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Mating Modifies Female Life History in a Haplodiploid Spider Mite

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ABSTRACT: Mating usually modifies females' resource allocation pattern, often as a result of conflicts between male and female partners. Can such a switch occur even in the absence of sexual conflicts? We addressed this issue in the haplodiploid spider mite *Tetranychus urticae*, whose biology and population structure considerably reduce conflicts between males and females over reproductive decisions. Comparing virgin and mated females, we tested the hypothesis that mated females modify their allocation pattern so as to maximize their probability of producing daughters. Mated females produced fewer but larger eggs, resulting in an overall similar reproductive effort but an increased probability of producing daughters, since in this species larger eggs are more likely to be fertilized and thus to become female. Moreover, mated females concentrated their reproduction early in life. Again, this might be a way to produce more daughters, since sperm is more abundant early in life. For virgins, spreading reproductive investment might be a way to save resources to extend life span, thus increasing their probability of encountering a sexual partner. Females with multiple opportunities for mating produced fewer eggs and a less female-biased sex ratio than once-mated females, raising the question of why multiple mating often occurs in this species.

Keywords: cost of mating, reproductive effort, offspring size, life history, haplodiploid species, *Tetranychus urticae*.

Introduction

As a consequence of the limited pool of energy available, organisms have to make choices in allocating their resources toward different fitness components (Stearns 1992). In this context, reproductive decisions are of key importance. Investing in reproduction or in maintenance,

reproducing now or later, how often to mate, making many small or few large offspring, and investing more resources in daughters or in sons are crucial decisions that can greatly affect reproductive success (Stearns 1992). In a number of species, mating has been shown to considerably alter the pattern of resource allocation to these traits, especially in females. Mated females often have a shorter life span than virgins, and in many insects females are less sexually receptive after mating (e.g., Eberhard 1996; Chapman et al. 1998; Arnqvist and Rowe 2005). In some insects, such changes in the female's reproductive behavior have been shown to be mediated by sperm or by compounds present in the male seminal accessory fluids (Chapman et al. 1995; Chapman 2001; Arnqvist and Rowe 2005).

The change in allocation strategy associated with mating is often interpreted as a result of sexual conflicts (Arnqvist and Rowe 2005). This hypothesis relies on the fact that the optimal pattern of resource allocation generally differs between males and females. For instance, in polygamous species, the interests of males and females diverge concerning the frequency of mating (Arnqvist and Rowe 2005). For males, mating with several females provides obvious benefits, since more mating events are expected to result in more offspring and to reduce the mating frequency of male rivals. For females, who are generally limited by the number of offspring that they can produce rather than by mating opportunities, multiple mating can nevertheless also be advantageous by increasing offspring genetic diversity (Jennions and Petrie 2000) or by allowing females to profit from cryptic sperm selection (Eberhard 1996; Simmons 2001; Whittingham and Dunn 2010). However, mating comes at some costs, because of time and energy expenditure, increased predation risk (including "sexual cannibalism"; Welke and Schneider 2010), and

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physical injuries caused by the male genital organs or potential toxic effects from accessory substances transferred with the male ejaculate (Chapman et al. 1995; Price et al. 1999; Arnqvist and Nilsson 2000; Davies et al. 2005). Therefore, the benefits of multiple mating for females are often less straightforward than those for males (Birkhead and Møller 1998; Snook 2001; Griffith and Immler 2009), so that the optimal mating rate is usually lower for females (Arnqvist and Nilsson 2000). Another important point of divergence between the interests of males and females in polygamous species concerns the quantity of resources allocated to reproduction. Males are selected to increase their mates' immediate reproductive rate because offspring produced later in life are likely to be sired by other males. For females, however, high investment in egg production at one point in life is likely to have negative pleiotropic effects, such as reduced life span (see Roff 1992; Stearns 1992). Hence, the optimal rate of investment by females in their current reproduction is generally lower from the female's perspective than from that of their mates. In haplodiploid species, an additional conflict opposes male and female partners. In such species, males arise from unfertilized eggs, whereas females arise from fertilized eggs. Hence, males pass genes only through daughters, whereas females pass genes through both daughters and sons, leading to a possible divergence of interests over the offspring sex ratio (Arnqvist and Rowe 2005; Kraaijeveld 2009).

The interpretation that any change in the life-history pattern of mated females is a consequence of sexual conflicts might be abusive. Indeed, a switch in mated females' behavior or physiology could also occur because the optimal allocation strategy for females differs according to mating status. For instance, in diploid species, the fact that the rate of egg production increases only once mating has occurred could be viewed as a strategy used by females to avoid production of sterile eggs, which may be energetically costly (Chapman et al. 1998). Hence, studying the consequences of mating for females in the absence of conflicts may shed light on the extent to which optima of mated and virgin females differ, irrespective of the male strategy, and may aid in testing adaptive hypotheses over females' behavior depending on their mating status. The haplodiploid spider mite *Tetranychus urticae* is an ideal model species for such an investigation, for three main reasons. First, in this species, females store sperm in a spermatheca and only the first insemination seems to be effective, unless copulation is interrupted (Potter and Wrensch 1978; Satoh and Takafuji 2001). Hence, both males' and females' interest should be that females invest all resources in the first mating event, which eliminates conflicts over mating frequency. Surprisingly, however, remating is frequently observed in this species (Oku 2010; personal observations of all the authors). Second, this spe-

cies adjusts sex ratio in response to local mate competition (LMC; Wrensch and Young 1978; Young et al. 1986; Roeder 1992; Roeder et al. 1996; Sato and Saito 2007; Macke et al. 2011*b*). Under weak LMC (several foundress females per patch), females are selected to produce an even sex ratio, whereas the optimal sex ratio from the point of view of males is highly female biased, since their genes are transmitted only through daughters. However, under strong LMC (a single foundress female per patch), the optimal sex ratio is highly female biased for both males and females. Hence, under LMC, sexual conflicts over sex ratio vanish. Third, females are likely to become sperm depleted over time, because only the sperm from the first male is supposed to be used by females and this sperm has limited survivorship. This hypothesis is supported by the fact that the proportion of sons increases with female age (Hamilton et al. 1986; Krainacker and Carey 1990; Roy et al. 2003). Hence, both males and females are expected to benefit from a higher reproductive investment early in life, when sperm is still abundant, thus minimizing sexual conflicts over reproductive investment. Spider mites present the additional advantage of being haplodiploid, so that virgin females can reproduce by laying unfertilized eggs that become male offspring. This allows one to investigate the consequences of mating for the overall resource allocation pattern, by comparing virgin and mated females not only in terms of longevity but also in terms of fecundity and egg provisioning. Indeed, survivorship represents only one part of fitness, and lifetime reproductive output might be enhanced, despite a reduction of longevity, if mating increases offspring size or number (Kemp and Rutkowski 2004). Moreover, it allows one to study an important aspect of reproductive investment that females have to deal with: the trade-off between offspring size and offspring number. There is ample knowledge of mating strategies and their consequences for the parents' fitness as well as of reproductive allocation into offspring size or number (Stearns 1992). Surprisingly, attempts to associate the two, by addressing the consequences of mating strategies for allocation of resources to offspring size or number, have been very timid or incomplete (but see Hahn et al. 2008; Sprenger et al. 2008).

In this study, we provide a complete analysis of how mating modifies the resource allocation strategy of females in *T. urticae*, by comparing the life histories of females with different mating statuses (virgin, once-mated, and exposed to multiple mating opportunities) in an LMC context, thus minimizing sexual conflicts. On the basis of the elements cited above, in particular the assumptions that both males and females are trying to maximize the number of daughters (because of LMC) and that only the first mating event leads to fertilization, precise predictions can be made about the strategy that females should adopt.

