when they 'need them'. For male *Utetheisa*, the PAs have the added function of serving as precursors for the sex pheromone they use in courtship[4].

In nature, of course, larvae might remedy systemic PA deficiencies chiefly by seeking out the seeds of their foodplant rather than pupae. In our field test only very few pupae were actually cannibalized. We attribute this to the special circumstance that seeds were in abundance on our experimental *Crotalaria* at the time, and that the larvae on these plants were therefore not sufficiently PA-deficient to be highly cannibalistically inclined. But pupal cannibalism may be rare as a matter of course in *Utetheisa*. *Utetheisa* larvae do not, as a rule, pupate on their foodplant. As we ourselves determined, and was earlier noted by others (W. J. Conner, personal communication), mature *Utetheisa* larvae crawl away from their *Crotalaria* host to pupate on shrubs and herbs nearby, or under the bark of trees. Cannibalism in *Utetheisa* is much more likely to befall the eggs. As we will be reporting elsewhere, consumption of eggs by *Utetheisa* larvae is subject to the same 'rules' as reported here for pupal cannibalism. Thus, for instance, larvae tend to feed on eggs only if these contain PA and the larvae themselves are PA-deficient (Bogner and Eisner, unpublished).

While we do not here provide supporting data, we know from chemical analyses that (−) larvae that cannibalize (+) pupae acquire substantial loads of systemic PA. Actual assays of moths which as (−) larvae had each eaten a (+) pupa, gave a mean value of 546±169 (SEM) μg monocrotaline/moth (N = 10) (J. Kopecky, unpublished data), a value closely matching that obtained with moths that acquired their PA naturally from seed-bearing *C. spectabilis*, or from our PA-containing laboratory diet (see 'Materials and methods').

Acquired PAs evidently play a dual role in the life of *Utetheisa*. While on the one hand they can convey protection against predation, they can on the other, increase vulnerability to cannibalism. One wonders to what extent, under changing natural conditions, the trade-off between these potentially conflicting functions may undergo shifts. Are there times when it 'pays' for *Utetheisa* to be deficient in systemic PA, times when the threat from cannibalism outweighs that from predation, times perhaps when foodplant seeds are scarce, larvae abundant, and PA to be found most concentrated in the body of conspecifics? In the absence of appropriate field data these questions remain open. What is clear is that the tendency of *Utetheisa* to pupate away from its hostplant provides a mechanism for minimizing the risks of cannibalistic attack from larvae.

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Male production induced by antibiotic treatment in *Encarsia formosa* (Hymenoptera: Aphelinidae), an asexual species

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Abstract. The production of large numbers of males in the thelytokous species *Encarsia formosa* was induced by feeding antibiotics to their mothers. The males induced by antibiotic treatment produce sperm and sometimes mate with females, but insemination does not occur.

Key words. Thelytoky; microorganisms; autoparasitoid; biological control; sex ratio; parthenogenesis.

Encarsia formosa Gahan (Hymenoptera: Aphelinidae) is a solitary, thelytokous, endoparasitoid of the greenhouse whitefly (*Trialeurodes vaporariorum* (Westwood)) (Homoptera: Aleyrodidae) used for whitefly control in commercial greenhouses worldwide[1]. Although males are occasionally produced at low frequencies, they appear to be non-functional[2-4] and rarely attempt mating[5]. Originally described from collections made in Ohio[6], most of the laboratory-reared *E. formosa* populations now distributed throughout Europe may have originated from a few individuals introduced to England[7]. This species seems to be very uniform genetically even relative to other Hymenoptera, which are characterized by low genetic variability[8]. In an electrophoretic study of esterase-isozymes of *E. formosa* from five European countries, only one isoesterase variant was found[7].

Thelytokous strains of four *Trichogramma* species have been permanently changed into arrhenotokous ones (producing functional males) by feeding several kinds of antibiotic to adult females. Antibiotic treatment presumably cures the strains of microorganisms that cause thelytoky[9]. In order to test the possibility that thelytoky in *E. formosa* is also caused by microbial symbionts and to try to produce arrhenotokous lines, we followed similar procedures. The induction of fertile male production would facilitate study of the genetics of *E. formosa* and provide a potential avenue to increase its genetic variability for the purpose of genetic improvement (for review see Beckendorf and Hoy[10]).

Materials and methods

A strain of greenhouse whiteflies was maintained in a growth chamber at 26 °C, 60% RH and 16:8 (L:D), on bean plants (*Phaseolus vulgaris* cv. 'slenderette'). The *E. formosa* strain originated in Ohio, where it was field-collected in the early 1970's and subsequently reared at Cornell University. To maintain the parasitoid colony, uninfested bean plants were placed for 3 days in the whitefly colony for whitefly oviposition, whereafter adult whiteflies were removed from the leaves and the plants placed in a cage in a second chamber (22–26 °C, 60% RH, 16:8 (L:D)). When host larvae had reached third instar, the most suitable stage for parasitism[11], the plants were exposed to adult wasps. About 10 days later, leaves with parasitized pupae ('black scales') were removed from the plants and placed on trays to dry for 24 h. The leaves were then transferred to plastic containers (0.47 l) covered with white polyester organdy. When *E. formosa* females started to emerge, the organdy was replaced with an inverted 10-cm diameter plastic funnel, which in turn was covered with a small (20 ml) scintillation vial containing honey. Newly emerged *E. formosa* adults would climb up the funnel and into the vial. The parasitoids were then either used in experiments or returned to the *E. formosa* colony.

