Fitness of arrhenotokous and thelytokous *Venturia canescens*

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Theory predicts that asexual reproduction has a competitive advantage over sexual reproduction because of the cost of producing males. One counterbalancing force may be reduced phenotypic fitness of asexuals as a consequence of mutation accumulation. We measured a number of fitness components of arrhenotokous and thelytokous *Venturia canescens*. Thelytokous females had higher egg loads and ovariole numbers and laid more eggs per host larva. Arrhenotokous females had a higher longevity. These differences are considered in the context of different life histories of both reproductive modes.

Keywords: sex, paradox of sex, arrhenotoky, thelytoky, phenotypic fitness, *Venturia canescens*

Sex is the exchange of genetic material between organisms. It occurs widespread in bacteria, and the plant and animal kingdom. Most 'higher' animals reproduce sexually (Barton & Charlesworth 1998), but asexual reproduction occurs at least sporadically in most groups. If the term is used in a broad sense, asexual reproduction can be divided into 'true' asexuality in which there is reproduction from somatic tissue (vegetative reproduction, budding) and parthenogenesis. Parthenogenesis is the development of an egg without fertilization.

Compared to asexual reproduction, sexual reproduction is expensive. Some costs that sexually reproducing individuals have to pay are: the time to find mates, dangers experienced during mating, the transmission of sexual diseases, the breakdown of favourable gene combinations by recombination and outbreeding depression. Most attention has however been paid to the 'cost of meiosis' (Williams 1975) and the 'cost of males' (Maynard Smith 1971, 1978). While the first refers to the fact that sexual individuals only provide half of their genes to each offspring, the second deals with the fact that males cannot give rise to offspring by themselves. Assuming a sex ratio of 1:1 (females:males) this means that half of the offspring cannot reproduce by themselves. This on its own makes sexual reproduction twice as expensive, which is described by Maynard Smith (1978) as the 'twofold cost of sex'. In contrast, asexual individuals do not pay the cost of meiosis and the cost of males. As a consequence they are expected to quickly outcompete sexual individuals. Nevertheless, sexual reproduction is the predominant mode of reproduction and this apparent disconcordance between theory and practice is known as the 'paradox of sex' (Maynard Smith 1978).

Many theories have been proposed to resolve the paradox of sex (overview in Kondrashov 1993, West *et al.* 1999). Some of these theories (Muller 1964, Kondrashov 1988) consider the accumulation of deleterious mutations to occur faster in asexuals than in sexuals, because asexuals cannot purge them from the genome through recombination. As a consequence asexuals are predicted to have lower fitness than sexuals. General problems encountered when trying to test this prediction is that sexuals and asexuals often differ in a number of other characteristics (*e.g.* ploidy level, geographical range) and that both reproductive modes typically do not occur within one and the same species. These problems are however absent in *Venturia canescens*. In this solitary parasitoid wasp both obligatory sexual and parthenogenetic individuals can be found in sympatry (Schneider *et al.* 2002). Like all Hymenoptera, *Venturia* has haplodiploid sex determination, *i.e.* males are haploid and females are diploid. The sexual mode is called arrhenotoky: males develop parthenogenetically from unfertilized eggs and females from fertilized eggs. The asexual mode is truly parthenogenetic: females develop from unfertilized eggs and there are no males. This system is ideally suited for testing alternative theories of the maintenance of sex. In this article we compare a number of fitness parameters of an arrhenotokous and a thelytokous *V. canescens* strain in the laboratory.

MATERIAL AND METHODS

Strains

We used an arrhenotokous and a thelytokous strain, both collected from Antibes (France) in 1999. Wasps were cultured with the pyralid moth *Ephestia kuehniella* as a host. The host larvae were cultured on a mixture of flower, semolina and yeast.

Lifetime reproductive success

Within 24 hours after emergence from the mass culture females were individually put in plastic containers (diameter 60 mm, height 65 mm) at 25°C and given a drop of honey for feeding. This design allowed arrhenotokous females to mate before the start of the experiment. Every second day until death females were provided with 22 moth larvae for oviposition for a period of three hours. The parasitized larvae were cultured at 29°C as described above, except for two per batch that were dissected to determine the rate of parasitism and superparasitism (see below). After about 3 weeks the number and sex of the offspring of each female was determined that emerged from the remaining 20 larvae.