First, virgins should delay the onset of reproduction and/or spread their reproductive effort over time in order to increase the likelihood of finding a mate and producing daughters. Conversely, because sperm has a limited life span, mated females should start laying eggs as soon as they are mated and should allocate most resources to reproduction early in life, before becoming sperm depleted. Thus, we predict that virgins should mature at a later age, have a longer reproductive life span, and have a lower reproductive effort (or at least a delayed reproductive effort) than mated females.

Second, since in this species larger eggs are more likely to be fertilized (Macke et al. 2011a), mated females should produce larger eggs than virgins throughout their lifetime, in order to produce female-biased sex ratios. Because of sperm mortality, egg size in mated females should increase through time, in order to keep a high probability of producing daughters.

Third, such an increase in egg size might be costly, in terms of daily fecundity, unless mated females have more resources than virgins, for instance, via nutrients contained in sperm. We thus predict that, compared to virgins, mated females either have more resources and pay no cost of increasing egg size or have a similar amount of resources and a reduced daily fecundity.

Fourth, if, as suggested by earlier studies, only the first mating event is effective while remating occurs frequently, remating might be costless, or even beneficial, for males and/or females. Thus, we predict that multiply mated females should live at least as long as once-mated females, that they should produce at least as many eggs, and that these eggs should be as large as or larger than those of once-mated females.

Fifth, if indeed only the sperm from the first male can be used, being multiply mated should not affect the offspring sex ratio, even in old females. Both types of females should become sperm depleted over time, thus producing a more male-biased sex ratio toward the end of their lives.

Material and Methods

To test the five predictions above, we compared age at first oviposition, size at sexual maturity (used here as a proxy for the quantity of resources that females have), life span, reproductive life span, fecundity, offspring size, total reproductive investment, and sex ratio of virgin females, once-mated females, and females with multiple opportunities for mating.

Spider Mite Populations and Rearing Conditions

The spider mites used in this study were reared in large numbers (>5,000) on cucumber plants (var. Ventura; Rijk

Zwaan, Aramon, France), under controlled conditions (25°C, photoperiod of 16L : 8D). They were originally collected from a cucumber greenhouse in Pijnacker, the Netherlands, in May 1994 and kept on the same variety in a climate chamber at the University of Amsterdam. In April 2004, a first population was established at the University of Montpellier from approximately 10,000 individuals sampled from the Amsterdam culture. This first population became extinct in September 2007 because of a predatory mite attack; hence, a second population was established from approximately 5,000 individuals sampled from the same Amsterdam stock. Both 2004 and 2007 Montpellier populations were used in the experiments.

Consequences of Mating for Females' Resource Allocation Strategy

To avoid any confounding factor, we compared females that had very similar life histories, except for their mating status. To this purpose, we first produced a pool of females that grew in a common environment and had the same age. Two hundred adult females were haphazardly sampled from the 2004 population and placed together on cucumber leaf fragments maintained on wet cotton under uncontrolled room temperatures (about 21°C) and a photoperiod of 16L : 8D (fig. 1). They were allowed to lay eggs for 24 hours, and these eggs ($n = 400$) were then individually placed on 1-cm² leaf fragments to complete their development. When larvae reached the quiescent-deutonymph stage, visual discrimination between males and females became possible. The sexes were then separated, and males to be used in the experiment were prepared by being isolated from females to facilitate sperm replenishment. Among the remaining females, quiescent deutonymphs ($n = 133$) were sampled and placed on leaf fragments sufficiently large to ensure that they had ad lib. food. To start the experiment, those females were randomly assigned to one of three treatments (fig. 1). In the first treatment, “virgin females” ($n = 43$), each female was left alone on its leaf fragment, remaining virgin throughout its lifetime. In the second treatment, hereafter “once-mated females” ($n = 44$), each deutonymph female was exposed to two males until the beginning of the egg-laying period, to ensure mating (usually, 1 day of exposure), and then left alone until death, with no further exposure to males. In the third treatment, each deutonymph female ($n = 46$) was exposed to two males until the beginning of the egg-laying period and then exposed to two new virgin males for 4-h periods twice a week until death. Although we have no direct evidence that females exposed to males more than once actually mated several times, our own observations in other experiments suggest that females frequently remate. Hence, this third treatment is

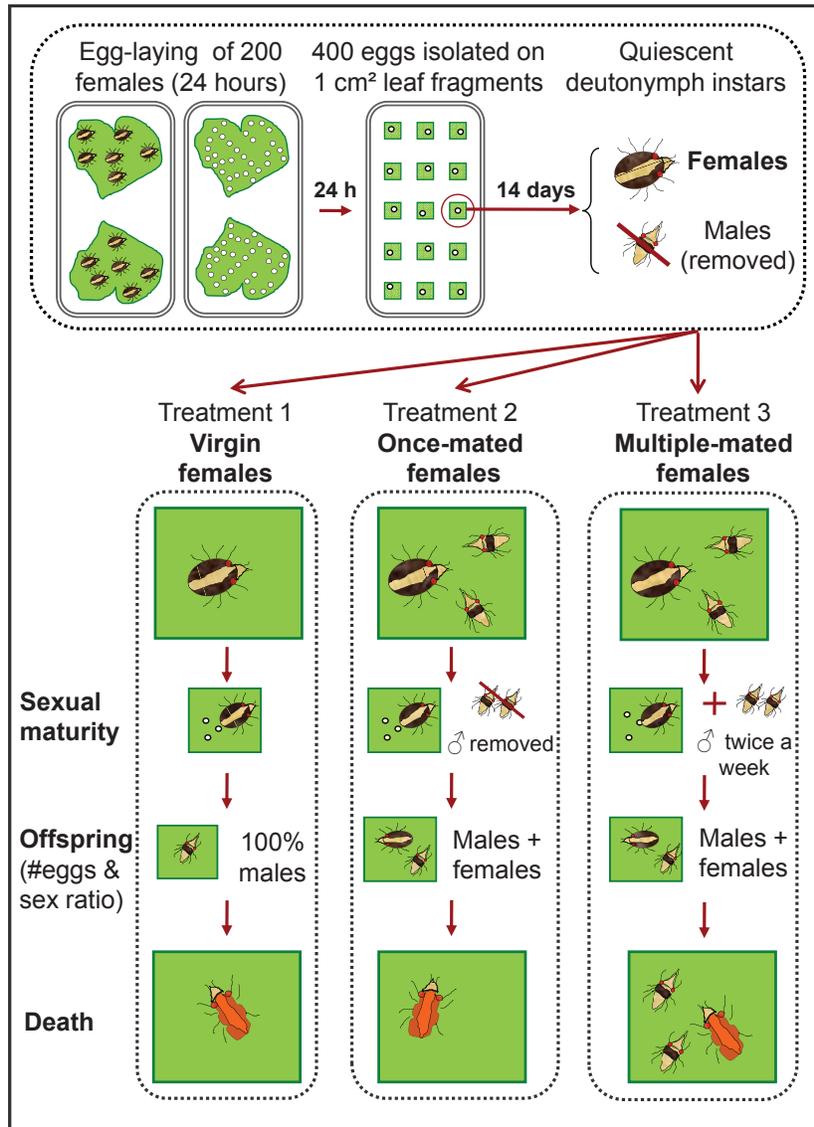


Figure 1: Protocol used in replicate 1 and part of replicate 2 of the experiment.

hereafter called “multiply mated females.” Treatments 2 and 3 thus differed only in the procedure applied after the beginning of the egg-laying period. Note that even in the once- and multiply mated treatments, females were alone on the leaf most of the time.

