Sex allocation strategies in response to conspecifics’ offspring sex ratio in solitary parasitoids

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Parasitoid females adjust their offspring sex ratio in order to maximize their fitness. The optimal sex ratio they produce varies with several factors but especially with competition level. In solitary species, only one adult can emerge from a given host, whatever the number of eggs laid. In some species, the mortality of supernumerary individuals could be due to larval combats. This ability to fight could vary from one sex to another within species. In this way, when females explore an already parasitized host patch, the sex ratio of previous eggs can influence their fitness. These 2 factors could thus strongly influence females’ sex allocation strategies. However, this prediction assumes that parasitoid females can assess the sex of eggs previously laid by conspecifics. We used host acceptance and sex ratio behavior to test this capacity, and our experimental data provide the first evidence for this capacity in a parasitoid species. Females of the solitary ectoparasitoid Anisopteromalus calandrae discriminated the sex of eggs already laid by a conspecific but only when these eggs had reached a certain developmental stage. They adapted their offspring sex ratio as predicted by Hamilton’s “sex ratio games” model, allocating the sex of their eggs differentially according to the sex of eggs already on the hosts on which they oviposited. In this way they prevented a lethal larval fight between their sons and the females they could potentially mate after their own emergence, increasing their own fitness and their sons’ reproductive success. Key words: discrimination capacities, intraspecific competition, oviposition behavior, sex ratio games, superparasitism. [Behav Ecol 21:107–112 (2010)]

Sex ratio and sex allocation strategies have a direct impact on the fitness of individuals. Whereas a number of organisms produce equal numbers of males and females (Fisher 1958), the females of a wide range of species have been shown to adjust their offspring sex ratio, for example, birds (Komdeur et al. 1997), protozoan parasites (West et al. 2001), and parasitoid wasps (Godfray 1994). In parasitoid Hymenoptera, females adjust their offspring sex ratio through arrhenotokous parthenogenesis, whereby fertilized (diploid) eggs develop into females and unfertilized (haploid) eggs into males. Females adapt their sex allocation strategies in response to several factors such as host quality (Charnov et al. 1981), environmental constraints (King 1987), overlapping generations (Charnov 1982), and competition level, which is one of the most important factors (Hamilton 1967; Werren 1980; West et al. 2000; King 2002). In his local mate competition (LMC) model, Hamilton (1967) showed that, in gregarious species, isolated females produce a female-biased sex ratio, producing the minimum number of sons able to inseminate their daughters. If the number of egg-laying females increases, they adapt their sex allocation strategies, producing more sons in order to inseminate the daughters of other females. This theory applies to a population of females, and the cues they perceive to evaluate the level of competition could be either direct (the presence of n females) or indirect (e.g., the presence of eggs previously laid in a patch) (Werren 1980; Shuker and West 2004; Darrouzet et al. 2008). As the presence of eggs on hosts has been shown to be the primary mechanistic cue of sex ratio adaptation in a competition context (Shuker and West 2004), the features of a previous clutch should thus influence the sex allocation strategy of a second female. One important parameter is the sex ratio of the first clutch; females should adjust the proportion of sons they produce according to the proportion of female eggs laid by other females. This is what is predicted by Hamilton (1967) in his “sex ratio games” model. This model was further adapted by Werren (1980) who incorporated the relative clutch size. The main predictions of these superparasitism models are that, for a given clutch size, females will lay a higher proportion of male eggs when a majority of females have already been laid on the host. The proportion of male eggs should then decrease as the number of female eggs on hosts decreases. More recently, these superparasitism models have been extended, incorporating the characteristics of specific species. In Melittobia spp., for example, the model has been extended to cover lethal combat among adult males to explain extremely female-biased sex ratios (Abe et al. 2003). Likewise, Shuker et al. (2005) included asymmetrical competition for mates between males because of asynchronous emergences that occur in the wasp Nasonia vitripennis. However, the discrimination capacity of the gender of eggs previously laid has never been demonstrated in any parasitoid species.