Rate of parasitism and superparasitism

As described above, two larvae per oviposition bout per female were dissected to determine the rate of parasitism (*i.e.* wasp eggs present or absent) and superparasitism (one or more than one wasp egg present). This was done for the first five

oviposition bouts, *i.e.* of days 1, 3, 5, 7, and 9 of the experiment. Larvae were torn apart with small forceps in 45% acetic acid and the number of eggs inside each larva was counted under a stereo binocular.

Egg load and number of ovarioles

For egg load we used the number of mature eggs in the ovaries (Fig. 1A). These could easily be distinguished by their characteristic plush at one end of the egg and a thick shell, which prevented water to enter (Fig. 1B). Similarly, ovariole number could easily be determined by counting the number of egg rows in the ovaries (Fig. 1C). One-day old females were dissected with small forceps in demi-water on a dark background under a stereo binocular. The tibia of both hind legs were measured and taken as indicator for body size (Fig. 1D).

Longevity

One-day old females were taken from the stock cultures and placed individually in plastic containers. Survival was tested at 15°C, 25°C and 29°C, with and without food. In the condition 'with food' a drop of honey was put on the lid of the container once at the start of the experiment. Containers were checked daily for death of females.
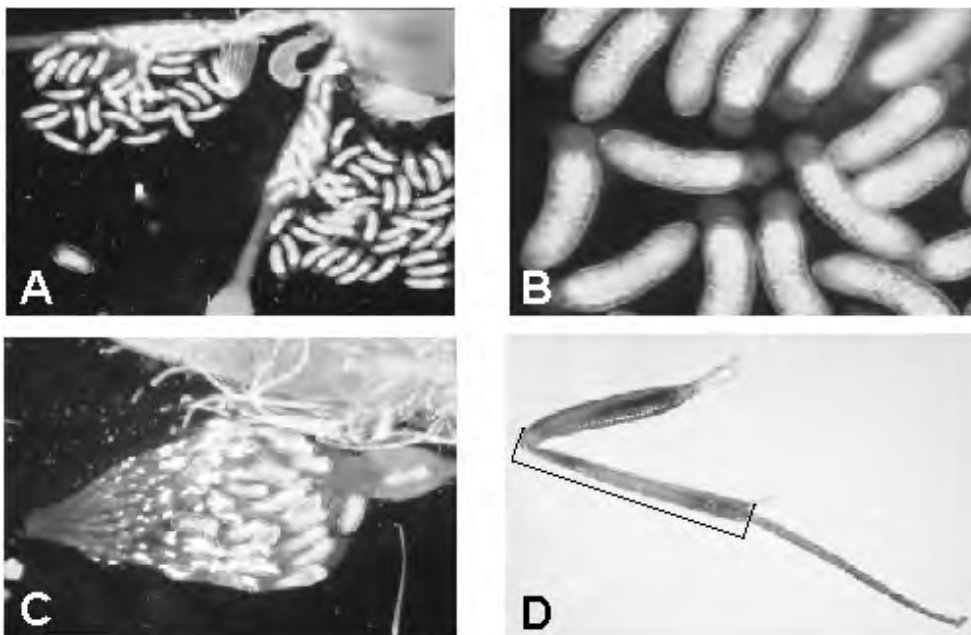

Figure 1. Dissection of ovaries showing (A, B) egg numbers and (C) ovariole numbers. Tibia length (D) was used as measure for size which is known to correlate with egg and ovariole number.

Data analysis

Data were analyzed statistically with SPSS 11.0 for Windows. Data were first checked for normality with the Kolmogorov-Smirnov-test and then tested parametrically with the *T*-test, or in case of no normality non-parametrically with the Mann-Whitney *U*-test (arrhenotoky *vs.* thelytoky).