Female survival was checked every day. To ensure sufficient resources, leaf fragments were changed twice a week, on which occasion eggs were counted (there were thus two “clutches” per week). A total of 10,630 eggs were counted. Every time a leaf was changed, the old leaves of treatments 2 and 3 were kept to determine the progeny

sex ratio 2 weeks later, when eggs had reached adulthood. As sex could be determined only in survivors, the sex ratios shown here correspond to tertiary (adult) sex ratios (West 2009). In this experiment, 79% of individuals survived from egg to adulthood. Each female was thus characterized for the following fitness components: (1) age at production of the first egg, (2) life span, and in particular reproductive life span (i.e., time between first and last egg produced), (3) total fecundity, (4) daily fecundity, and (5) daily offspring sex ratio (defined here as the proportion of males; fig. 1). The mean daily fecundity was calculated by dividing

the total fecundity by the reproductive life span. The overall (tertiary) sex ratio was equal to the number of sons divided by the total number of daughters and sons.

To increase statistical power and to have a more precise estimation of females' reproductive investment, we repeated this experiment and included the measurement of two additional traits: (1) female size at sexual maturity (used here as a proxy for the quantity of resources that females have at the beginning of their reproductive life) and (2) egg size at different times in the female's reproductive schedule. For this replicate, we used spider mites from the 2007 population, and we performed the experiment in a growth chamber at 25°C, under continuous light.

As in the first replicate, three treatments were created: (1) virgin females ($n = 48$), (2) once-mated females ($n = 48$), and (3) multiply mated females ($n = 44$). When all females had reached sexual maturity, they were measured. For this purpose, females were individually placed on a glass slide under a binocular microscope (lens $2\times, \times 40$) and photographed with the AVT SmartView software. The outline of the body was traced manually with OPTIMAS 6.5 software, which then automatically calculated the area. After being photographed, females were individually replaced on their leaf fragments and surveyed as in replicate 1. In addition, 1–4 eggs per female were measured (on average 3.08 ± 0.95 [SE] eggs/female/clutch) every time a leaf was changed (i.e., approximately every 4 days), with the same procedure as was used for females. The eggs were then placed back on their leaves, and their sex was determined later. The pictures were analyzed with OPTIMAS 6.5, which automatically calculates the projected area covered by each egg. From this area (A), we calculated the egg volume (V), assuming a spherical shape; hence, $V = (4\pi/3)(A/\pi)^{3/2}$. In this way, we obtained the average size of eggs produced at different times in the females' life, which provided information about possible changes in egg size with female age. Of 11,382 eggs laid in this experiment, a total of 1,425 eggs were measured ($n = 526, 466, \text{ and } 433$ for virgin, once-mated, and multiply mated females, respectively).

The total reproductive investment was defined as the total volume of eggs produced by a female during her whole life span and was calculated as follows. First, for each clutch (here, one clutch corresponds to eggs laid between two leaf changes), we calculated the reproductive investment of females by multiplying the number of eggs they produced by the average size of those eggs (calculated from the measure of 1–4 eggs per female per clutch). This provided the daily reproductive investment. Then, values obtained for all clutches were added to obtain the total reproductive investment. The mean daily reproductive investment (while fertile) was obtained by dividing the total

investment by the reproductive life span. The average size of eggs produced by females throughout their life span was calculated by dividing the total reproductive investment (i.e., the total volume of eggs) by the total fecundity.

Statistical Analysis

We compared females from the different treatments for (1) age at sexual maturity, (2) life span and reproductive life span, (3) fecundity, (4) egg size, (5) total reproductive investment, (6) the relationship between egg size and egg number, and (7) sex ratio. Furthermore, to determine whether the pattern of resource allocation varied with time differently among treatments (e.g., whether mated females increased their reproductive investment early in life), we examined how fecundity, egg size, and reproductive investment varied through time in the different types of females. We also compared how sex ratio varied through time in once-mated and multiply mated females.

Statistical analyses were performed with the SAS 9.2 software. All mating treatments were analyzed together, but the results of the comparisons between once-mated and virgin females and between once-mated and multiply mated females are presented separately in "Results."

Age at sexual maturity, total fecundity, mean daily fecundity, and overall sex ratio were analyzed with generalized linear models, with mating treatment and replicate as fixed factors (using the GLIMMIX procedure). Body size, mean egg size, and overall and mean daily reproductive investment were studied in experiment 2 only, and were thus analyzed without replicate effect. We added time since sexual maturity as a covariate in the analysis of body size, because at the time of measurement some females had been mature for longer than others, which might affect their size. We specified a Poisson probability distribution for fecundity, a binomial one for sex ratio (number of males over number of offspring), and a normal one for the other variables. For age and size at sexual maturity, there were only two levels of mating treatment to consider (virgin females and mated females), since all mated females had had a single opportunity for mating at the time of first egg laying. There were also two levels of mating treatment to consider for sex ratio (once-mated and multiply mated females). For fecundity, egg size, and reproductive investment, there were three levels (virgins, once-mated, and multiply mated females), so we performed pairwise comparisons between mating treatments whenever relevant (CONTRAST statement). We obtained the least squares means estimates of all traits per mating treatment, as well as their 95% confidence limits.

Reproductive life span was analyzed with a Cox proportional-hazards model regression (PHREG procedure), with mating treatment and replicate as fixed factors. In-

dividuals that died by accident—for example, because of experimental manipulation—were coded as censored. We performed relevant pairwise comparisons between mating treatments by using the CONTRAST statement. All survival curves were drawn with the LIFETEST procedure, which also provides summary statistics of survival time (mean and standard error).

We analyzed changes over time in sex ratio, daily fecundity, daily reproductive investment, and egg size and compared these changes between mating treatments. To this purpose, we performed regressions with mating treatment and replicate (when appropriate) as fixed factors, time since sexual maturity as a random covariate, and individual as a random blocking factor because of the repeated measures through time on individual females. For the analysis of fecundity, reproductive investment, and sex ratio, second-order polynomial regressions were performed, because in spider mites these traits are expected to vary nonmonotonously through time (i.e., fewer eggs and more males at the beginning and at the end of the reproductive period; Krainacker and Carey 1990; Roy et al. 2003; A. Janssen, personal communication), because inspection of data confirmed this expected trend and because it provided the best Akaike Information Criterion values. Because second-order terms are correlated to first-order ones (e.g., see Wold et al. 1984), we minimized the effect of collinearity by first performing linear regressions of time^2 over time, and we used the residual of these regressions as a new, orthogonal variable for time^2 in the regressions of daily fecundity, daily reproductive investment, and sex ratio over time. For sex ratio, the model did not converge when we specified a binomial distribution of the response variable, and the arcsine–square root transformation did not improve normality, so we performed the analysis on nontransformed data, specifying a normal distribution. Similarly, fecundity was analyzed after log transformation. A linear regression was performed for egg size. Because previous results showed that egg size affects the probability of fertilization (Toyoshima 2010; Macke et al. 2011a) and because there may be physiological constraints on maximum egg size, we also investigated changes in minimum and maximum egg size separately. To this purpose, we noted the minimum and the maximum egg size per mother for each clutch. We then performed a linear regression of the minimum and maximum egg sizes as a function of time, with mating treatment as a fixed factor and individual as a random blocking factor.

Using data from experiment 2, we examined whether there was a trade-off between egg size and egg number by performing a linear regression of the overall fecundity on average egg size, with mating treatment as a fixed factor. Upon finding a significant interaction between mating

treatment and egg size, we repeated the same analysis per treatment, adding female size as a covariate.

Since all treatments were studied in both experiments, we analyzed the data as a single data set. However, only relevant pairwise comparisons were performed when the overall model indicated a significant effect of the mating treatment or of its interaction with another factor.

