Effects of multiple mating and adult nutrition on longevity and fecundity in two Yponomeuta species
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Many male Lepidoptera transfer a large ejaculate, containing nutrients and/or secondary plant compounds. In principle this ‘nuptial gift’ could help to increase female survival and longevity. Here we investigated the effects of both multiple mating and extra nutrition in the adult stage on males and females in two species of *Yponomeuta*. Mated females had significantly shorter live spans than unmated females and similar fecundity. Females therefore do not appear to benefit from remating, which suggests that the considerable weight transfer of the males to the females during mating does not provide nutritional value. Females of *Y. cagnagellus*, which received honey throughout their adult lifetime, lived significantly longer and laid larger egg batches than females, that did not receive honey. In *Y. padellus* this effect was not found. It is concluded that neither species benefits from multiple mating and that in *Yponomeuta cagnagellus* adult nutrition rather then mating increases female reproductive output and longevity, which contrasts with many studies of Macrolepidoptera.

**Keywords:** nuptial gift, honey, reproductive output, Macrolepidoptera

Female butterflies and moths require significant amounts of energy for pheromone production, calling, courtship, and oviposition. Many empirical studies in Macrolepidoptera have found that, in addition to adult nutrition, multiple mating can have a positive effect on female longevity and fecundity. A single mating can provide a female with enough sperm to fertilize all her eggs (Lederhouse 1981). Remating is therefore not needed for fertilisation. However, males can transfer an ejaculate to the female that contains a ‘nuptial gift’, accessory substances, which provide nourishment to the female, increasing her fecundity (Cook & Wedell 1996).

In addition to direct nutritional effects that increase fecundity, nuptial gifts might also increase female lifespan. This benefits the female, presuming that the longer a female lives the more opportunities she has to produce offspring and find suitable partners that can provide a genetic benefit to her offspring in the form of...

Most studies look at the reproductive behavior of butterflies, few have focused on moths. An exception is the study by De Jong (1988) who found in small ermine moths that females who do not receive honey before mating had stunted growth, small egg number, produce less attractive pheromones, and yet were more successful in mating repeatedly. We therefore designed an experiment in Yponomeuta moths with the following questions in mind:

(1) How do multiple mating and adult nutrition affect female longevity, mating frequency, and egg production in two closely related species of Yponomeuta?

(2) Can nutritionally stressed females increase their longevity and reproductive output by mating more frequently?

Both species feed on their own specific host-plant: Yponomueta padellus can be found feeding on plants from various species of Rosaceae including Prunus spinosa while Yponomeuta cagnagellus exclusively feeds on Euonymus europaeus belonging to the Celastraceae (Menken 1992). In late spring females deposit up to 150 eggs in clusters near a bud or side branch of their food plant and eggs hatch three weeks later (Povel 1984).

We tested the potential benefits of nuptial gifts on fecundity and longevity by mating females with one or two males or by providing continuous access to males. In addition, the effect of honey was investigated. We quantified the total number of matings, the insect’s total lifespan, and the total surface of all egg batches laid over a female’s complete lifetime.

**MATERIAL AND METHODS**

**Mating and feeding treatments**

A total of 389 Y. cagnagellus and 396 Y. padellus males and females were assigned to unmated (‘virgin’), once-mated, twice-mated, and unlimited mating groups. Half of the insects were fed honey throughout their lifetime and half were not.

Both mating and courtship were recorded once per hour between 9:00 and 18:00 hours. A mating was recorded when the male and female remained in copula for more than 1.5 hours. Females that did not want to mate were supplied with a new virgin male twice per week.

**Egg production analysis & longevity**

Most studies examining the importance of male investment for female reproduction assume that the mass of the male ejaculate is positively correlated with female output. Spermatophore size is however not the best measurement of male investment, since it is the content and not the size of the spermatophore that determines its true value (Marshall 1989, Delisle & Bouchard 1995). For this reason we measured lifetime female fecundity rather than spermatophore size. A host-plant stem was placed with every female in a Petri-dish and was checked
3-5 times per week for egg batches. Batches were photographed, and the egg batch surface was measured using the program ‘ImageJ’ (Rasband 1997). Longevity was measured from the moment a moth enclosed till its death for all moths in both experiments.

RESULTS

Adult Nutrition

The lifespan for *Y. cagnagellus* was approximately one month and for *Y. padellus* it was approximately two months. *Yponomeuta cagnagellus* females did utilize honey to increase their lifespan (Fig. 1; ANOVA: df=1, F=15.3, p<0.001). Honey did not increase the lifespan of the shorter lived *Y. padellus* (Fig. 2; ANOVA: df=1, F=1.82, p=0.18).

*Yponomeuta cagnagellus* females with access to honey laid significantly more egg batches and also produced a significant larger egg batch surface (Fig. 3; ANOVA: df=1, F=10.3, p<0.001).
ANOVA: df=1, \(F=7.78, p<0.01\) than females without this extra source of energy. 
Yponomeuta padellus females on the other hand did not benefit significantly from their access to honey and both groups produced about equal egg batch surfaces (Fig. 4; ANOVA: df=1, \(F=0.14, p=0.91\)).

Multiple Mating

Multiple mating did not increase female longevity in either species of Yponomeuta. Mating not only failed to extend female lifespan but in fact significantly reduced it (Figs. 1 and 2; ANOVA, df=1, \(F_{cag}=17.49\) and \(F_{pad}=43.05\), \(P<0.001\)).

Total egg production (both egg batch number and batch surface) did not change significantly when females were mated multiple times (Figs. 5 and 6; ANOVA, df=1, \(F_{cag}=0.08\) and \(F_{pad}=3.13\), \(P=ns\) for both species). Unmated females of both species in all cases laid only very few (unfertilized) eggs.

DISCUSSION

Our results show that contrary to expectation (based on literature data for Macrolepidoptera), multiple mating has a negative effect on longevity and females did not convert the content of the male spermatophore into eggs.
Lederhouse et. al (1990) observed that *Papilio glaucus* ingest nectar, sodium, and other electrolytes and transfer this extra source of nutrition to females during mating in order to increase female reproductive output. Arnqvist & Nilsson (2000) suggested that multiple mating may increase female fitness by stimulating egg production, replenishing sperm supplies, and offering accessory substances that contribute to female survival and/or reproduction. Here we found no support for these ideas as mating multiply had a significant negative effect on female longevity and did not affect reproductive output. It should be noted however that the lack of effect of the nuptial gift as an energy source does not rule out that it might contain secondary plant compounds that increase the survival of the female and/or the offspring.

In contrast to the lack of a nutritional benefit from multiple mating, honey can be used as an energy source by *Y. cagnagellus* to extend lifespan and produce larger eggs batches. *Yponomeuta padellus* on the other hand did not profit from honey. This is possibly due to time constraints related to its shorter lifespan. It seems that *Y. cagnagellus* has the ability to use external carbohydrate sources in its adult life, while *Y. padellus* females are relying exclusively on their larval resources.
REFERENCES