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Sex Allocation and the Evolutionary Transition between Solitary and Gregarious Parasitoid Development

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Parasitic wasps (parasitoids) may be classified as solitary or gregarious depending on how many offspring are able to successfully complete development on a single host. Solitary species are those where at most one offspring successfully completes development on a host regardless of how many eggs were initially laid. When more than one larva is present per host, supernumerary individuals are eliminated by physical attack (frequently with enlarged, sclerotized mandibles), physiological suppression, or intense "contest" competition for resources (Clausen 1940; Salt 1961; Fisher 1971; Vinson and Iwantsch 1980; Strand 1986). Gregarious species, in contrast, are those where more than one offspring is able to complete development on a host. Larvae of gregarious species are generally not siblicidal, and any reduction in initial clutch size is typically the result of "scramble" competition for limited host resources (Godfray 1994).

While phylogenetic data are extremely limited for the parasitic Hymenoptera, existing evidence suggests that solitary development is ancestral to gregarious development and that the transition to gregarious development has occurred on numerous occasions (Rosenheim 1993; Mayhew 1998). One possible scenario for the evolution of gregarious development starts with a female of a solitary species that lays more than one egg per host during a single host visit (multiple egg clutches). The oviposition of multiple-egg clutches appears to be a widespread reproductive behavior among solitary parasitoids (Rosenheim and Hongkham 1996). Multiple egg clutches may be selected for if the additional eggs serve as "insurance eggs," thereby increasing the probability that one offspring will successfully emerge from a host (Rosenheim 1993; Rosenheim and Hongkham 1996). Once multiple egg clutches are routinely produced, alleles coding for nonsiblicidal, gregarious behavior become exposed to selection, and under appropriate conditions may spread through populations.

Despite the apparently large number of times that gregarious, nonsiblicidal behavior has arisen, Godfray (1987) has shown that the conditions required for an allele for nonsiblicidal behavior to spread within a population of siblicidal individuals are very stringent; such an allele will spread only when the per capita fitness of an individual that develops while sharing the host resource with a sibling exceeds the fitness of an individual developing alone. When more than one egg is laid per host, at least two sex allocation patterns of an ovipositing female can relax these conditions by increasing the chances that two nonsiblicidal individuals are placed in the same host: an overall female-biased sex ratio (Godfray 1987) and single-sex broods (Rosenheim 1993). Haplodiploidy results in relatedness asymmetries between brothers and sisters; both female-biased sex ratios and single-sex broods increase the levels of within brood relatedness, thereby favoring altruistic behavior such as nonsiblicide.

Sex Allocation Decisions of *Comperiella bifasciata*

To determine if the production of female-biased sex ratios or single-sex clutches could be important in shaping the evolution of gregarious development in nature, we must move from the predictions of genetic models to the direct evaluation of sex allocation decisions by ovipositing females. Solitary parasitoids that lay multiple-egg clutches have been identified as likely intermediates in the transition from solitary to gregarious development (Godfray 1987). Until now, however, no one has studied within-host sex allocation decisions by these solitary parasitoids. This is due in part to difficulties in determining the sex of each egg that is laid in a host (the primary sex ratio). Because only one offspring per host emerges, ex-

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amination of adult offspring sex ratios (the secondary sex ratio) gives little or no indication about sex ratio decisions made at oviposition.

Here, we analyze sex allocation and its consequences for successful development in *Comperiella bifasciata* Howard (Hymenoptera: Encyrtidae), a solitary parasitoid we believe possesses a strong potential to develop a gregarious life history. *Comperiella bifasciata* is an endoparasitoid of armored scales (Homoptera: Diaspididae), including the California red scale (*Aonidiella aurantii* [Maskell]) and the yellow scale (*Aonidiella citrina* [Coquillet]). Although *C. bifasciata* is solitary, ovipositing females frequently lay more than one egg per host during a single host visit (Blumberg and Luck 1990; Rosenheim and Hongkham 1996). We are able to determine accurately both the number of eggs and the sex of each egg laid per host by carefully observing the abdominal gestures of ovipositing female *C. bifasciata.* Similar to several other species (e.g., Cole 1981; van Dijken and Waage 1987; Strand 1989), a pause in the abdominal movements during oviposition correlates with the fertilization of the egg and, therefore, the production of a daughter (P. J. Ode, unpublished manuscript).