These models were primarily developed for gregarious species in which several individuals could emerge from one host. Whereas it appears that it is less relevant for solitary species that generally lay single eggs, it could be extended to those that reproduce on an aggregate of hosts. In fact, an aggregate of hosts could be considered as a single host on which several parasitoids develop at its expense (even if, in fact, only one individual emerges from a given host of this aggregate). The fact that only
one adult can emerge from a host, whatever the number of eggs initially laid, is another important constraint in solitary species. This mortality is due either to lethal larval combats or physiological suppression (Godfray 1994). In species where larval fights occur, the first instars actively search for eggs and larvae on the hosts and try to eliminate these competitors by attacking them with their mandibles (van Albreck et al. 1993). However, this ability to fight varies from one sex to another within species (van Baaren et al. 1999). Differences in competitive abilities could be explained by morphological differences between male and female larvae, such as the number of bristles involved in mobility, an important feature in larval fights (van Baaren et al. 1997, 1999). This asymmetry could strongly affect the oviposition strategy of a female performing superparasitism (laying an egg on an already parasitized host). We could thus expect that ovipositing females would avoid laying eggs on hosts already parasitized by an egg belonging to the better sex competitor and/or have a tendency to lay their better sex competitor on already parasitized hosts.

In this study, we investigated the ability of females of the solitary ectoparasitoid Anisopteromalus calandrae Howard (Hymenoptera, Chalcidoidea, Pteromalidae) to distinguish the sex of eggs previously laid by a conspecific. Assessing the exact nature of previously laid eggs is especially important in A. calandrae because females, depending on their physiological state, tend to select already parasitized hosts (Lebreton, Darrouzet, et al. 2009). We investigated: 1) the larval combat ability of males and females in this species and 2) whether ovipositing females could assess the sex of eggs previously laid and how they adjust their sex allocation strategies based on both the “sex ratio games” model and lethal larval fights between sexes.

**MATERIALS AND METHODS**

**Rearing conditions**

Anisopteromalus calandrae Howard (Hymenoptera, Chalcidoidea, Pteromalidae) is a solitary ectoparasitoid of a wide range of coleopteran pest larvae and pupae (Ahmed 1996), used throughout the world for biological control of pests in seed stocks. Two homozygous strains of A. calandrae were isolated: one red-eyed mutant (noted as R) and one black-eyed wild type (noted as B) (Do Thi Khanh et al. 2005). The use of these 2 strains enabled us to monitor the offspring of a given female. Parasitoids were mass-reared on larvae of the bruchid Callosobruchus maculatus in a climatic chamber: 12 h light at 28 °C, 12 h dark at 22 °C, and 65% relative humidity.

**Sex competition in larval combat**

To obtain eggs, A. calandrae females were allowed to lay on an artificial seed system composed of gelatine capsules (Gauthier and Monge 1999; Darrouzet et al. 2003). The bruchid larvae were placed inside the artificial seeds after removal from seeds by dissection and selection by size (9.12 ± 0.20 mg). The gelatine capsules mimic the bruchid pupal chamber in the seed and are accepted for oviposition by females.

To analyze whether females or males were better competitors in larval combat, virgin R and mated B females were allowed to lay eggs on C. maculatus larvae for 2 h. In a previous study, no differences were found in the competitive abilities of the 2 strains (Lebreton, Labarussia, et al. 2009), and therefore, only one combination was performed in this study. Mated females were obtained by placing a 2-h-old virgin female with a 24-h-old virgin male of the same strain in a Petri dish. Mating was confirmed by direct observation. Random eggs of the B strain (males or females) were deposited individually with a random egg of the R strain (male) on the same host (n = 284) and placed in a separate cell in a Plexiglas sheet closed by a Plexiglas cover slide until emergence of the parasitoid adults (Darrouzet et al. 2003, 2007). At emergence, the phenotype and sex of the surviving individual was noted.

