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Parker, K.; Roessingh, P.; Menken, S.B.J.

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Do Female Life Span and Fecundity of Small Ermine Moth Increase with Multiple Mating and Adult Nutrition?

Katherine Parker · Peter Roessingh ·
Steph B. J. Menken

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Abstract Several evolutionary explanations for multiple mating in insects have been proposed, and it has been suggested that Lepidoptera benefit from the energy contained in nuptial gifts. In two closely related species of small ermine moths, *Yponomeuta cagnagellus* (Hübner) and *Yponomeuta padellus* (L.), we experimentally controlled the number of matings and access to honey as an energy source, and assessed the effects of these factors on female life span and lifetime fecundity. No positive effects of mating were found in either species. In fact, mating significantly shortened life span. The effect of access to energy in the form of honey differed between the two species. In the shorter-lived *Yponomeuta padellus*, access to honey did not increase the life span of mated females and did not increase fecundity. In *Yponomeuta cagnagellus* however, access to energy in the form of honey counteracted the negative effects of mating and females with access to honey lived significantly longer and laid significantly more eggs during their lifetime.

Keywords Nuptial gift · longevity · Lepidoptera · Yponomeutidae · life history

Introduction

Multiple mating of females over a single reproductive cycle is commonly observed in a wide range of animal taxa and is almost ubiquitous in insects (Birkhead and Møller 1998), but until now no consensus about the underlying mechanisms of this behaviour has emerged (Jennions and Petrie 2000; Zeh and Zeh 2003; Hosken and Stockley 2003; Simmons 2005; Parker 2006). Benefits as well as costs of multiple mating have been identified (Hosken et al. 2009), and as a consequence selective forces controlling mating behaviour can be antagonistic. This is particularly the case in the insects,

K. Parker · P. Roessingh (✉) · S. B. J. Menken
Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, Science Park 904,
Amsterdam 1098 XH, The Netherlands
e-mail: roessingh@uva.nl

in which substantial nuptial gifts are common (Gwynne 2008), and in which multiple mating is often associated with fitness advantages for the female. Nutrients acquired via mating can provide direct benefits to females, and meta-analyses of studies on polyandrous species have demonstrated that fecundity and longevity are positively correlated with number of matings (Cook and Wedell 1996; Arnqvist and Nilsson 2000; Gwynne 2008). In addition to such direct nutritional benefits, males of several insect species transfer substantial amounts of secondary plant compounds during mating, and these compounds can be deployed to protect the receiving female as well as her progeny against natural enemies (Eisner et al. 2002). In addition to the direct fitness consequences accruing from the acquisition of extra sperm, food, or defensive chemicals, there are also genetic benefits associated with multiple mating. These include a reduction in the risk of producing unfit progeny with an unsuitable partner as well as an increase in genetic variability (Simmons 2005; Cornell and Tregenza 2007; Garcia-Gonzalez 2008).

It is also clear that multiple mating poses substantial risks to females. A frequently observed effect of mating is the reduction of a female's life span. This might simply be a direct consequence of the physiological changes related to egg-maturation (Chapman et al. 1998; Rogina et al. 2007; Partridge et al. 2005). However, evidence has been found that ejaculates may contain chemical compounds (Gillott 2003; Colonello and Hartfelder 2005; Wedell 2005) that males use to actively influence and manipulate female reproductive physiology in order to maximize their own reproductive fitness.

The outcome of cost-benefit analyses of multiple mating can be further affected by the breeding strategy of a species. In general, females can be either “capital breeders”, using resources that were acquired earlier in life, or “income breeders”, which also invest newly acquired resources into the production of their progeny (Drent and Daan 1980). Such differences in life history need to be taken into account when assessing the potential benefits to the female from nuptial gifts and explaining the evolution of mating behaviour. In a capital breeding organism, with a physiology geared towards the use of previously stored energy reserves, the use of newly acquired nutrients might be more constrained than in an organism which is already adapted to the use of additional resources in adult life.