Sex allocation decisions within a host were tested by presenting each of 486 females a single, standard-sized, virgin third instar host. All oviposition events were carefully observed to identify the number and sex of the eggs that were oviposited. While the majority of hosts received one egg (151 hosts received one male egg and 165 hosts received one female egg), 170 hosts (34.98%) received two eggs during a single visit by an ovipositing *C. bifasciata* female. Overall, the primary sex ratio of eggs laid in two-egg clutches was very female biased (23.82% male; fig. 1). Furthermore, even given the strongly female-biased sex allocation, there was a significant underproduction of mixed-sex broods (fig. 1).

The influence of clutch size and sexual composition on parasitoid developmental success was tested by presenting each of a second group of 450 females a single, standard-sized, virgin third instar host. Because we wanted to generate many clutches composed of two male eggs, some of the females in this group were virgin (and therefore constrained to produce only males). In addition, we increased the proportion of mixed-sex broods in this experiment by discarding some of the single-sex broods that were produced. These discarded broods were not counted as part of the sample size of 450. Again, the number and sex of each egg laid were recorded. Parasitized hosts were isolated for 40 d, a sufficient time for *C. bifasciata* to complete development, at which time each host was checked for the emergence of an adult wasp. Hosts from which no wasp emerged were dissected, and, in most cases (143 out of 165), *C. bifasciata* eggs were found to have been encapsulated by the immune system of the host. Emergence rates were significantly greater in two-egg clutches than in one-egg clutches (fig. 2). Furthermore, the positive effect of clutch size on emergence was greater for all-female broods than for all-male broods (fig. 2).

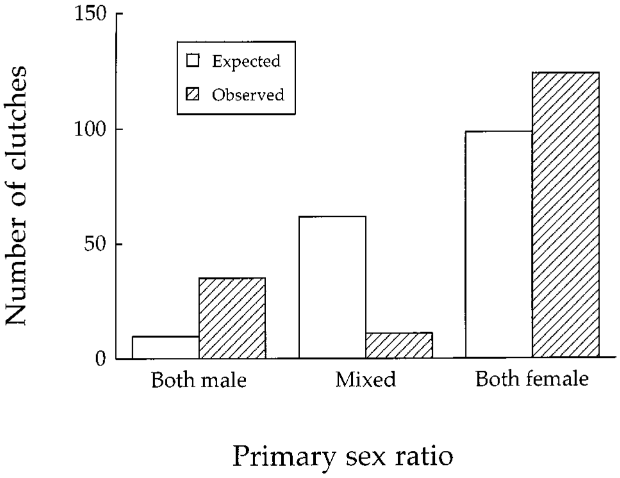

Figure 1: Sex allocation decisions of the 170 females who laid two eggs per host during a single host visit. Overall, females laid 35 all-male broods, 11 mixed-sex broods, and 124 all-female broods. Expected values were generated from a binomial distribution with $P = .2382$ (the proportion of male eggs laid across all two-egg clutches) for each of the three categories of broods. Ovipositing females laid significantly more single-sex clutches and fewer mixed-sex clutches than expected (goodness of fit, $G_{adj} = 108.60$, df = 2, $P < .001$).

Joint Effects of Female-Biased Sex Ratios and Single-Sex Broods

Female *Comperiella bifasciata* exhibit both of the sex-allocation traits that, when analyzed in isolation, have been shown to facilitate the spread of a rare nonsiblicidal allele (Godfray 1987; Rosenheim 1993). We can show with a simple extension of Godfray's (1987) model that these two factors act in a complementary fashion to facilitate the evolution of gregarious parasitoid development. We retain the simplest-case assumptions described in Rosenheim (1993) and evaluate the simultaneous roles of x, the sex ratio (the proportion of male eggs laid), and m, the proportional reduction in the production of mixed-sex broods compared to that expected under binomial sex allocation (for the underproduction of mixed-sex broods analyzed here, $0 \leq m \leq 1$). The critical condition for the successful spread of a rare dominant allele coding for nonsiblicidal behavior is framed in terms of the threshold fitness value of a parasitoid developing in a