To test if microorganisms in the wasps influence fecundity and the thelytokous mode of reproduction, 30 *E. formosa* females, 0–2 days old, were starved for one day and then placed in a vial. Females were fed either honey mixed with tetracycline (50 mg/ml) or pure honey as a control. After 24 h, the females were released on a bean plant heavily infested with second to fourth instar whitefly nymphs for oviposition. In order to differentiate eggs that were laid shortly after the antibiotic treatment from those laid later, a second infested bean plant was introduced to the wasps three days after the first one. Twelve days after the beginning of oviposition, the first bean plant was taken out of the cage and the total number of black (parasitized) pupae counted to assess fecundity. These comprise the whitefly nymphs attacked during the first five days of oviposition because parasitized hosts take seven days to turn black at the temperatures used. In order to observe the sex ratio produced, 100 of these black pupae were randomly sampled and placed individually in #4 gelatin capsules. Three days later, the same procedure was applied to the second plant. The number and gender of the emerging parasitoids was recorded daily. The experiment was repeated a month later.

Mating potential of the antibiotic-induced males was tested at four levels: 1) sperm production, 2) mating, 3) transfer of sperm and 4) viable female offspring. The reproductive tracts of *E. formosa* males were dissected in saline and examined for the presence of sperm. Mating behavior was observed under three different conditions: a) a male and a female in a vial, b) one pair in a petri dish with a piece of an uninfested bean leaf on filter paper, and c) five males and 15 females in a vial. All *E. formosa* in this experiment were 0–24-h-old virgins, and their behavior was observed for 30 min. Females that appeared to be mating were removed from the arena and examined for sperm in the spermatheca. These females were CO_2-anesthetized, placed in a drop of distilled water and covered with a coverslip, then gently squashed with a probe. The transparent spermatheca was generally visible to one side of the abdomen, and was observed at 400–1000 × phase contrast. Also, the five males and 15 females from the mating behavior experiment were left in the vial with honey for 48 h and then females were dissected for the presence of sperm in the spermatheca.

Unfertilized arrhenotokous hymenopteran females will lay only male eggs, whereas fertilized females will potentially produce males and females. To test for egg fertilization, 10 *E. formosa* females were fed antibiotic as described above. After 24 h, they were numbered and caged individually on bean leaves with whitefly numphs for oviposition for 48 h, and then placed individually in vials with one male for 24 h. The 'mated' females were caged again on fresh leaves to oviposit for 48 h and then removed. Twelve days after oviposition, the black pupae were counted, placed in gelatin capsules and the sex of the emerging wasps recorded.

Results

Antibiotic treatment had a dramatic effect on the sex ratio produced by female *E. formosa* (table 1). The progeny sex ratios of treated vs untreated females

Table 1. Sex ratio produced by *Encarsia formosa* females fed with tetracycline ('Early'-0-5 d after feeding on antibiotic, 'Late'-3-8 d after feeding on antibiotic). Sample size for % males was 100 immature wasps (black scales) for each of two replicates.

Treatment	No. Females/replicate	Mean No. of offspring	% Males Early	Late
Antibiotic	30	711	71	100
Control	30	409	0	5

Table 2. The number of mating attempts made by *Encarsia formosa* males when confined with females for 30 min under different conditions as explained in the text.

Arena	No. of males per arena	No. of females per arena	No. of replicates	Total no. of females tested	Mating attempts
Petri dish	1	1	20	20	4
Vial	1	1	20	20	4
Vial	5	15	4	60	18

were significantly different in both replicates ($\chi^2 > 224$, $p < 0.0001$). For up to five days after antibiotic treatment ('early'), the females continued to produce female offspring, but from the fifth day on ('late'), no females developed (table 1). All of the males that appeared on the late control plant were found in the first replicate and we suspect that these resulted from a treated female that contaminated the control cage. Large numbers of whitefly pupae produced adult whiteflies, demonstrating that the hosts were not limiting to the reproduction of the *Encarsia*. *E. formosa* females fed antibiotic appeared to produce more offspring than the control wasps (table 1), but there was too much variation between the replicates to achieve statistical significance.