The wide range of factors that might influence mating behaviour, their many possible interactions, as well as the observation that closely related species often show differential effects of mating on fecundity and receptivity (Blanckenhorn et al. 2002; Martin and Hosken 2004), suggest that comparing the consequences of multiple mating on female fitness is a good approach to improve our understanding of the evolution of mating behaviour. Therefore, in this study, we evaluate the effects of multiple mating (with ejaculates possibly containing male-provided nutrients), and access to an external food source (honey-agar) on fecundity and female life span in two species of lower Ditrysia, viz., the small ermine moths *Yponomeuta padellus* (L.) and *Y. cagnagellus* (Hübner). These species are closely related (Menken et al. 1992; Turner et al. 2010) and polyandrous (Bakker et al. 2008; Bakker et al. 2011), but their mating frequencies, life span, and sexual maturation time differ significantly. *Yponomeuta padellus* becomes sexually mature within a week after eclosion and tends to have a shorter life span than *Y. cagnagellus*, which may take up to 2 weeks to mature and may live for over 2 months (Hendrikse 1979; Bakker et al. 2008). Such life

history differences make these congeners a good choice for comparing the consequences of multiple mating for female fitness. In addition, the study will enhance what we know about multiple mating in the lower Ditrysia, that have often only a limited representation in meta-analyses (e.g., Arnqvist and Nilsson 2000).

We mated females of both species with one or two males, or gave them the opportunity to mate serially with as many different males as they choose. We hypothesized that if females receive material benefits by mating, females that mate multiple times can be expected to show an increase in their fecundity and/or longevity compared to females that mated only once. The same prediction can be made with respect to adult feeding: female moths that are able to use resources gained by additional feeding will be expected to live longer and have a higher lifetime reproductive output than those without access to food.

Materials and Methods

Insect Collection and Rearing

Approximately 40 egg batches of *Y. cagnagellus* (containing hibernating first instars under protective egg shields) were collected in early spring from *Euonymus europaeus* in Overveen, the Netherlands (52°23'57"N, 4°34' 03"E), and approximately 55 egg batches of *Y. padellus* were collected from *Prunus spinosa* in Arnhem, the Netherlands (51°58' 28" N, 5°54' 30"E). Egg batches were difficult to locate and were collected from numerous plants over a half a mile radius and for this reason it is likely they came from different females and give a good representation of the population. All egg batches were stored in a cold room at 4 °C until they were used in the experiments. Rearing started by lifting the protective shield from the egg batch. The larvae were counted and placed in a 10-cm-diameter Petri dish between two newly flushed leaves, picked from greenhouse-grown plants of the species from which they were collected. A small piece of wet cotton wool was placed over the petiole to minimize desiccation. This cotton wool was slightly wetted with demineralized water every other day (excluding weekends). The larvae were fed ad libitum with host leaves until pupation. The Petri dishes were placed in a climate box under constant environmental conditions of 60 % r.h., 21 °C, and L17:D7 photoperiod. Approximately 2 weeks after pupation, insects were weighed on an OHAUS Analytical Standard Scale (d=0.00005) to the nearest 0.1 mg, after which they were placed in an 8×2 cm glass tube capped with a cotton plug and placed back into the climate box. The cotton plug closing the tube was slightly wetted with de-mineralized water to provide the emerging moths with a drinking source. Humidity and light cycle within the climate boxes remained constant, but the temperature was changed to 18 °C during the dark cycle and 21 °C during the light cycle. This new temperature regime more closely resembles the conditions in the field. Since larval development times are equal, and the new regime was started before the moths emerged, it is unlikely that this change had a differential effect in the two species.

Male and female *Yponomeuta* become sexually active during the end of the scotophase and at dawn (Hendrikse 1979). To facilitate our observations of mating, the light cycle was shifted forward by about 8 h. A 40-W light source was switched

on from 13:00 until 14:15 h to simulate pre-dawn conditions, and a 1,500-W light source was used from 14:15 until 21:00 h to simulate daytime conditions. From 21:00 until 13:00 h the climate box was dark.