Results

Table 1 gives the results of the overall statistical analyses, with all treatments and both replicates. Interaction terms were included whenever relevant. Below, we give only the results of the contrast analyses aimed at testing specific hypotheses, that is, when comparing either virgins with once-mated females or once-mated with multiply mated females. Table 2 gives the least squares mean estimates for fitness traits and their 95% confidence intervals.

Do Virgin Females Delay the Onset of Reproduction and Have a Longer Reproductive Life Span and a Lower Reproductive Effort than Mated Females?

Contrary to our prediction, virgin and mated females started to reproduce at the same age (about 15 days; tables 1, 2), but, as predicted, virgins had an extended life span compared to once-mated females (fig. 2; tables 1, 2; $\chi_1^2 = 12.25$, $P = .0047$). This resulted in an extended reproductive life span in virgins: on average, they lived and reproduced for 5.8 more days (23.4% longer) than once-mated females (tables 1, 2; $\chi_1^2 = 8.51$, $P = .0035$). Taking into account egg size in experiment 2 (see below), we found that the total reproductive investment was similar for virgins

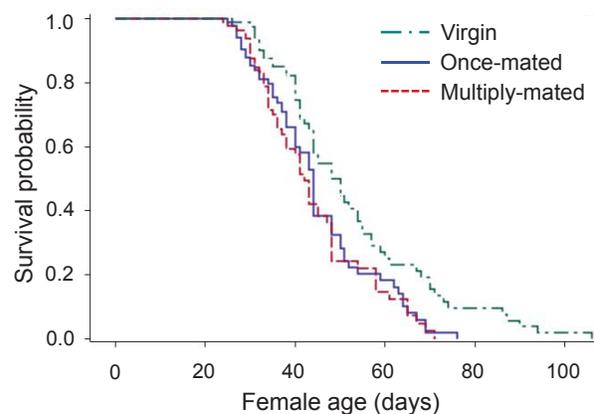


Figure 2: Survivorship of virgin, once-mated, and multiply mated females. The graph shows the probability of survival as a function of age.

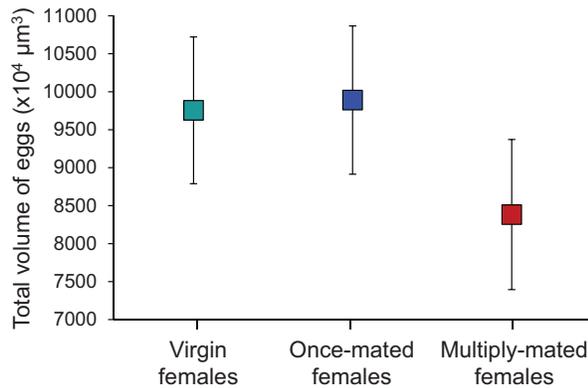


Figure 3: Total reproductive investment of virgin, once-mated, and multiply mated females. Symbols correspond to least squares means. Error bars correspond to 95% confidence limits.

and once-mated females (fig. 3; table 1; $F_{1,126} = 0.04$, $P = .85$). Hence, once-mated females invested the same amount of resources in reproduction as virgins but over a shorter period of time, since their reproductive life span was shorter. The mean daily reproductive investment during the reproductive life (egg size \times egg number per day of reproductive life span) in mated females was thus significantly (14%) larger ($F_{1,126} = 3.23$, $P = .043$) than that in virgins (tables 1, 2). Hence, although mated females invested a similar amount of resources into reproduction overall, they had a larger daily reproductive effort than virgins, as predicted. Such an increased reproductive effort early in life was probably responsible, through a physiological trade-off, for their shorter life span.

Do Mated Females Produce Larger Eggs than Virgins, and Do They Increase Egg Size through Time?

As predicted, once-mated females produced larger eggs than virgins (fig. 4; table 1; $F_{1,129} = 17.84$, $P < .0001$). Moreover, egg size gradually increased over time in once-mated females, whereas it remained constant in virgins (fig. 5A; table 1; coefficient in the regression of egg size over time: 0 in virgins and 0.32 in mated females; $t_{120} = 2.27$, $P = .025$). Further analyses revealed that the increase in egg size in mated females concerned mostly the smallest eggs of the distribution (fig. 5B, 5C; table 1).

Is the Increase in Egg Size Costly, and Do Mated and Virgin Females Have Similar Amounts of Resources?

At sexual maturity, mated females were significantly larger than virgins (tables 1, 2). Since mated and virgin females

were in the same condition (similar amount of food available to each), the larger size of mated females can have come only from the fact that they were mated (and possibly had modified their feeding behavior as a result of mating).

However, even though they were initially larger (and thus potentially had more resources), once-mated females overall produced about 14% fewer eggs than virgins (fig. 6A; table 1; $F_{1,266} = 81.64$, $P < .0001$). Such a lower total fecundity in once-mated females, compared to virgins, mostly resulted from their shorter reproductive life span, since mean daily fecundity (total fecundity divided by reproductive life span) did not differ among mating treatments (tables 1, 2). However, there was a significant interaction between mating treatment and time for daily fecundity (fig. 6B, 6C; table 1). Inspection of figures 6B and 6C suggests that the decline of fecundity with age was steeper for once-mated females than for virgins, and indeed the coefficients of time and of time² in the regression of log (daily fecundity) over time were significantly smaller for once-mated females than for virgins ($P = .0066$ and $.0327$ for the coefficients of time and time², respectively, of mated females). This is consistent with the increasing size of eggs through time in once-mated females but not in virgins. Indeed, the daily reproductive investment through time was similar between virgins and mated females ($t_{129} = 1.13$, $P = .26$). Thus, it is likely that there is a trade-off between egg size and egg number, which the larger size of mated females did not allow them to overcome. To test this, we performed a regression analysis of total fecundity on mean egg size. We found that overall, egg size and egg number were negatively correlated (fig. 7; table 1). However, there was a significant interaction

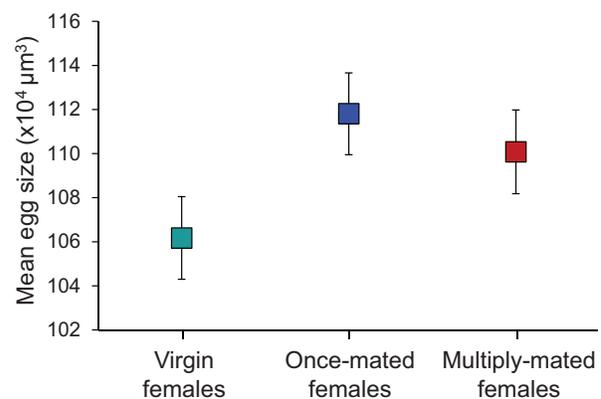


Figure 4: Mean egg size of virgin, once-mated, and multiply mated females. Symbols correspond to least squares means. Error bars correspond to 95% confidence limits.