The survival rate of an egg laid on an already parasitized host decreases sharply as the time between the 2 ovipositions increases (Lebreton, Labarussia, et al. 2009). To investigate how the fighting abilities of male or female larvae vary with the time interval, random eggs of the B strain (males or females) were deposited individually with a random egg of the R strain (male) laid with a 28-h time interval (the first egg about to hatch) on the same host as described above (R egg laid first: n = 217; B egg laid first: n = 217). At the same time, we determined precisely the proportion of each strain winning a male/male combat by placing 2 eggs laid by a virgin female (one of each strain) on the same host for the 2 intervals described above (eggs laid at the same time: n = 79; eggs laid with a 28-h interval: n = 86).

When eggs laid by B mated and R virgin females are placed together, 2 categories of larval competition occur: one between a B female and an R male, the second between 2 male larvae (one R and one B). Individuals emerging after larval fights are thus B females, B males, or R males (Table 1). As we were unable to determine precisely the proportion of R males emerging from each category of fight, we had to make an estimate, as shown in Table 1. From the number of females and the estimated number of males emerging from a female/male combat, it was then possible to determine which sex was the better competitor in larval competitions.

**Table 1**

<table>
<thead>
<tr>
<th>Emerging strain</th>
<th>B female/R male</th>
<th>B male/R male</th>
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<td>B</td>
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<td>R</td>
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<td>r&lt;sub&gt;2&lt;/sub&gt; B&lt;sub&gt;male&lt;/sub&gt;</td>
<td>R&lt;sub&gt;tot&lt;/sub&gt;</td>
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In bold, the observed number of emerging individuals. P<sub>B</sub> represents the proportion of B male winning a male/male fight with an R male, obtained with competition experiments between offspring of virgin females.

**Egg sex discrimination**

Isolated mated R females were exposed to 2 hosts in a choice test experiment, using the same artificial seed system as previously described. One host had previously been parasitized by a male egg (laid by a virgin B female) and the other by a male or female egg (laid by a mated B female). To examine whether females’ oviposition strategies evolved with the embryonic development of the egg already on the host, 2 series of experiments were performed, one with 2-h-old eggs (obtained just after oviposition) and the other with 28-h-old eggs (just before hatching). The choice tests took place in a climatic chamber (12:12 h light:dark, 30:22 °C, and 71% relative humidity) and ended as soon as the females laid an egg, with a maximum time of 2 h. The oviposition sequence was observed: the first capsule visited, the capsule selected (corresponding to the capsule on which the female settled after examining the patch), and the capsule with oviposition (at the end of the choice test, eggs have not been necessary laid in all the selected capsules). After oviposition, the female was removed.
and each egg was transferred onto a different host and deposited in a separate cell in a Plexiglas sheet in order to avoid larval fights. After emergence, the phenotype (R or B) and sex of individuals were noted, enabling us to differentiate between eggs offered to females during the choice tests and those laid by tested females. Female/male (2 h, \( n = 23; 28 h, n = 43 \)) and male/male (\( n = 32 \)) choice tests were then analyzed separately. For each category of test, the hosts selected for oviposition were noted, and the sex ratio of eggs laid by tested females (proportion of males) was calculated. Because other recent models incorporate characteristics of other parasitoid species or parameters that are not applicable to \( A. \) \( calandrae \), we compared these sex ratios with those predicted by Werren’s model \((X)\), which included asymmetry in clutch size:

\[
X = \sqrt{2X_0(T + 1) - 2X_0} \div 2T
\]

where \( X_0 \) is the sex ratio of the first clutch and \( T \) the ratio of the eggs laid by the first and second females (Werren 1980).

**Statistical analyses**

To analyze which sex is the better competitor in larval fights, we used a \( \chi^2 \)-test to compare the proportion of females and males emerging from each category of fight to a theoretical proportion of 50:50. To compare the offspring sex ratios laid by tested females on each category of host (previously parasitized by a male or female egg), a \( \chi^2 \)-test was also performed. Next, to compare these sex ratios with those predicted by Werren’s model, we performed a Fisher’s Exact test. Analyses were carried out with R software (R 2.1.1, R Development Core Team, Free Software Foundation Boston, MA) and a threshold level of \( P < 0.05 \) was set.