Experimental Treatments and Measurements

Yponomeuta cagnagellus and *Y. padellus* females matched for size were assigned to two nutritional treatments (honey vs. no honey,) and one of four mating treatments (the control treatment of no-mating, once-mated, twice-mated, and a free-mating treatment, in which females had constant access to a virgin male). We used a complete block design (see Table 1). Females assigned to the “honey” treatment received one cube of honey-agar [approximately 10 mm³, containing 10 % honey (wt/vol) and 1 % agar (wt/vol)] per week throughout their lifetime. All males had access to honey as well. Females that refused to mate received a new virgin partner twice a week until they reached their assigned number of matings, but were discarded if they did not reach their pre-assigned target. In the free-mating treatment, females were completely free to mate as often as they wanted, but mated on average more than two times (Fig. 1). Moths assigned to the free-mating group received a new virgin partner immediately following the removal of the previous partner at the end of a mating. A mating was scored as having occurred when a male and female were observed in copula for more than 1.5 h (Bakker et al. 2008). Mating was checked once per hour between 9:00 and 18:00 h.

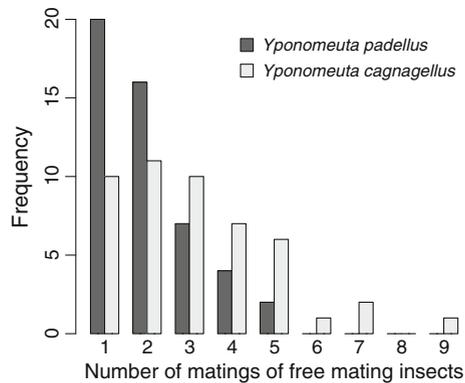
As an oviposition substrate, a 5-cm-long piece of host plant stem was placed in a Petri dish containing a female moth, and it was checked for egg batches 3–5 times per week. *Yponomeuta* egg batches contain on average 50–100 eggs that are difficult to count individually. Therefore egg batches were photographed, and for each batch the total surface area was measured using the software program ImageJ (Rasband 1997). Egg batch surface-area is a good proxy of fecundity: in both species, eggs form a continuous single layer of similar sized eggs, lacking open spaces. Longevity was measured as the number of days between eclosion and death.

All statistical tests (ANOVA and Welch’s Two Sample *t*-test) were carried out using the “R” statistical package (R Development Core Team 2011). A Bartlett test of homogeneity was performed to determine whether the variances in treatment groups were equal. Because this assumption was not met for total egg batch surface area, this data was rank-transformed. Normality of the residuals in the linear models was checked using normal quantile-quantile plots in R.

Table 1 Numbers of *Yponomeuta cagnagellus* and *Yponomeuta padellus* females assigned to the different treatments

	<i>Yponomeuta cagnagellus</i>		<i>Yponomeuta padellus</i>	
	Honey	No honey	Honey	No honey
No mating–control	31	30	30	30
Once mated	23	24	24	25
Twice mated	21	22	18	20
Free mating	26	22	24	25

Fig. 1 Distribution of the number of times females of *Yponomeuta cagnagellus* ($n=48$) and *Yponomeuta padellus* ($n=49$) mated in the free mating treatment



Results

Effects of Mating and Adult Feeding on Female Longevity

The average life span for *Y. cagnagellus* females was 50.2 ± 1.0 days (mean \pm SE), and the average life span of the shorter lived *Y. padellus* moths was 31.0 ± 0.7 days. Contrary to the expectation that mating, and the related spermatophore transfer, would benefit the females, a significant reduction in life span was observed for both species (Fig. 2). A two-way ANOVA was carried out to test for the significance of the effects of mating and access to honey on life span (Table 2). Access to honey allowed *Y. cagnagellus* females to largely offset the reduction in life span caused by mating, while mated *Y. padellus* females did not benefit from honey feeding. This species