RESULTS

Lifetime reproductive success

A total of 17 arrhenotokous and 44 thelytokous females were given hosts every other day for life. Lifetime offspring production for arrhenotokous females was on average 35.9 and for thelytokous females 26.4, but did not differ significantly between both groups (Fig. 2). Ten of the arrhenotokous females produced at least one daughter and thus proved to have mated. The other females produced only sons, whereas all thelytokous females produced only daughters.

Rate of parasitism and superparasitism

In order to determine the rate of parasitism, two host larvae of each of the first five oviposition bouts were dissected. Arrhenotokous females parasitized 43 of 94 (45.7%) larvae analyzed. In the remainder no eggs could be found. Thelytokous females parasitized 111 of 228 (48.7%) larvae analyzed. The parasitation rates are not statistically different (Chi²=0.13, df=1, P=0.721). The degree of superparasitism was determined among those larvae that contained at least one egg. Nineteen of the 43 (44.2%) parasitized larvae of the arrhenotokous group
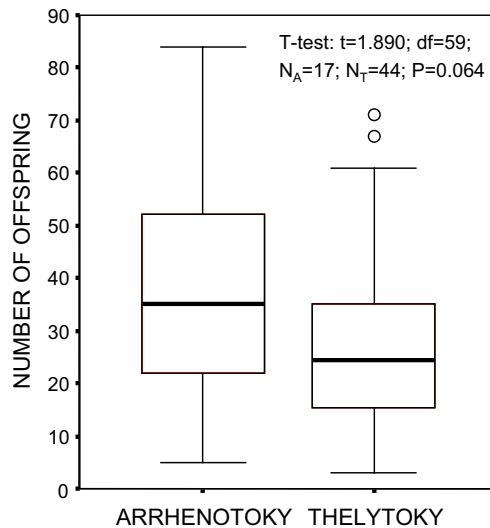

Figure 2. Total number of offspring produced during life for arrhenotokous and thelytokous wasps. The box plot shows the median (bold line), the interquartile range (length of box), the 1.5 interquartile range (vertical line) and extreme data points.

contained more than one egg. The remainder contained only a single egg. Seventy-two of the 111 (64.9%) larvae of the thelytokous group were superparasitized, which is significantly more than the arrhenotokous group (Chi²=4.66, df=1, P=0.031). Thelytokous females lay on average 2.87 eggs per larva *vs.* 1.91 eggs for arrhenotokous females (estimate based on all dissected larvae, N=11 and 43, respectively; Fig. 3).

Egg load and number of ovarioles

Thelytokous females had on average 49.0 mature eggs in their ovaries (N=52), which is more than three times the average of 15.5 of arrhenotokous females (N=11) (Fig. 4). Three thelytokous females that had no eggs at all were excluded. Since egg load is known to be positively correlated with body size, we took tibia length as a measure of body size. Tibia lengths of the left and right hind femur were highly correlated (data not shown) and we took the mean of both measures if they could be obtained. For both reproductive modes slightly positive, but non-significant, correlations were found between tibia length and egg number. Moreover, mean tibia lengths between both groups did not differ (T-test, df=62, P=0518). Therefore, the observed differences in egg load between both reproductive modes were not due to differences in wasp size.

Thelytokous females had on average 21.6 ovarioles (N=33), which is significantly more than the 15.6 of arrhenotokous females (N=10) (Fig. 5). The three thelytokous females that had no eggs at all were excluded from the analysis. A positive but not significant correlation was found between mean tibia length and ovariole number for the thelytokous, but not the arrhenotokous group.
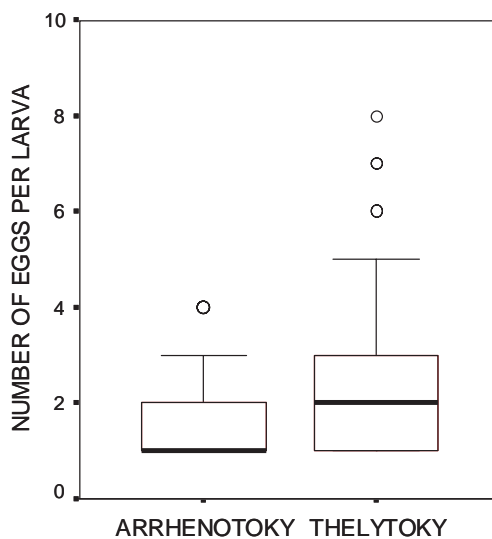

Figure 3. Number of eggs oviposited per host larva for arrhenotokous and thelytokous wasps. See legend of figure 2 for box plot details.