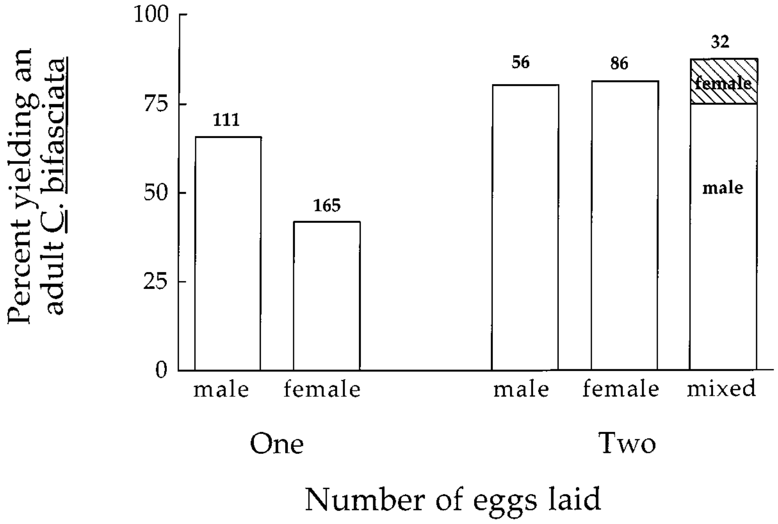

Figure 2: Percentage of broods that successfully produced a single adult parasitoid as a function of clutch size and sexual composition. Numbers above each column represent the total number of broods in that category. An adult *Comperella bifasciata* emerged from two-egg clutches more frequently than from one-egg clutches (females: likelihood ratio $G = 38.11$, $\mathrm{df} = 1$, $P < .001$; males: $G = 3.99$, $\mathrm{df} = 1$, $P < .05$). The effect of clutch size on emergence probabilities was significantly stronger in female broods than male broods (Mantel-Haenszel odds ratio, $\chi^2 = 4.02$, $\mathrm{df} = 1$, $P < .05$). Mixed-sex broods showed similar emergence rates as the single-sex two-egg clutches (likelihood ratio, $G = 0.84$, $\mathrm{df} = 2$, $P > 0.50$). Males emerged significantly more often from mixed-sex two-egg broods that yielded a *C. bifasciata* adult (goodness of fit, $G_{\mathrm{adj}} = 19.94$, $\mathrm{df} = 1$, $P < .0010$.

clutch of two eggs, $f(2)$. If we define $f(1) = 1$, then when $x = 0.5$ and $m = 1$ (i.e., binomial allocation of an equal proportion of male and female eggs to clutches), the critical condition is $f(2) > 1$ (Godfray 1987); that is, the fitness of a parasitoid developing in a clutch of two eggs must exceed the fitness of a parasitoid developing alone, as discussed above. Allowing the proportion of female eggs laid and the proportion of single-sex broods to vary, the critical condition is

$$f(2) > \frac{4}{1 + \sqrt{17 - 16xm}},$$

as plotted in figure 3. Thus, these factors work in a complementary fashion to relax the conditions under which a nonsiblicidal allele can invade.

This result can be understood intuitively by considering the within-brood relatedness for each of the three types of two-egg broods: $r = 0.5$ for all-male broods, $r = 0.375$ for mixed-sex broods, and $r = 0.75$ for all-female broods. Therefore, underproduction of male offspring and mixed-sex broods will both increase the mean within-brood relatedness within the population. For *C. bifasciata,* the overall mean within-brood relatedness for two-egg clutches is influenced by both the observed underproduction of males ($x = 0.2382$) and the observed underproduction of mixed-sex broods ($m = 0.1783$), yielding $r = 0.6743$. This value is 34.9% higher than the value expected under binomial sex allocation with an