Fig. 2 Effect of access to honey and mating [once-mated, twice-mated, or free-mating (labeled 3+)] on longevity for *Yponomeuta cagnagellus* and *Yponomeuta padellus* females

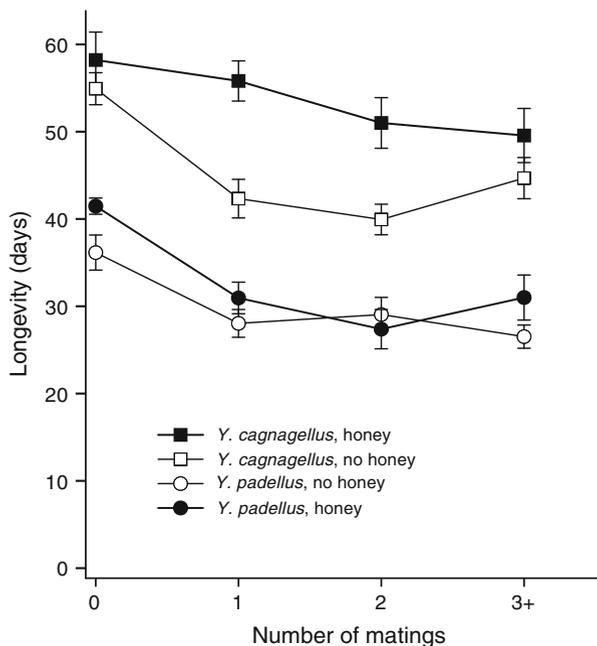


Table 2 Results of ANOVA on longevity of *Yponomeuta cagnagellus* and *Yponomeuta padellus* females as a function of species, number of matings, and access to honey

Treatment	Df	Sum Sq	Mean Sq	F value	P
Species	1	29984	29983.7	284.9	<0.01
Mating	3	6157	2052.3	19.5	<0.01
Honey	1	2319	2319.2	22.03	<0.01
Species*Honey	1	500	500.4	4.75	0.0299
Mating*Honey	3	223	74.4	0.707	0.5485
Residuals	321	33787	105.3		

The significant interaction between species and honey reflects the fact that *Y. cagnagellus* was able to utilise this energy source much more effectively than *Y. padellus*

difference is reflected in the significant interaction between species and honey. No interaction between mating and honey was found. For each species we estimated the size of the effect of mating and access to honey, and included the possible confounding factors insect size (measured as pupal weight) and fecundity (measured as total egg batch surface) as covariates (Table 3). Inspection of the coefficients showed that mating and access to honey had large and significant effects on longevity in both species. The effect of pupal weight was more than an order of magnitude smaller and only significant in *Y. padellus*. Fecundity, which in principle could be viewed as a cost, had an extremely small effect on longevity, and the positive coefficient showed that higher fecundity was associated with a longer life span rather than lowering it.

Table 3 Contribution to longevity from the factors mating group and access to honey for *Yponomeuta cagnagellus* and *Yponomeuta padellus*

Treatment	Coefficient	SE	t value	P	Significance
<i>Yponomeuta cagnagellus</i>					
Mating once	-13.4	3.6	-3.73	<0.001	***
Mating twice	-18.4	3.7	-4.98	<0.001	***
Mating free	-13.9	3.2	-4.34	<0.001	***
Honey	7.2	2.0	3.52	<0.001	***
Pupal weight	0.28	0.21	1.32	0.188	
Fecundity	7.2×10^{-5}	3.3×10^{-5}	2.19	0.029	*
<i>Yponomeuta padellus</i>					
Mating once	-11.2	2.2	-5.06	<0.001	***
Mating twice	-13.3	2.3	-5.69	<0.001	***
Mating free	-11.0	2.2	-5.01	<0.001	***
Honey	3.65	1.5	2.48	0.014	*
Pupal weight	0.44	0.19	2.43	0.017	*
Fecundity	8.4×10^{-5}	5.4×10^{-5}	1.5	0.121	