**RESULTS**

**Sex competition in larval combat**

After larval combats between males laid at the same time, each individual had an equal probability of winning the competition, as 53% of \( B \) males and 47% of \( R \) males emerged (\( \chi^2 = 0.26, \) degrees of freedom [df] = 1, \( P = 0.61; \) Figure 1). Thus, the proportion of \( R \) males surviving \( R \) male/\( B \) male combats with eggs from \( B \)-mated females should also reflect about 47% of these combats. We can thus estimate, as described in the Materials and Methods section (Table 1), that the number of emerging males represents only about 23.5% of male/\( L \) female combats (Figure 1). These results demonstrate that female larvae have supremacy over male larvae in larval fights (\( \chi^2 = 35.5, \) df = 1, \( P < 0.001 \)) and have a higher probability of winning than a male laid under the same conditions (0.74 vs. 0.53; \( \chi^2 = 15.3, \) df = 1, \( P < 0.001; \) Figure 1). Under our experimental conditions, in 8.8% of these male/\( L \) female combats, no individuals reached adulthood.

When eggs were laid with a 28-h interval, the probability of the second male winning the competition against a first male was only 25.75% (Figure 1). The estimated percentage of females laid 28 h after a male egg that emerged from these combats is thus 53.12%. Under these conditions, females lose their supremacy against a male laid 28 h before (\( \chi^2 = 0.0017, \) df = 1, \( P = 0.979 \)) but have a higher survival rate than a male egg laid under the same conditions (0.25; \( \chi^2 = 7.48, \) df = 1, \( P = 0.006; \) Figure 1). When the female is laid first, the estimated survival probability of a second male is 18.7%, which is not different from its probability of winning the fight when the 2 eggs are laid at the same time (23.5%; \( \chi^2 = 1.34, \) df = 1, \( P = 0.26 \)). Under our experimental conditions, in 16.0% of cases, no individuals reached adulthood.

**Egg sex discrimination**

With regard to host selection, when confronted with mixed patches containing hosts previously parasitized by a male and a female egg, \( A. \) \( calandrae \) females first visited an equal number of each category of capsules, whatever the developmental stage of the eggs provided (2-h old: \( \chi^2 = 0.03, \) df = 1, \( P = 0.87; \) 28-h old: \( \chi^2 = 0.01, \) df = 1, \( P = 0.90 \)). Whereas they selected an equal number of hosts parasitized by male and female eggs when confronted with 28-h parasitized hosts (\( \chi^2 = 0.0, \) df = 1, \( P = 1.00 \)), they selected more hosts parasitized by a female egg when confronted with 28-h parasitized hosts (65.4% of selected hosts; \( \chi^2 = 4.90, \) df = 1, \( P = 0.03 \)). Nevertheless, the number of eggs laid on hosts parasitized by a female and by a male egg is not statistically different, whatever the developmental stage of these first eggs (Figure 2; 2-h old, \( \chi^2 = 0.04, \) df = 1, \( P = 0.83; \) 28-h old, \( \chi^2 = 2.80, \) df = 1, \( P = 0.09 \)).

With regard to sex allocation, when confronted with a host parasitized by 2-h-old male or female eggs, the females allocated the sex ratio (proportion of males) of their offspring (sex ratio = 0.10) in the same way as those confronted with a host containing only male eggs (sex ratio = 0.06) (Figure 2; Fisher test, \( P = 0.93 \)). However, females allocated sexes differentially on hosts parasitized by a male or a female egg when these eggs were 28-h old (about to hatch, Figure 2). In this case, they produced more sons on hosts already parasitized by a male egg (sex ratio = 0.55) than on those parasitized by a female egg (sex ratio = 0; Figure 2; Fisher test, \( P < 0.001 \)).

When \( A. \) \( calandrae \) females were confronted with patches containing 2 hosts, one parasitized by a female and one by a male egg about to hatch (\( X_0 = 0.5 \)), they laid eggs with a sex ratio of 0.17, which is similar to the value predicted by Werren’s model \((X = 0.22; \) Fisher test, \( P = 1.00 \)). When there were only male eggs on hosts \((X_0 = 1)\), the observed sex ratio (0.06) was also similar to that predicted by the model \((X = 0; \) Fisher test, \( P = 1.00 \)).