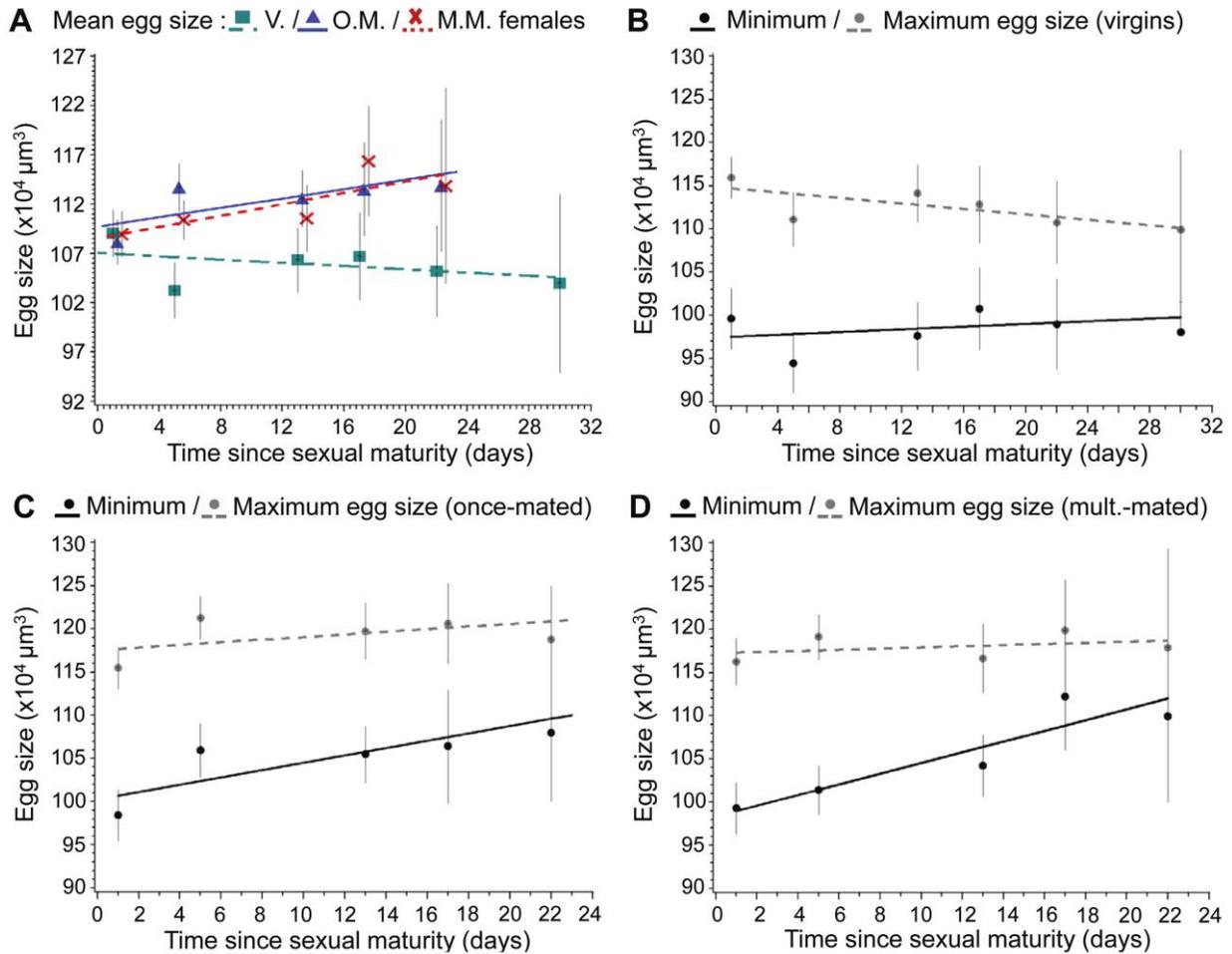


Figure 5: A, Mixed linear regression of mean egg size as a function of time since sexual maturity in virgin (V), once-mated (O.M.), and multiply mated (M.M.) females. B–D, Mixed linear regression of minimal and maximal egg volume as a function of time since sexual maturity in virgin (B), once-mated (C), and multiply mated (D) females. Lines correspond to mean predicted values; individual symbols correspond to observed mean values per female.

between egg size and mating treatment (table 1), suggesting that the slope of the trade-off was contingent on the mating status of females. Indeed, figure 7 reveals that the negative relation between egg size and egg number was more pronounced in virgin females than in mated females. When the analysis was performed separately for each treatment, the negative relationship was still significant in both treatments ($F_{1,41} = 67.85$, $P < .0001$ and $F_{1,41} = 6.08$, $P = .018$ for virgin and once-mated females, respectively). Similar results were obtained when female size was taken into account in the analysis ($F_{1,40} = 80.91$, $P < .0001$ and $F_{1,40} = 6.13$, $P = .018$ for virgin and once-mated females, respectively; table 2).

Is Remating Beneficial?

As predicted, multiply mated and once-mated females had similar life spans, including reproductive life span (fig. 2; tables 1, 2; $\chi_1^2 = 0.052$, $P = .82$ and $\chi_1^2 = 0.0075$, $P = .93$ for life span and reproductive life span, respectively), and there was no difference in egg size between the two types of females (fig. 4; table 1; $F_{1,129} = 1.66$, $P = .20$). However, multiply mated females produced significantly fewer eggs than once-mated females (fig. 6A; table 1; $F_{1,266} = 11.80$, $P = .0007$). Overall, the total reproductive investment was smaller in multiply mated females than in once-mated females (fig. 3; table 1; $F_{1,126} = 4.61$, $P = .034$). Thus, remating actually seems to be costly.

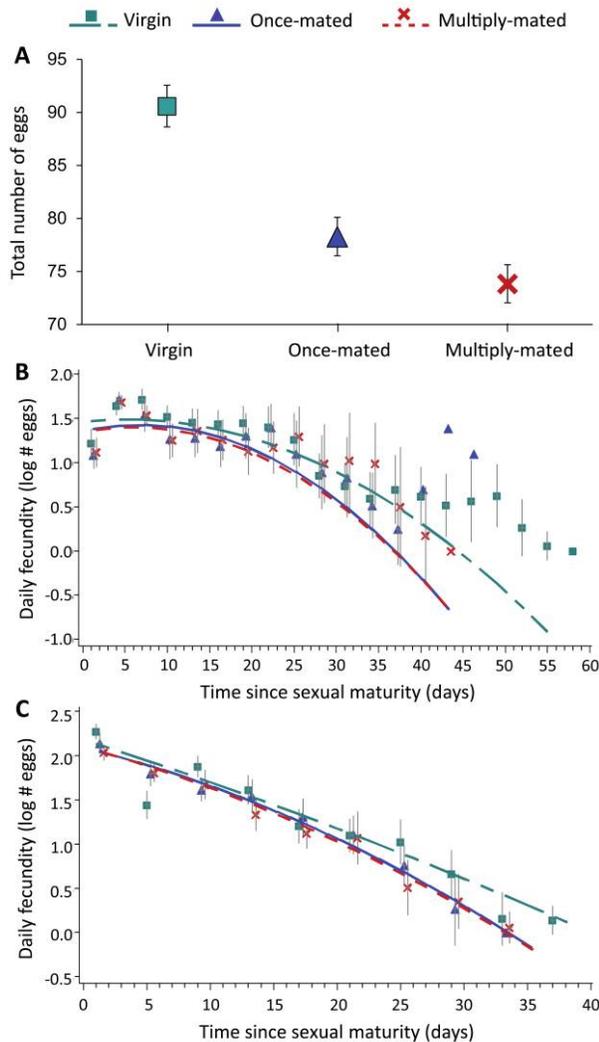


Figure 6: A, Total fecundity (total number of eggs) of virgin, once-mated, and multiply mated females in experiments 1 and 2. Symbols correspond to observed mean values. Error bars correspond to 95% confidence limits. B, C, Mixed linear regression of daily fecundity (number of eggs per day and per female) as a function of time since sexual maturity for virgin, once-mated, and multiply mated females, in replicates 1 (B) and 2 (C). Lines correspond to mean predicted values; individual symbols correspond to observed individual data.

Is Sex Ratio Increasingly and Similarly Male Biased for Once- and Multiply Mated Females?