However, since mean tibia lengths between both groups did not differ, the difference in ovariole number cannot be attributed to wasp size.

Longevity

Longevity was determined by measuring the number of days that an individual female stayed alive. In general, survival was much higher in the treatment 'with food' compared to 'without food' and increased with decreasing temperature. Wasps lived on average about 50 days at 15°C with food, but only 2 days at 29°C without food. Arrhenotokous females lived significantly longer in the 'with
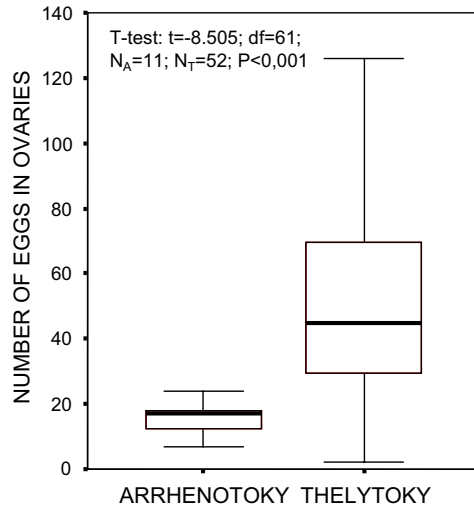

Figure 4. Number of eggs in ovaries for arrhenotokous and thelytokous wasps. See legend of figure 2 for box plot details.
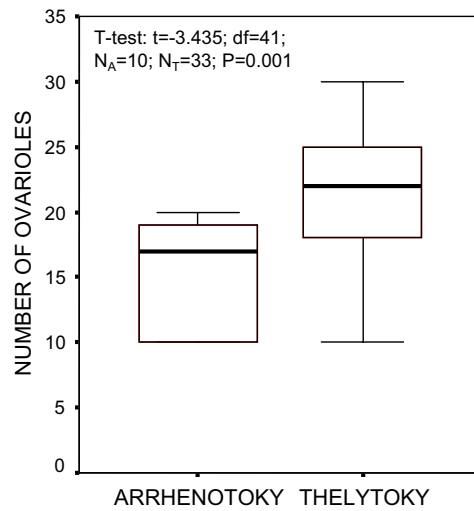

Figure 5. Number of ovarioles for arrhenotokous and thelytokous wasps. See legend of figure 2 for box plot details.

food' treatment at 15°C and 25°C than the thelytokous females (Fig. 6). At 29°C the difference is not significant, but the trend is in the same direction. In the 'without food' treatments no differences were found between both reproductive modes at any temperature. Note that the sample size of the arrhenotokous group is somewhat low in this treatment.

DISCUSSION

Theory predicts that asexual individuals that only produce daughters have a twofold reproductive advantage over sexuals that waste half of their reproductive effort on males that cannot reproduce by themselves. This idea is based on the assumption of 'all else being equal' (Williams 1975). For example it assumes that sexual and asexual females have equal phenotypic fitness, such as longevity and fecundity. We measured a number of phenotypic fitness parameters of arrhenotokous (sexual) and thelytokous (asexual in the broad sense) *V. canescens* females. We found clear deviations from the 'all else being equal' assumption.