![Figure 1](image-url)
Although the supremacy of females in larval fights could be mainly attributed to fighting abilities, we cannot exclude the possibility that an intrinsic differential mortality between sexes during their egg or larval development partly explains this competitive asymmetry. However, without fight, under our experimental conditions, the mortality of males was generally low (less than 10%, Lebreton, personal observation) and not significantly higher than the mortality of clutches containing both male and female eggs. This cannot therefore be the only factor responsible for the asymmetry.

Nevertheless, females did not avoid laying eggs on hosts parasitized by female eggs (the better sex competitor) as might be expected. They even had a tendency to select a capsule containing a host parasitized by a female egg when the eggs were about to hatch. However, when female eggs were laid on a host which had been parasitized for a long time by a male egg (28 h), they lost their supremacy in larval fights, with only 50% of females emerging from a male/female competition, although they still had a higher probability of winning the competition than a male egg laid under the same conditions (50% vs. 25%). Consequently, being laid 28 h after a first male reduces the probability of winning the competition by 25%, whatever the sex of the second individual. This decrease is mainly due to the previous hatching of the first egg as the larva from this first egg could kill the second egg before it hatches (Godfray 1994). But when the first individual laid is a female, the probability of a second individual winning the fight if it is laid 28 h later seems to decrease only slightly (less than 5% for a male). This result could thus be explained either by an inability of females to localize other eggs or by a weaker aggressiveness of females toward these eggs. In any event, this result could partly explain why ovipositing females have a tendency to select more hosts parasitized 28 h before by a female egg. In fact, on these hosts, their offspring have little risk of being killed before hatching.

Nevertheless, hosts which have been parasitized for 28 h, especially by a male egg, could thus be considered as worse quality hosts, which could explain the higher proportion of sons laid on these hosts, as predicted by the “host quality model” (Charnov et al. 1981; Werren 1984a). Moreover, the loss of female supremacy in larval fights could induce less benefit in laying only female eggs. Under this condition and if the second individual has a nonnull probability of winning the fight, it could be better for the female to lay a male egg, which is less costly to produce as it does not use spermatozoa. Indeed, Anisopteromalus calandrae females mate only once, and after successive matings, males do not transfer enough spermatozoa to females to produce female eggs throughout their reproductive life (Do Thi Khanh 2005). In this case, it is possible that females become sperm limited. It could thus be more profitable for females to adjust their sex allocation strategies according to the sex ratio of the first clutch (in order to maximize their offspring’s reproductive success) rather than to their offspring’s fighting abilities. Anisopteromalus calandrae females have previously been shown to respond to both LMC and host quality (Nishimura and Jahn 1996; Choi et al. 2001; Ji et al. 2004).

Previous experimental studies failed to demonstrate an egg sex discrimination ability in parasitoids (Werren 1984b; van Baaren et al. 1999), in spite of a number of theoretical studies dealing with this topic (Hamilton 1967; Suzuki and Iwasa 1989; Werren 1980; Abe et al. 2003). Werren (1980) suggested that the relative size of the 2 clutches is more important in sex ratio adaptation than the sex ratio of the previous clutch. However, according to this model, for a given relative clutch size, the sex ratio of the first clutch plays a primary role in sex allocation strategies. van Baaren et al. (1999) were unable to demonstrate this discrimination capacity in a solitary

DISCUSSION

In some solitary parasitoids, because of asymmetries in larval combats (van Baaren et al. 1999), the survival probability of an egg laid under superparasitism conditions depends largely on the sex of other eggs laid on that host. Therefore, in these species, the ability of a female to assess the sex of eggs previously laid could have a direct impact on her fitness. The present study provides evidence of this capacity in a parasitoid species.