Overall, the sex ratios obtained in our experiments fell within the range of values usually observed in spider mites (25%–35% males; Carey and Bradley 1982; Helle and Sabelis 1985; Vala et al. 2003). When investigating changes in sex ratio over time, we found that for both once-mated and multiply mated females, the proportion of males de-

creased during the first days after sexual maturity and then gradually increased until the end of the reproductive life span (fig. 8; table 1). In the first experiment, where females lived longer lives, the sex ratio of multiply mated females was slightly more female biased during the second part of the life than that of once-mated females (fig. 8A). In the second experiment, this difference was not observed. Instead, and unexpectedly, multiply mated females produced a significantly higher proportion of male offspring (resulting from unfertilized eggs) than once-mated females throughout their life (fig. 8B; table 1). Overall, the sex ratio (i.e., proportion of males) of multiply mated females was higher than that of once-mated females (fig. 8C; table 1).

Discussion

Mating Modifies the Pattern of Resource Allocation in a Way That Increases the Chances of Producing Daughters

Our study suggests that, in spider mites, mating modifies the female's reproductive strategy, independently of sexual conflicts. Indeed, we found that in a context of LMC, which minimizes conflicts between sexual partners, the pattern of resource allocation differed between virgin females and females that had been mated once. The two types of females invested a similar amount of resources in reproduction. However, the way in which those resources were allocated was very different.

The main difference concerned the sharing of resources between eggs. Mated females produced fewer but larger eggs than did virgins. Indeed, in contrast to previous studies with spider mites (Wrensch and Young 1975; Bonato and Gutierrez 1999), we found that the total fecundity of mated females was lower than that of virgins. Had we not

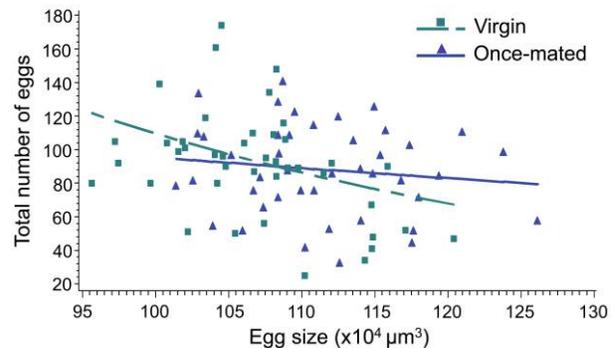


Figure 7: Trade-off between egg size and total fecundity for virgin and once-mated females. Lines correspond to mean predicted values; individual symbols correspond to observed individual data.

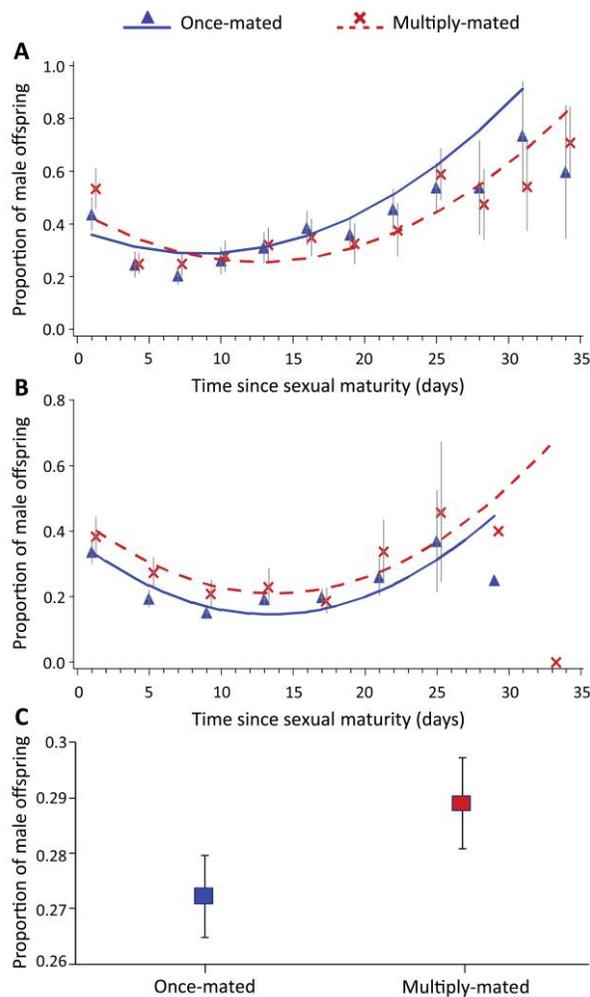


Figure 8: A, B, Mixed polynomial regression of sex ratio (proportion of male offspring) as a function of time since sexual maturity in once-mated and multiply mated females, for replicates 1 (A) and 2 (B). Lines correspond to mean predicted values; individual symbols correspond to observed individual data. C, Total sex ratio (proportion of male offspring) for once-mated and multiply mated females. Symbols correspond to least squares means. Error bars correspond to 95% confidence limits.

measured egg size, we would have erroneously concluded that once-mated females paid a cost in terms of reproductive output. However, we found that the reduced fecundity in mated females was compensated for by an increase in egg size. Assuming that egg size properly reflects egg provisioning, this means that mated females allocated more resources to each of their eggs than did virgins. These results constitute one of the first findings that mating affects egg provisioning. The other study tackling this issue was performed in the diploid fly *Sarcophaga crassipalpis*,

but it did not reveal any difference in egg size at oogenesis between mated and virgin females (Hahn et al. 2008).

The increase in egg provisioning did not take place immediately after mating. Rather, it occurred gradually. Contrary to previous studies, which have shown that mothers generally reduce egg provisioning as they age (Blanco et al. 2003; Giron and Casas 2003), we found that in spider mites, mated females increased the size of their eggs over time, in particular the size of their smallest eggs, possibly because of morphological and/or physiological constraints on their ability to produce very large eggs (Fox and Czesak 2000). By contrast, virgin females kept their egg size constant through time. These results are consistent with those found by Toyoshima (2010) in *Tetranychus urticae*.

Taken together, these findings suggest two things. First, a trade-off governs the allocation of resources into offspring size and number, and mated and virgin females are at different positions on the trade-off curve. The fact that the slope of the trade-off was steeper in virgins than in mated females could suggest that the trade-off is not linear, although this would not be consistent with the fact that the overall egg volume was not significantly different between virgins and once-mated females (fig. 9). Second, mated females seem to adjust their allocation strategy in order to increase their production of daughters, as expected under LMC and as we predicted. Indeed, in spider mites, the quantity of sperm stored in the spermatheca of mated females decreases over time (Helle and Sabelis 1985). Since larger eggs are more likely to be fertilized (Macke et al. 2011a), increasing egg size over time might be a way to increase the probability that those eggs will encounter sperm and thus to maintain as high a ratio of females to males as possible, which benefits both male and female partners. It could also be that a large egg size is more beneficial to daughters than to sons, for example, if males need fewer resources to develop and reproduce because they are smaller and rarely disperse (Helle and Sabelis 1985). In this case, increasing egg size would be advantageous in mated females but not in virgins. However, under this hypothesis, it is unclear why mated females increased mostly the minimum egg size, that is, the size of those eggs initially most likely to develop into males. Another possibility to account for changes in egg size through time might be that females have evolved in an environment that deteriorates as females age. The main dispersing stage in spider mites is young females; hence, old females probably typically occur in more depleted patches. Under such circumstances, older females are expected to invest in egg size instead of egg number (Allen et al. 2008). However, under this hypothesis, we would expect to find a similar pattern in the progeny of virgin females, which was not the case. Hence, overall, the hypothesis that the increase in egg size is a way (under either

Table 1: Results of the overall statistical analyses (all treatments and replicates analyzed together) for each trait

