resource per offspring is not related to host size, sex ratio manipulation in response to host size will not be directly correlated.

M. acasta is a potential biocontrol agent for dipteran fly pests and understanding its interactions with its hosts is a first step towards designing a biocontrol strategy for the control of these fly pests. In this work, we test the effect of host size on the sex ratio of the parasitoid.

Materials and Methods

Stocks of the host, *C. erythrocephala*, were obtained from commercial sources as larvae and reared in honey jars on a diet of commercial yeast and milk soaked in cotton wool. The adults were kept at 25°C. Before use, host puparia were viewed with transmitted light and those in which a distinct outline of the head, thorax and abdomen of the pupa within the puparium was seen and thus likely to be alive were selected for the experiments.

Stocks of immature stages of *M. acasta* were obtained in *C. erythrocephala* puparia from Wye College, University of London. They were then kept at 25°C, 65% relative humidity (RH) and a photoperiod of 12D:12L regime until adult eclosion. The resulting progeny were propagated in *C. erythrocephala* puparia for all the experiments. *C. erythrocephala* puparia were exposed to *M. acasta* daily for twenty-four hour duration in specimen tubes at ratios of one parasitoid to two hosts. This ensured that an adequate supply of suitably aged *M. acasta* adults were available when required on a daily basis after the first eighteen days.

All observations and measurements were made using a stereoscopic microscope with an eye piece graticle. Light sources included the low voltage illuminating base of the microscope and a Volpi 150H fibre optics with cold light illumination [Micro instruments (Oxford) Ltd].

A pilot study was conducted to select three puparial sizes with which to test the effects of host size on aspects of maternal behaviour (i.e. egg laying behaviour and sex ratio). One hundred, randomly selected *C. erythrocephala* puparia from the stock, were measured and grouped into seven classes with the smallest falling in the bottom class range of 5.0 - 5.9 mm and the largest falling in the equal to or greater than 11mm class.

The result of the pilot study showed that there were significant difference in the number of eggs laid within the classes (Table 1, $P < 0.05$). A further investigation using Duncan's multiple range test revealed the differences to be within classes 1and 6, 1 and 7, 2 and 6, 2 and 7, 3 and 6, and 3 and 7. On the basis of these results and the percentage distribution of the puparia in the classes within the stock, three classes were chosen, namely; 6 - 6.9 mm, 8 - 8.9 mm, and 10 - 10.9 mm, because for idiobionts in general, host size is a critical factor in their development in that the host is 'mummified'

Table 1 Pilot study to determine the number of eggs laid by *M. acasta* on *C. erythrocephala* of various puparial sizes but uniform age per 24 hour exposure.

Class	Pupal length (mm)	Percentage distribution in stock	Eggs laid / 24 h. Replicates	Mean number laid	Percentage of total
1	5.0-5.9	9	4, 7, 11, 10, 14	9.2a	8.47
2	6.0-6.9	19	6, 11, 7, 9, 11	8.8ad	8.10
3	7.0-7.9	6	9, 3, 12, 7, 16	9.4ae	8.66
4	8.0-8.9	21	12, 19, 20,14, 19	16.8af	15.47
5	9.0-9.9	11	14, 9, 19, 26, 21	17.8ag	16.39
6	10-10.9	29	17, 21, 25, 30, 26	23.8bfg	21.19
7	≥ 11.0	5	15, 27, 26, 24, 22	22.8cfg	20.99

Values with the same letter do not differ significantly at P> 0.05 (one way Anova and Duncan's multiple range test).

with venom when parasitized and no further development takes place.

This index (host size) has been used by several other workers as a measure of host quality (Godfray, 1994).

From results obtained in the pilot study, (Table 1), three *C. erythrocephala* puparium sizes (A,B and C) with dimensions as shown in Table 2 were used in this study.

Table 2: Dimensions of *C. erythrocephala* puparia employed in the study

Puparial (host) size grade	Length ± SE (mm)	Width ± SE (mm)
A	10.1 ± 0.2	3.6 ± 0.2
B	8.1 ± 0.2	3.1 ± 0.2
C	6.6 ± 0.2	2.4 ± 0.2

All were puparia aged between 48 and 72 hours. *M. acasta* females of uniform size (head width: 0.36 - 0.37 mm) which had emerged less than twenty-four hours previously from *Calliphora erythrocephala* puparia of uniform size (grade A above) were transferred to clean specimen tubes (∅ 10 mm) and fed for twelve hours on 0-18h old *Calliphora erythrocephala* puparia, then given 1:1 honey-water solution (smeared on the inside of the tubes) for another twelve hours. The females were then individually given five puparia of a similar age from one of the experimental host sizes for a period of twenty-four hours for oviposition. Because the sex of *Melittobia* sp. can only be determined from the pupal stage onwards (Browne 1922), the parasitized puparia treated were reared at 25°C for fourteen days and then dissected to count the number of male and female parasitoid pupae.