In A. calandrae, when 2 eggs are laid during the same time interval, female larvae win more larval combats than males, with about 75% of fights ending in a female victory. Therefore, when the previous eggs have been recently laid, females often lay a female egg (which has a higher probability of winning the competition) under superparasitism conditions. Interestingly, in this study, females never laid a male egg on a host already parasitized by a female egg. Because males are poorer competitors in larval combats, a male egg laid on a host already parasitized by a female egg has a low probability of reaching adulthood. Therefore, a female should avoid laying a male egg in this situation. Moreover, if the male wins the competition, it means that it killed the female larva and by killing females before their emergence it reduces its own reproductive success at the adult stage. Therefore, by not laying male eggs on hosts already parasitized by female eggs, A. calandrae females avoid a lethal fight between their sons and females that they could possibly mate after their own emergence. In this way, females increase both their sons’ reproductive success and their own fitness. However, the loss of fitness induced by killing a female would be negligible compared with the low probability of winning the fight. This would therefore be the main factor explaining why females did not lay any male eggs on hosts previously parasitized by a female egg.

![Figure 2](image-url)

Adjustment of the females’ offspring sex ratio according to both sex and the developmental stage of the eggs of a previous clutch. Proportions of male and female eggs laid on previously parasitized hosts obtained in a male/female choice test with 24-h-old and 28-h-old (about to hatch) eggs or a male/male choice test (in male/male choice tests, as results are similar whatever the developmental stage of first eggs [Fisher test, $P = 1.0$], results with 24-h-old and 28-h-old eggs have been pooled). Marks show a statistical difference between the sex ratio of eggs laid on hosts previously parasitized by a male egg and by a female egg ($*** P < 0.001$). The values in each bar represent the number of tested females oviposited on each category of host.
parasitoid species (*Anaphes vicius*), but the hosts offered to the second females in their study had been parasitized less than 1 h before. It is possible that eggs have to reach a certain developmental stage for females to be able to distinguish their sex. The present study demonstrated that *A. calandrae* females adjust their sex allocation strategies only when they are confronted with 28-h-old eggs (about to hatch) and not when eggs are 2-h old (recently laid). Although we showed that females may have no benefit in adjusting their offspring sex ratio when they are confronted with newly laid eggs, this lack of laying adjustment could also be due to an inability to discriminate the sex of newly laid eggs. Ovipositing females could thus perceive a cue that evolves as the eggs develop. However, there are no visible morphological differences between male and female eggs in *A. calandrae* prior to hatching (Sébastien Lebreton SL, personal observation). It is therefore possible that eggs produce a sex-related cue during their development that could be perceived by ovipositing females. However, to our knowledge, differences in the chemical profile of eggs according to their sex have never been shown.

Although sex ratio games have already been identified in some experimental studies (Werren 1980, 1984b; Shuker et al. 2005; Abe et al. 2007), the present study confirms the model first described by Hamilton, that is, an adjustment of offspring sex ratio according to those of conspecifics. In line with the models of Hamilton (1967) and Werren (1980), *A. calandrae* females only laid a higher proportion of male eggs when females were laid in the vicinity. In fact, females have no benefit in laying male eggs if there are no female eggs nearby to inseminate after their emergence. At the same time, when females compete with virgin conspecifics, they are not expected to adjust their offspring sex ratio in the same way as when confronted with only mated competitors. This hypothesis is supported by the constrained model (Godfray 1990), which predicts a more female-biased offspring sex ratio when females are confronted with constrained conspecifics (virgin, sperm-depleted or mated with a sterile male) in order to compensate for the excess of males in the population. This factor could be important in *A. calandrae* as females mate only once, the proportion of constrained females could be significant. The present study suggests that, if this theory is valid in this species, the main mechanistic cue perceived by mated females could be the sex of eggs laid by virgin females rather than their mating status. Indeed, they do not produce more males when confronted with male offspring of mated females; our results show that ovipositing females responded to the sex of eggs previously laid, not to the mating status of their mother.

To conclude, sex allocation has already been shown to be adjusted in response to several factors, such as the number of females, relative brood size, dispersal status, etc. (West et al. 2005). Here, we demonstrated an extra level of complexity, showing that females can adapt their offspring sex ratio in response to those of clutches laid by a previous conspecific. Through their high discrimination capacity, females can detect the reproductive strategy of conspecifics, thereby increasing their sons' mating success, and at the same time their own fitness.

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