Fitness component, factor	Where illustrated	df	<i>F</i> (or χ^2)	Pr > <i>F</i> (or Pr > χ^2) ^a
Age at sexual maturity	Table 2			
Mating treatment		1, 270	.14	.71
Replicate		1, 270	1,174.63	<.0001
Life span:	Figure 2			
Mating treatment		2	15.63	.0004
Replicate		1	29.50	<.0001
Reproductive life span (RLS)	Table 2			
Mating treatment		2	10.79	.0045
Replicate		1	.35	.55
Total reproductive investment	Figure 3			
<i>Mating treatment</i>		2, 126	2.82	.0635
Mean daily investment (total investment/RLS)	Table 2			
Mating treatment		2, 129	5.58	.0047
Mean egg size	Figure 4			
Mating treatment		2, 129	9.33	.0002
Mean egg size through time	Figure 5			
Time		1, 120	5.80	.00176
Mating treatment		2, 129	1.45	.2375
Time × mating treatment		2, 120	4.18	.0177
Minimum egg size through time				
Time		1, 120	26.15	<.0001
Mating treatment		2, 128	1.00	.37
Time × mating treatment		2, 120	5.22	.0067
Maximum egg size through time	Figure 5			
Time		1, 120	.10	.75
Mating treatment		2, 128	1.41	.25
<i>Time × mating treatment</i>		2, 120	2.48	.088
Size at maturity	Table 2			
Mating treatment		1, 132	4.65	.0328
Age at maturity		1, 132	13.52	.0003
Total fecundity	Figure 6A			
Mating treatment		2, 266	83.78	<.0001
Replicate		1, 266	.76	.384
Replicate × mating treatment		2, 266	14.17	<.0001
Mean daily fecundity (total fecundity/RLS)	Table 2			
Mating treatment		2, 267	.58	.56
Replicate		1, 267	13.04	.0004
Daily fecundity through time	Figure 6B, 6C			
Mating treatment		2, 266	.46	.63
Replicate		1, 266	117.68	<.0001
Time		1, 260	479.61	<.0001
Time² (orthogonal)		1, 255	53.41	<.0001
Time × replicate		1, 260	33.23	<.0001
Time ² × replicate		1, 255	9.73	.0020
Time × mating treatment		2, 260	5.93	.0030
Time² × mating treatment		2, 255	3.07	.0480
Daily investment through time:				
Mating treatment		2, 129	5.58	.0047
Time² (orthogonal)		1, 476	34.24	<.0001
Time		1, 131	559.37	<.0001
Trade-off between egg size and egg number	Figure 7			
Egg size		1, 122	15.15	.0002
Mating treatment		2, 122	28.49	<.0001
Egg size × mating treatment		2, 122	26.22	<.0001

Table 1 (Continued)

Fitness component, factor	Where illustrated	df	<i>F</i> (or χ^2)	Pr > <i>F</i> (or Pr > χ^2) ^a
Sex ratio through time	Figure 8A, 8B			
Replicate		1, 175	.13	.72
Mating status		1, 175	6.02	.0015
Time		1, 170	74.80	<.0001
Time × time (orthogonal)		1, 166	117.84	<.0001
Time × mating status × replicate		3, 170	6.17	.0005
Overall sex ratio	Figure 8C			
Mating treatment		1, 178	9.10	.003
Replicate		1, 178	71.64	<.0001

Note: For each factor, the table indicates the degrees of freedom (df), the *F* statistic (or χ^2 for the analysis of RLS), and the *P* value. Results for contrasts are given in the text. Boldface indicates significant effects ($P < .05$) of relevant independent factors, and italics indicate marginal significance ($P < .10$). Significant random factors or their interactions with other factors are not highlighted.

^a Probability of exceeding *F* or χ^2 .

female or male control or both) to increase the fertilization rate is the most likely explanation. We also found that the sex ratio was nonmonotonously related to female age, being more male biased toward the end the life. Hence, increasing egg size to maximize fertilization probability seems to be efficient in young but not old females, possibly because sperm depletion is too limiting in old females.

The second important difference in the pattern of resource allocation between virgin and once-mated females is that virgins tended to spread their reproductive effort over a longer period of time. This might be a way for virgins to increase the probability of finding a mate and for mated females to maximize daughter production, by reproducing while live sperm is still abundant. Similarly, Toyoshima (2010) found that virgins of *T. urticae* lived longer than mated females. Alternatively, virgin females could have delayed the onset of their oviposition period, but we found that this was not the case. Similarly, Hahn et al. (2008) found no delay in oogenesis in virgin females of *S. crassipalpis*. The longer reproductive life span of virgins could also simply result from their lower mean daily reproductive investment.

Mating thus modified the females' reproductive strategy but not their total reproductive investment, because once-

mated females produced fewer but larger eggs than virgins and did so during a shorter period of time. Such changes in the pattern of resource allocation might benefit both male and female partners, since it is likely to maximize the number of daughters produced, and they could be under either female or male control. Interestingly, we found that mated females were larger than virgins. This could result from acquisition of a higher amount of resources by mated females, through either increased feeding or nutrients provided by sperm. This larger amount of resources might allow mated females to increase their egg size through time and thus to maximize the fertilization probability of those eggs. This hypothesis is reinforced by the positive and significant relationship we found between female size and average egg size (data not shown). It might also be that sperm contain substances that stimulate vitellogenesis, as found in some insects (Arnqvist and Rowe 2005).

Multiple Exposures to Males Entail a Cost for Females

In most organisms, including predatory mites, females benefit from multiple mating in terms of increased life span or offspring production (Arnqvist and Nilsson 2000;

Table 2: Summary of results for traits not shown in figures

Fitness trait	Virgin	Once-mated	Multiply mated
Age at sexual maturity (days)	15.3 (15.0–15.5) ^A	15.3 (15.2–15.5) ^A	...
Size at sexual maturity (μm^2)	89,919 (88,345–91,492) ^A	92,060 (90,885–93,235) ^B	...
Life span (days) (see also fig. 2)	52.85 (SE = 2.25) ^A	45.17 (SE = 1.62) ^B	44.18 (SE = 1.56) ^B
Reproductive life span (RLS, days)	30.32 (SE = 1.44) ^A	24.57 (SE = 1.09) ^B	24.61 (SE = 1.08) ^B
Mean daily reproductive investment ($10^{-4} \mu\text{m}^3$) ^a	442.58 (404.33–480.83) ^B	504.75 (466.06–543.45) ^A	445.40 (406.25–484.55) ^B
Mean daily fecundity (egg number/RLS)	3.96 (3.57–4.40) ^A	3.90 (3.52–4.33) ^A	3.66 (3.29–4.09) ^A

Note: For each trait, values correspond to the least squares means, with 95% confidence interval in parentheses. Within a line, different letters indicate significant differences between means at the 5% level.

^a Egg size × egg number per day of RLS.

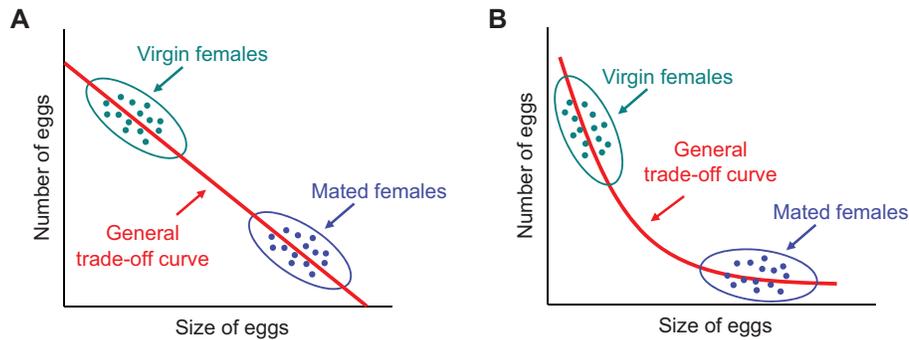


Figure 9: Two possible representations of the trade-off that governs allocation into egg size and egg number. Whatever the shape of the trade-off, mated and virgin females will be at different positions on the trade-off curve: mated females produce few large eggs, whereas virgin females produce many small eggs. However, the shape of the global trade-off curve will determine the intensity of the trade-off in each mating treatment. If the global trade-off is linear (A), a strong negative relation between egg size and egg number should be observed in both mated and virgin females. However, if the global trade-off is concave (B), the negative relation between egg size and egg number should be more intense, and thus more easily detectable, in virgins than in mated females.