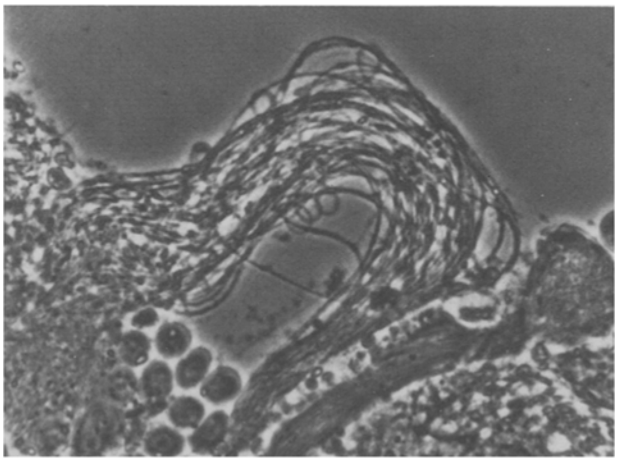
In these experiments, females were introduced to unparasitized whitefly nymphs, and their sons began to emerge concurrently with their daughters, suggesting that both females and males developed as primary parasitoids. These observations are consistent with other studies[4,5], but do not support an earlier report by Gerling[12] that *E. formosa* was one of the aphelinids defined as 'obligate autoparasitoids'[13] in which males develop only as hyperparasitoids of immature conspecifics.

Sperm were found in the seminal vesicles of dissected males (fig.). Only 4 out of 20 males that were confined with a female in a petri dish made any attempt to mate; 4 out of 20 males also attempted mating when single pairs were placed in a vial (table 2). There were 4–5 mating attempts on each occasion when five males were put in the same vial with 15 females; however, it was not possible to tell whether it was one or more male(s) that approached the females. The higher number of mating attempts in the more crowded vial may be explained by an elevated excitement level in the males due to a higher concentration of some female-derived chemical signal. Alternatively, the results may simply reflect variation in the receptivity of females; more females were tested in this experiment (table 2).

No sperm was found in the spermathecae of 20 females following the 48-h period with the males, nor could we find sperm in the spermathecae of the females that were seen mating. The failure of males to inseminate females was also demonstrated in a separate experiment, in which females treated with antibiotic did not appear to reproduce sexually following confinement with a male. For the first two days after antibiotic treatment, 56% of the progeny of the 10 virgin females were daughters (83 of 148 offspring), consistent with earlier observations (table 1) that females treated with antibiotic would continue to produce thelytokous daughters for a few days after treatment. When these females were confined with males for 24 h in a vial, and then replaced on plants, however, they produced only sons (out of 81 progeny), suggesting that their eggs were cured of microorganisms but not fertilized.

Discussion

As with some species *Trichogramma*[9], microorganisms appear to be involved in causing thelytoky in *E. formosa*. Our data suggest that female *E. formosa* lay unfertilized eggs in the whitefly host and that microorganisms cause, by a mechanism as yet unknown, the restoration of diploidy in the eggs to produce females. As these organisms are presumed to be transmitted exclusively in or on eggs, elimination of males increases their rate of transmission[9].



Squash of reproductive tract of an *Encarsia formosa* male, showing sperm.

Whether parthenogenetic reproduction is advantageous to individual females of any species has long been debated as an evolutionary problem[14, 15], but the elimination of males may not be advantageous to biological control. Although it has long been assumed that habitual parthenogenesis in *E. formosa* is an enormous advantage in rearing the parasite for economic purposes[2], the microorganisms causing parthenogenesis may be pathological. Thus, curing parasitoids may actually increase their fertility, as tended to happen in our observations and was also reported for *Trichogramma* spp.[16]. Moreover, thelytoky in a biological control agent may confer disadvantages such as low genetic variability, difficulties in studying the genetics and, thus, limitations in genetic improvement. These problems could be solved if an arrhenotokous line of *E. formosa* could be established and visible genetic markers, for example, induced by mutagenesis and recovered from males, could be introduced into the population. Genetic markers and other possible improvements would be especially useful if arrhenotokous females could then be manipulated back to thelytoky to prevent loss of desirable characters through outcrossing.

Nonetheless, even though males produce sperm and 70 males were tested in four different sets of conditions with at least 26 attempts by the males to mate, successful insemination did not appear to occur. Thus, it seems that the population of *E. formosa* investigated has been thelytokous for so long that behavioral, physical or mechanical pre-zygotic barriers now exist in either the males or females that prevent fertilization (as can be seen from the empty spermathecae and the failure to produce female progeny). As there are no known arrhenotokous populations sufficiently similar to be considered conspecific with *E. formosa* (M. Rose, pers. comm.), we could not use arrhenotokous males from another population or species to determine the exact nature of that reproductive barrier. In contrast, all curable thelytokous populations of *Trichogramma* are known or suspected to be sufficiently similar to be considered conspecific with arrhenotokous species[17]. In many cases, males of these species have already been successfully crossed with 'cured' females from the thelytokous populations[9]. All known populations of *E. formosa* have been asexual since this species was described in 1924. Studies of *Drosophila* have shown that sexual characters may degenerate rapidly in parthenogenetic populations under laboratory conditions[18]. Thus, one of the most striking results of this study is that males produced sperm at all. Although the retention of genetically or environmentally suppressed characters has been explained by essential plieotropic effects of the genes that control them[19], it is not clear that this explains sperm production in *E. formosa*. Spermatogenesis would seem to be a complex process involving a number of genes with no obvious functions in females.

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