Table 3: Mean sex ratio of *M. acasta* from *Calliphora erythrocephala* hosts of different sizes parasitized by one ovipositing female for a period of 24 hours (n = 12). Egg mortality in all host sizes less than 6.25%

Host Size (mm)	Mean Sex ratio (♂ / ♀) $\bar{x}$ ± SE*
A Length=10.085 ± 0.158 Width = 3.624 ± 0.158	0.0375 ± 0.0048[a]
B Length= 8.115 ± 0.158 Width = 3.073 ± 0.158	0.0400± 0.0041[a]
C Length= 6.618 ± 0.158 Width = 2.442 ± 0.158	0.1175± 0.0206[b]

**Values in a given column with different letters are significantly different (P<0.05).*

The control puparial batches were counted at the egg stage and the results compared with the total parasitoid pupae obtained from test experiments to decide whether mortality affected the numbers that reached the pupal stages.

Results

Table 3 shows the sex ratio of *M. acasta* from *Calliphora sp.* hosts of different sizes but of the same age parasitized for twenty four hours by one ovipositing female. The results show that *M. acasta* laid a similar proportion of male to female eggs on large (group A) as well as small hosts. In all the host sizes, mortality was less than 6.25% but it was not possible to determine whether the few eggs that did not mature were male or female.

Discussion

In most wasp species in which host size influences their ovipositional decisions, the sex ratio is biased in favour of females in large hosts as has been recorded in *Lariophagus distinguendus* Foerster (Charnov et al., 1981), *Anisopteromalus calandrae* Howard (Assem et al.,1984), *Heterospirus prosipoides* Viereck (Jones 1982) and *Asobara tabida* Nees (van Alphen and Nell 1982). Explanations for this bias have always been based on host quality models (Charnov et. al. 1981). Werren (1984) presented a model that explored the combined effects of host quality (host size) and Local mate competition (LMC) on the sex ratio of parasitic wasps and still concluded that for species in which females benefit more (in fitness) than do males from large (good) hosts, when a single host is parasitized, the Hamiltonian sex ratio (i.e. bias in favour of females) should be produced. When *M. acasta* were individually exposed to *Calliphora sp.* hosts of different sizes for 24 h, the sex ratios though variable (Table 3) were still biased in favour of the females. Observations of female bias sex ratios in this parasitoid show that this does not occur entirely as a result of LMC and host quality effects but because of a fixed mechanism with which *M. acasta* determines what eggs to lay. Within one oviposition, the sex ratios were variable (0.04 - 0.12, Table 3) but lifetime offspring sex ratios have been reported to be constant at 0.04 (Imandeh, 1998). Browne (1922) and Dahms (1984b) reported similar ratios. A fascinating deviation from the norm is shown in Table 4. When subjected to local mate competition between two, three, four or five mothers per host, the sex ratio still did not deviate from the lifetime offspring sex ratio of a single ovipositing mother on a similar host (Imandeh, 1998). The reason for this is male antagonism. *M. acasta* males are known to be extremely competitive and pugnacious, fighting each other until a single (fittest) male remains (Browne 1922, Graham-Smith 1919). In such situations, mothers would be expected to produce just enough sons and avoid waste.

Table 4: Mean head width, sex ratio and number of *Melittobia acasta* (parasitoid) produced by various combinations of hosts (*C. erythrocephala*) to ovipositing parasitoid females for their lifetime [Extracted from Imandeh, N.G. (1998)]

Host : Parasitoid	Number Produced (n)	Head width ± SE (mm).	Sex ratio
1:1	129	0.322 ± 0.005	0.0320
1:2	124	0.325 ± 0.007	0.0323
1:3	115	0.322 ± 0.004	0.0348
1:4	118	0.330 ± 0.009	0.0440
1:5	139	0.321 ± 0.008	0.0530
5:1	311	0.322 ± 0.005	0.0130
5:2	321	0.318 ± 0.010	0.0355
5:3	326	0.326 ± 0.007	0.0415
5:4	320	0.337 ± 0.005	0.0456
5:5	506	0.330 ± 0.014	0.0433

This behaviour satisfies the optimality principle in evolutionary stable strategy. Hardy (1994) agreed with this idea, that not all parasitoids conform to Charnov's theory of sex allocation and Fisher's theory of frequency dependent selection. It will still be interesting to see if this pattern is retained when more than five ovipositing females utilise a single host.

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