Ji et al. 2007; Gotoh and Tsuchiya 2008), although a few studies have found that polyandry reduces fecundity (e.g., Jennions et al. 2007; Sakurai and Kasuya 2008; Oku 2010; Ronkainen et al. 2010). In spider mites, such benefits would explain why multiple mating occurs frequently, although only the first mating contributes to the fertilization of eggs. However, our study did not reveal any benefit of multiple mating. Instead, we found that multiply mated females had a life span similar to that of once-mated females but a lower reproductive output. Indeed, multiply mated females produced eggs as large as those of once-mated females, but their fecundity was reduced, which corroborates the findings of Oku (2010). Such a decrease in fecundity could arise either from a cost of copulation itself or from a cost of sexual harassment, as found in the adzuki bean beetle (Sakurai and Kasuya 2008). It might also come from competition for resources with males (very unlikely, given that mites were fed ad lib.) or from the fact that they spent more time copulating and less time laying eggs. As a result, the total and daily reproductive investments of multiply mated females were less than those of once-mated females.

In addition to reducing fecundity, remating did not allow females to fertilize a larger proportion of their eggs. Indeed, overall we found that multiply mated females produced a smaller proportion of daughters than did once-mated females. The fact that in the first experiment multiply mated females produced a larger proportion of daughters during the second part of their life, compared to once-mated females, could indicate that sperm from subsequent males could be used, contrary to our initial hypothesis and to what is generally admitted in this species

(Potter and Wrensch 1978). Alternatively, since these females produced a less female-biased sex ratio during the first part of their life than did once-mated females, it could be that a larger quantity of sperm from the first male was available later. Therefore, assuming that producing a highly female-biased offspring sex ratio is optimum, neither males nor females seem to benefit from female remating in our study species. As expected, in *T. urticae*, males appear to be able to recognize the mating status of females and noticeably prefer virgin females (Oku 2010). However, in the absence of virgin females, Oku (2010) showed that a majority of males copulated with mated females. Hence, remating is likely to have occurred in our experiment. Why males and females remate in natural or experimental conditions is still unclear. We see five possible (nonexclusive) explanations:

1. “*Lack of discrimination*” hypothesis. Helle (1967) found that when the intervals between the first and second copulation events were shorter than 24 h or when the first copulation was disturbed, the second copulation was sometimes effective (see also Potter and Wrensch 1978; Satoh and Takafuji 2001). Possibly, *T. urticae* males may not be able to discriminate between females that have recently copulated and those that had copulated earlier, which may motivate the male copulation behavior (Oku 2010).

2. “*Increased relative fitness*” hypothesis. When few virgins are available, males could either invest in survival and wait for more virgins to become available or try to increase their relative fitness by decreasing the reproductive success of other males. Preventing other males from transmitting their genes is one way of achieving that. For example, the

second male could displace the sperm of the first male or transfer products that kill it (as suggested by Chapman et al. 1995), such that fewer eggs would be fertilized. In this way, males could benefit from remating by reducing the frequency of eggs fertilized by competitors and hence increasing their own genetic representation in future generations. Such strategy has been proposed by Harshman and Prout (1994) for *Drosophila*, although recent data run counter to this hypothesis (Manier et al. 2010). Our finding that in *T. urticae* females with multiple mating opportunities produced fewer female offspring than females mated once is just the opposite of what would be expected if females were able to use the sperm of several males to avoid sperm depletion. On the contrary, this result is in line with the increased-relative-fitness hypothesis. If something in the seminal fluids reduces the survival of sperm of the previous male, mating with mated females might be an efficient way to decrease the number of offspring of the first male. Furthermore, decreasing the fertility of females fertilized by another male is also advantageous for the second male. Consistent with this hypothesis, we found that females with several mating opportunities produced fewer eggs.

3. “Correlation” hypothesis. It could well be that males that tend to remate are (genetically or simply phenotypically) different from males that do not tend to copulate with already-mated females. For instance, such “remating males” might be more aggressive in general and more likely to win contests for access to virgin females. Such positive correlations between the tendency to copulate in general and the ability to “win” virgins would lead to the indirect selection of a remating behavior, even though such behavior might appear useless and even costly.

4. “Manipulation by female” hypothesis. Remating might be to the benefit of females rather than to that of males, for example, if females can benefit from nutrients in seminal fluids. In this case, however, we would expect an increase in either longevity or fecundity in remated females. Furthermore, assuming that the optimal sex ratio for females is less female biased than it is for males, remating could allow mated females to produce more male offspring when LMC does not occur, turning the male-male competition to their own advantage. However, this hypothesis contradicts the hypothesis that females increase egg size in order to maintain a constant sex ratio.

5. “Cost of avoidance” hypothesis. Assuming that remating actually benefits males, females might accept remating simply because the cost of avoiding it would be larger than its benefit.

Is It Better to Remain Virgin?

In our study, we found that virgin females had a longer life span and a higher fecundity (although with smaller

eggs) than mated ones. Hence, virginity might be advantageous for females in this species. Yet in either natural or laboratory populations of populations of *T. urticae*, virgin (or sperm-depleted) females are rare (about 5% in our population; H. D.-T. Khanh and E. Macke, unpublished data). Two hypotheses could be formulated to explain this apparent paradox. First, in *T. urticae*, females are guarded by males during their quiescent-deutonymph stage and fertilized as soon as they emerge, which gives them few opportunities to remain virgin. Moreover, Godfray (1990, 1994) predicted that the benefits of virginity, and thus the motivation of constrained (i.e., virgin or sperm-depleted) females to engage in mate finding, should depend on the mating structure of the species. Whereas virginity might be advantageous for females and thus spread in panmictic species with balanced sex ratios, it is expected to be more detrimental in nonrandomly mating species, such as *T. urticae*, in which local mate competition (LMC) is likely to occur (Helle and Sabelis 1985). Hence, under LMC, higher selective pressure to engage in mate finding has been predicted (Godfray 1990, 1994). Consistent with this, Steiner and Ruther (2009) found that females of the parasitoid wasp *Nasonia vitripennis*, in which LMC is likely to occur, were highly motivated to invest in mate finding. In our study, although we found that virgins did not delay the onset of oviposition compared to mated females, we found that they had an extended reproductive life span, which might be a way to increase their probability of finding mates before their egg complement has been depleted.

Conclusion

In light of this study, mating, or exposure to males, appears to considerably affect the pattern of resource allocation in females. In particular, it reduces female fecundity and induces an increase in egg size over time. We recently showed that in *Tetranychus urticae*, larger eggs were more likely to be fertilized (Macke et al. 2011a). Hence, the increase in egg size found in the present study might be a way to increase fertilization probability. Our results raise fascinating evolutionary questions about sexual conflicts. For instance, does mating induce the same changes in female reproductive strategy in a situation where sexual conflicts are more intense—for example, in the absence of LMC? Are those reproductive strategies under male or female control? Interesting questions also arise about the reasons why individuals continue to mate multiply, although doing so seems to be useless, or even detrimental, for both males and females. The first step to answer such questions will be to investigate the genetic determinism of reproductive allocation. It will also be necessary to determine how mating, in particular multiple mating, actually affects overall individual fitness.

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