Pupal weight and fecundity (measured as total egg batch surface) were used as covariates

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

The number of female matings in the free mating groups was not influenced by access to honey in either species (Welch Two Sample *t*-test; *Y. cagnagellus*: $t=0.608$, $df=41.04$, $p=0.54$; *Y. padellus*: $t=0.374$, $df=46.01$, $p=0.71$); however, *Y. cagnagellus* females mated on average 3.04 ± 0.27 times, which is significantly more than the average of 2.02 ± 0.16 times found in *Y. padellus* (Welch Two Sample *t*-test: $t=3.27$, $df=78.8$, $p<0.002$), a result that is in close agreement with the findings of Bakker et al. (2008) and Bakker et al. (2011).

Effects of Mating and Adult Feeding on Lifetime Fecundity

Unmated females of both species produced very few (unfertilized) eggs (Fig. 3) and therefore only mated females were analysed to assess the effects of mating and honey feeding. The ANOVA showed a significant interaction between honey and species, reflecting the fact that *Y. padellus* did not appear to benefit from access to honey, while *Y. cagnagellus* females produced significantly more eggs when they had access to honey. A significant interaction between mating and honey was also found, reflecting the observation that multiple mating did not benefit *Y. padellus*, and only contributed to fecundity in *Y. cagnagellus* if honey was not available (Table 4). When both species were evaluated independently, it became clear that the positive effect of honey on fecundity in *Y. cagnagellus* was not significant (Table 5). However, this analysis also showed that the effect of mating a second time had a positive effect on fecundity, while in the free mating group the coefficient indicated a negative effect. The covariates pupal weight and longevity either had no significant effect on fecundity, or a very small effect size.

Fig. 3 Effect of access to honey and number of matings [once-mated, twice-mated, or free-mating (labeled 3+)] on lifetime fecundity (measured as egg batch surface) for *Yponomeuta cagnagellus* and *Yponomeuta padellus*

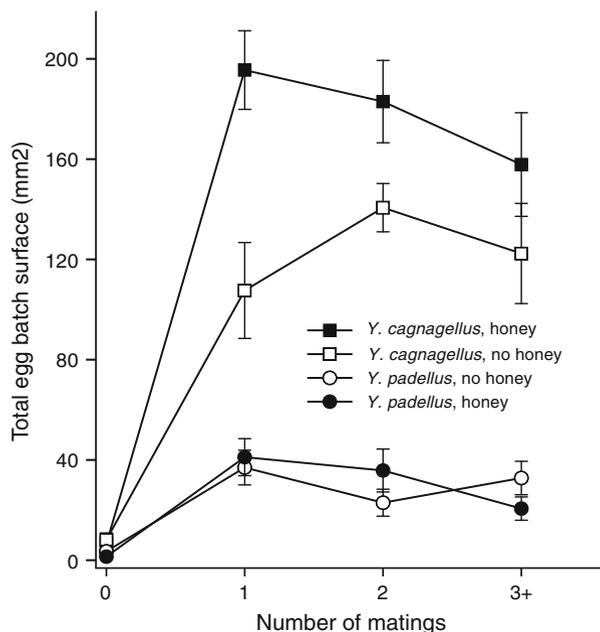


Table 4 Results of ANOVA on lifetime fecundity (measured as total egg batch surface) of mated *Yponomeuta cagnagellus* and *Yponomeuta padellus* females as a function of species, number of matings, and access to honey

Treatment	Df	Sum Sq	Mean Sq	F value	P	Significance
Species	1	819461	819461	270.0	<0.001	***
Mating	2	12163	6081	2.00	0.137	
Honey	1	22153	22153	7.30	0.007	***
Species*Honey	1	19163	19163	6.31	0.012	*
Mating*Honey	2	19984	9992	3.29	0.039	*
Residuals	266	807429	3035			