Fecundity of thelytokous females was much higher than that of arrhenotokous females. This was measured as a higher number of mature eggs present in the ovaries of one-day old females, as well as a higher number of ovarioles. Thelytokous females laid on average more eggs per host larva, although rates of parasitism did not differ between both groups. This means that thelytokous females lay more eggs per time unit, but do not spread their eggs over more lar-
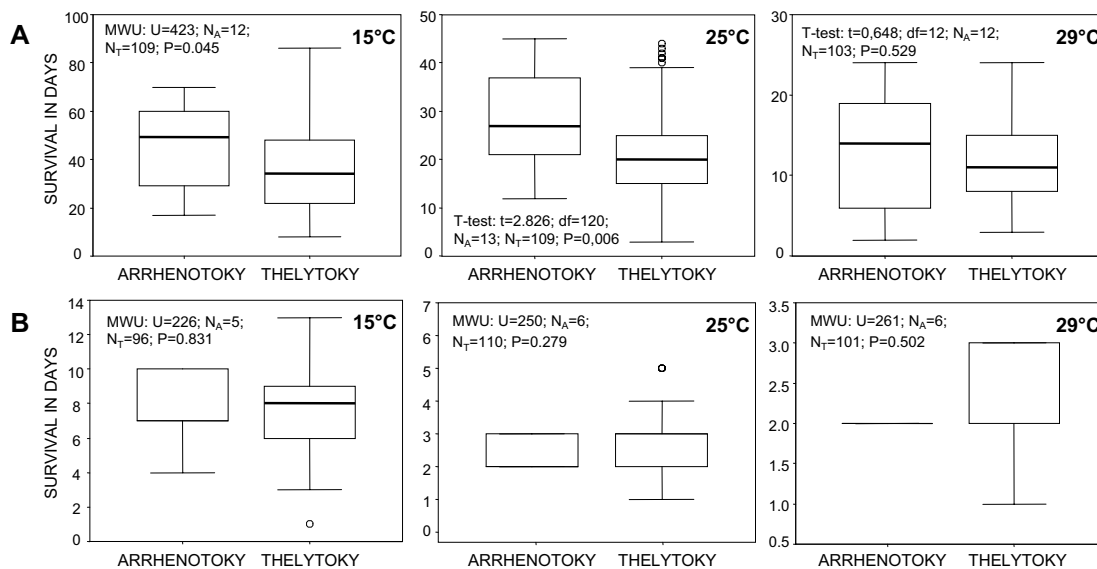

Figure 6. Survival in days (A) with and (B) without food at three different temperatures for arrhenotokous and thelytokous wasps. Note the different scales along the y-axes. See legend of figure 2 for box plot details.

vae. Since always only a single offspring emerges from a superparasitized host (see below) the total number of offspring produced over life did not differ between both reproductive modes. On the other hand, arrhenotokous wasps lived longer than thelytokous ones, at least when kept with food (honey). Schneider *et al.* (2001) found similar lifespans under the same conditions, but did not find any differences in longevity between both reproductive modes. The reason that we did not find a difference in longevity in the 'without food' treatment may be partly due to the low sample size of the arrhenotokous group, but also due to lack of discriminating power. If wasps live only for a few days, the intervals at which they were checked should have been shorter, for example twice or four times per day instead of once a day.

Our data are consistent with information that is available about differences in life history between both reproductive modes. Thelytokous females are generally found in rich environments (*e.g.* bakeries) with high host density (references in Schneider *et al.* 2002). Sexuals on the other hand appear more adapted to field situations with fewer hosts at larger travel distances (Driessen *et al.* 1995). This is consistent with the fact that sexuals appear to invest relatively more in fat than in eggs (Schneider 2003). It can also explain why arrhenotokous females lived longer than thelytokous ones in our experiments. *V. canescens* is a solitary parasitoid which means that always only a single offspring emerges from a host. Is has been shown that multiple larvae per host result in cannibalism whereby the youngest instar typically kills the older instars because it has mandibles that are absent in later instars (Marris *et al.* 1996). The higher rate of superparasitism by thelytokous females observed in our experiments may be due to a reduced tendency of such females to migrate in search of new hosts. Obviously, laying on average 2.5 eggs per host larva in only 50% of available hosts is not a very adaptive behaviour. However, this rate of self-superparasitism may well be induced by artificial experimental conditions used.

In conclusion, we have shown that thelytokous females are twice as fecund as sexual females. Given the cost of males and assuming an equal sex ratio, this would put them at a fourfold advantage over the sexuals. Some of this competitive lead is however counterbalanced by the lower survival rate of thelytokous females. However, one should also bear in mind that conditions of direct competition between both reproductive modes may be limited because they partly occupy different niches.

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