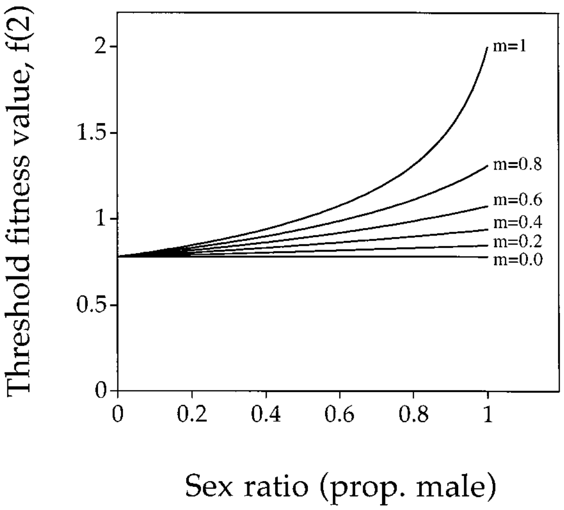

Figure 3: Simultaneous influence of sex ratio (x: proportion males) and the proportional reduction in the production of mixed-sex broods (m) on the conditions under which a rare dominant allele coding for nonsiblicidal behavior would successfully invade a population of siblicidal parasitoids. For parameter combinations above the solid lines, a rare nonsiblicidal allele will successfully invade. Below the lines, siblicide is evolutionarily stable. The y-intersect (where sex ratio = 0) has the value of 0.7808.

equal sex ratio ($r = 0.5$). The sex ratio effect considered alone would have increased mean within-brood relatedness by 20.0%, whereas the single-sex brood effect considered alone would have increased mean within-brood relatedness by 20.6%. Thus, these two variables make approximately equal contributions to enhancing opportunities for altruistic behavior within two-egg clutches of *C. bifasciata.* The two parameter estimates lower the critical condition for nonsiblicide to $f(2) > 0.7937$: in other words, the per capita fitness of an individual developing in a clutch of two may be reduced by up to 20% because of the need to share host resources, and an allele for nonsiblicidal behavior will still spread successfully through the population.

Discussion

In this study, we have shown that both conditions (female-biased sex ratios and single-sex broods) thought to favor the spread of an allele for nonsiblicidal behavior are present in a solitary parasitoid that commonly produces multiple-egg clutches. We also examined whether these allocation patterns have any adaptive function in their own right. Specifically, we asked: Why should ovipositing females lay multiple-egg broods, produce female-biased sex ratios, and lay predominately single-sex broods when laying more than one egg per host? The answers to this multipart question will help us to understand how common these brood- and sex-allocation patterns may be within the parasitic Hymenoptera and, thus, how likely they are to function as important facilitators of evolutionary transitions to gregarious development.

The production of multiple-egg clutches by *Comperiella bifasciata* females appears to be favored because it increases the probability that one offspring will avoid the host's encapsulation response and successfully emerge (see also Blumberg and Luck 1990). The heavy production of all-female clutches suggests that females benefit more from developing in multiple egg clutches than do males. There is clear evidence that this is the case. Female offspring were 3.1 times more likely to emerge when clutch size was two as compared to when clutch size was one; males were only 1.9 times as likely to develop successfully in two-egg clutches compared to single-egg clutches (fig. 2). The production of single-sex rather than mixed-sex broods allows ovipositing females to retain control over which sex emerges from a given host. A variety of environmental conditions favor maternal control of offspring sex ratio (e.g., variation in host quality [Charnov 1982] or population mating structure [Hamilton 1967]). Furthermore, the competitive superiority of sons largely precludes mothers from producing daughters via mixed-sex broods (see fig. 2). Therefore, in order to produce a daughter when the risk of encapsulation is high, a mother ought to lay two-egg female clutches. This is precisely the type of allocation pattern that facilitates the transition to gregarious development.

The production of single sex broods is very rare among gregarious parasitoids (e.g., Salt 1931; Askew and Ruse 1974; Bryan 1983). Once gregarious development has arisen, individuals in finite populations may experience selection to reduce variance in their offspring sex ratios and, especially, to reduce the production of single-sex clutches (Verner 1965; Taylor and Sauer 1980; Green et al. 1982). However, single-sex brood production may be much more common during the transitional phase between solitary development and gregarious development (Rosenheim 1993), and the results presented in this note show that solitary species are capable of making sex allocation decisions that include both female-biased sex allocation and single-sex brood production.

In sum, there are distinct fitness advantages for *C. bifasciata* females who produce multiple egg clutches and in particular clutches composed of two female eggs. This pattern of brood production creates an environment favorable to the spread of nonsiblicidal behavior. Nevertheless, gregarious development has not yet evolved in *C. bifasciata.* It is interesting to note that on very rare occasions, two *C. bifasciata* adults have been observed to develop successfully in the same host (T. Unruh, personal communication). We suggest that resource levels in the hosts currently attacked by *C. bifasciata* are not sufficient for the development of more than one offspring. If *C. bifasciata* ever expands its host range to include larger hosts, gregarious behavior may very well evolve.

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