A significant interaction between species and honey was found. *Yponomeuta padellus* did not appear to be able to profit from either multiple mating or access to honey. *Yponomeuta cagnagellus* on the other hand did only profit from multiple mating when no honey was available, as indicated by a significant Mating*Honey interaction

Discussion

This study addressed two questions: (1) Do adult nutrition and multiple mating affect female longevity and fecundity in two species of *Yponomeuta*, and (2) do these two potential resources interact. Results showed that multiple mating had a significant negative effect on female longevity (unmated females of both species lived significantly longer than mated females). Only females of the longer-lived *Y. cagnagellus* were able to utilize honey to (partly) compensate for the negative effects of mating on longevity. Fecundity showed a more complex response. In *Y. padellus*, neither mating nor honey increased egg batch surface. In *Y. cagnagellus* however, females with access to honey produced more eggs than “no honey” females. In addition, this

Table 5 Contribution to fecundity (measured as total egg batch surface) from the factors mating group and access to honey for mated *Yponomeuta cagnagellus* and *Yponomeuta padellus* females

Treatment	Coefficient	SE	t value	P	Significance
<i>Yponomeuta cagnagellus</i>					
Mating twice	15.3	8.6	1.77	0.078	
Mating free	-1.22	8.2	-0.15	0.881	
Honey	23.2	7.6	3.07	0.003	**
Pupal weight	0.28	0.05	0.15	0.875	
Longevity	7.9×10^{-3}	3.3×10^{-5}	3.42	<0.001	***
<i>Yponomeuta padellus</i>					
Mating twice	-13.4	8.4	-1.60	0.112	
Mating free	-14.2	7.7	-1.84	0.067	
Honey	2.57	6.6	0.39	0.700	
Pupal weight	1.60	0.8	1.97	0.051	
Longevity	0.18	0.7	0.68	0.496	

Pupal weight and longevity were used as covariates

species appeared to benefit from multiple mating, but only under the somewhat artificial low energy conditions of the “no honey” treatment. Independent evaluation of *Y. cagnagellus* showed that only females that mated a second time tended to increase fecundity. In the free mating group that on average mated some three times, fecundity was again lower. It should be noted however that due to the difficulties of constructing a large enough group that mated three or more times, females in this group were really free to mate as often as they wanted, and some individuals mated only once. It cannot fully be excluded that this explains the lack of significance in the honey treatment of *Y. cagnagellus*. The lack of a clear benefit from multiple mating that we observed is inconsistent with previous studies on Macrolepidoptera, which provided good examples of positive effects of adult nutrition and multiple mating on female longevity and reproductive output. Lederhouse et al. (1990), for instance, found that *Papilio glaucus* males supplement their adult diets with nectar, sodium, and other electrolytes and then transfer this extra source of nutrition to females during mating. By doing so, they increase female reproductive fitness as well as their own. Arnqvist and Nilsson (2000) and Gwynne (2008) concluded that in insects, multiple mating may increase female fitness by stimulating egg production, and also by providing accessory substances that contribute to female survival and/or reproduction. We found no real support for the occurrence of such fitness benefits in *Yponomeuta*, as mating had a negative effect on female longevity, and reproductive output after multiple mating did not show a significant increase. Comparable with the situation in *Yponomeuta*, McNamara et al. (2008) showed that in the almond moth, *Cadra cautella*, extra mating opportunities did not increase fecundity and significantly reduced life span. Such negative effects of mating are commonly observed. In many insect species, males have evolved genitalia with barbs and spines, some inject toxins during copulation, and others exhibit male aggression which physically damages female genitalia in an attempt to dissuade females from re-mating with other males (Johnstone and Keller 2000). Damages to the female genitalia can lead to increased female mortality, and females may act to reduce the extent of this damage by shaking off the male (Crudgington and Siva-Jothy 2000; Blanckenhorn et al. 2002). Since mated *Yponomeuta* females of both species showed a decreased longevity compared to virgin females, it is clear that there were costs associated with mating. Further experiments on the genitalia of males and females would shed more light on this phenomenon and may help to explain the decrease in female longevity.

In addition to a crude mechanical damage strategy, more sophisticated male manipulation has also been documented. In *Drosophila melanogaster*, males transfer accessory substances to females in their ejaculate; such ejaculates increase female fecundity, but decrease female longevity, and this confronts females with a trade-off between number of matings and longevity. For this reason, small-sized nutritionally stressed *D. melanogaster* females cannot compensate for their size by increasing mating frequency (Chapman et al. 1995). We expected that unfed *Yponomeuta* females would attempt to compensate for their lack of adult nutrition by increasing their mating frequency in the free-mating groups. However, contrary to our expectations the “no honey” females did not mate more often than the “honey” females and females that mated once lived just as long as females that mated up to nine times. It is possible that this was due to negative effects of mating imposed by the males. The fact that there was no further increase in the negative effects of mating

after mating once (and some *Y. cagnagellus* males mated up to nine times) could be interpreted as evidence against male manipulation, and as support for the hypothesis that in *Yponomeuta* mating simply speeds up development (Chapman et al. 1998; Partridge et al. 2005; Rogina et al. 2007). It can however not be excluded that male manipulation is not dose dependent, or caused by indirect effects like male suppression of the female immune system which would make them more prone to viral infection after physical damage, and in this way decreases their longevity (Rolff and Siva-Jothy 2002).

Yponomeuta cagnagellus females can be classified as ‘income breeders’ (Drent and Daan 1980), as they take advantage of nutrients during their adult life. *Yponomeuta padellus* females, on the other hand, were unable to utilize these nutrients to the extent *Y. cagnagellus* did: “honey” females produced similar numbers of eggs as “no- honey” females, and both treatment groups lived on average about 30 days. *Yponomeuta padellus* therefore appears to be more of a ‘capital breeder’, which does not utilize nutrients other than those acquired during the larval stages for the production of their eggs. It should be noted however that it is possible that in nature this species uses nutrient sources that were not available during our experiment.

The fact that honey feeding could offset the negative effects of mating in *Y. cagnagellus* but not in *Y. padellus* might mean that mated females start to convert all their body mass to eggs, and under low nutrient conditions this shortens life span. However, as a relative long-lived species, *Y. cagnagellus* has evolved physiological mechanisms that enable it to use carbohydrates from its environment more efficiently than *Y. padellus*.

To gain a deeper insight into the evolution of mating behaviour in *Yponomeuta*, more research should be carried out on the contents of the spermatophore. Both *Y. cagnagellus* and *Y. padellus* males transfer a substantial proportion of their body weight to the females during mating (up to 15 %; Bakker et al., unpublished results). Although our experiments demonstrated no positive effects of multiple mating in *Y. padellus*, and only a hint of influence in *Y. cagnagellus*, it is possible that the large volume of ejaculate transferred during copulation by *Yponomeuta* males contains secondary plant compounds (Eisner et al. 2002). Such substances might confer fitness effects that will not be detected in the type of experiment we conducted.

In summary, we have shown that in *Y. cagnagellus* extra nutrition in the form of honey led to an increase in fecundity and longevity, whereas multiple mating under low nutrient conditions only showed a trend in increased fecundity in this species. In *Y. padellus*, neither feeding nor multiple mating had a significant positive effect. Since unfed and mated females showed a decreased longevity compared to virgin females, it is clear that there are costs associated with mating, but the present result do not allow us to determine the exact origin of this effect. Future investigations should therefore look at damage to female genitalia and willingness of females to copulate with males. Research along these lines may shed more light on the mechanisms that govern the evolution of multiple mating in Lepidoptera.

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