

Male Mating History in *Antheraea mylitta* and its Effect on Ejaculation Size and Female Reproductive Fitness

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Reproductive activity in the economically important insect, *Antheraea mylitta* is very important because the female reproduces only once in her life time and the aging is very rapid and costly. It is a capital breeder and strictly a monoandrous species. To know how strategically the insect behaves and the male allocates its ejaculate resource more prudently during its successive mating with virgin females to maximize its own fitness and the fitness of the female with whom it mated, both being most important to sericulture industry. So, the present study was undertaken and the results revealed fresh and virgin females always prefer to mate with fresh virgin males (84%) and receives high dose of ejaculates leading to higher hatchability than to virgin males of one day old (13.7%) and one day old males with mating experience (2.3%). The ejaculation size (as referred to eupyrene sperm count in the ejaculation) declined significantly over successive mating ($r = -0.9931$, $P < 0.001$), so also the male body weight ($r = -0.9560$, $P < 0.001$). The quantity of ejaculate passed to female also dramatically declined during aging ($r = -0.9982$, $P < 0.001$). It was found that male weight contribute substantially to the quantum of ejaculate ($r = -0.9519$, $P < 0.001$), so also higher fecund females receive relatively more ejaculate than the lower group to reach higher reproductive fitness. The life time fecundity was found to be 334 ± 31 .

Key words: *Antheraea mylitta*, Mating history, Ejaculation size, Reproductive fitness

Introduction

The fact that male contribute substantially to reproduction as female does was become very clear from studies carried out in the past (Boggs, 1990; Gwynne, 1984; Vahed, 1998). Ejaculates serve to maximize the male fitness in several ways (Gillott, 2003), the primary function being to ensure male fertility. Males allocate its sperm in a most adaptive way among ejaculate when it mate more than once (Cook and Wedell, 1996) and in some species males are able to vary the number of sperms ejaculated according to circumstance. Male mating with large females ejaculated more spermatozoa than when mating with small females (Engqvist and Sauer, 2003; Gage, 1998; Gage and Barnard, 1996; Wedell and Cook, 1999). The ejaculate weight decreases over successive matings is very common in insects (Damiens and Boivin, 2006; Eady, 1995; Lauer, 1996; Lewis, 2004; Savalli and Fox, 1999a, b). Many empirical studies have indeed shown that ejaculate allocation patterns are markedly plastic within species (Eddy, 1995; Engqvist and Sauer, 2001; Ofuya, 1995; Wedell *et al.*, 2002).

In *Antheraea mylitta* the aging is rapid and costly as the insect is very short-lived, and the female reproduce only once in their lifetime (Rath, 2000; Rath *et al.*, 1997; Rath *et al.*, 2002, 2007). The aging has an immense effect on reproductive performance in this insect where a decline in mating success, fecundity and fertility with aging was reported (Rath, 2000; Rath *et al.*, 1997; Rath *et al.*, 2002, 2007). Adult moths are sexually ripened at the time of emergence and the act of mating starts 2-3 hrs after the emergence. Females have full complement of eggs and mating does not stimulate egg production; male ejaculates sufficient sperms (2570-3500 sperms per one egg; personal communication of the author) during coupling to fertilize the eggs of the female and no re-mating in this insect is observed (Rath *et al.*, 1997, 2007). Thus *A. mylitta* is monoandrous. It is a capital breeder too in the

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absence of adult feeding. Adult of this species do not need to feed to reproduce successfully as reported in other species also (Savalli and Fox, 1999b; Wightman, 1978) and the ejaculate size may therefore affect female fitness to a great extent which ultimately influence the viable egg production in this economically important insect.

The present study was under taken to understand the effect of male mating history on male reproductive fitness (production of fertile sperms and maintenance of ejaculation size) and female reproductive fitness (life time fecundity and hatching) which is largely not studied in the species. This might be helpful in making strategic approach to augment the egg production to meet the ever increasing demand.

Materials and Methods

Male mating history and ejaculation size

Male moth of weight range 3.1 g to 3.3 g were used in this experiment and the females used were of 6.5 g to 7.0 g. Male moths soon after eclosion were separated and made three mating groups- repeated mating (same male was used for 4 successive mating with a resting period of 16 hrs between two matings), alternate mating (the same male was used in alternate days, where the resting period was 40 hrs between two successive matings) and single mating (separate male was used but of different ages, day 0 to day 4). The moths were released inside the nylon net enclosure (separate for each mating group) for mating for 8 hrs (Rath *et al.*, 2002). After recording the mating success five mated females were randomly selected from each lot of the mating type for eupyrene sperm count and the rest were kept in the earthen cups for life time fecundity (Rath *et al.*, 1997) and hatching. There were 10 replications each with 100 moths of either sex.

Body weight loss during repeated mating

Ten number of male moths were weighed 2-3 hrs after eclosion (2.7 to 3.5 g weight with separate identifying marks for a definite weight) and then released inside a nylon net for mating with fresh females (weighing 6.4 - 6.5 g) for 8 hrs (Rath *et al.*, 2002). After 8 hrs of mating the mated males were weighed and then rested for 16 hrs and then again released inside the net for its second mating for 8 hrs with fresh females after that the weight of males were noted. Thus, male moths were mated 4 times and after each mating the weight of males was noted.

Correlation between body weight and ejaculation size

Fresh male moths of various weights (2.65 g to 3.5 g) were mated with fresh females (2- 4 hrs old, fresh weight-

6.4-6.5 g) for 8 hrs to note any variation in the ejaculation size along the weight range of male moth. After the desired period of mating eupyrene sperm estimation in the ejaculate was made.

To find out whether the female weight has any relation to have a larger ejaculate we have performed an experiment where fresh males having particular body weight 3.30 to 3.33 g were allowed to mate with fresh females of three different body weight ranges (5.8 ± 0.158 g, 6.53 ± 0.156 g and 7.34 ± 0.102 g) for a period of 8 hrs. There were 10 replications each with 50 moths. Ten females were randomly selected from each body weight range for eupyrene sperm count while other samples were kept for finding out the realized fecundity and fertility.

Female mating preference

To find out female mating preference we conduct an experiment where 150 fresh females were released in side a nylon net enclosure along with 150 fresh males, 150 virgin males of 1 day old and 150 earlier mated males of 1 day old in the night. Virgin males of 1 day old and earlier mated males of 1 day old were given identifying marks so that it can be easily identified to find out whether it was mated or not. In the morning mating success with respect to different male categories were noted. After required period of mating (8 hrs) mated females were kept for oviposition to find out the realized fecundity and fertility. There were 7 replications.

Eupyrene sperm count

The sperm preparation was made by opening the spermatheca and bursa copulatrix of a mated female in 1 ml of Belar's saline (Flint and Kressin, 1969; 6 g NaCl, 0.2 g KCl, 0.2 g CaCl₂, 0.2 g Na₂CO₃ and water to make 1 liter) which serve as the diluting medium for sperms. After thorough mixing the sperm suspension was loaded to improved Neobaur's chamber to fill the volume 1/50 mm³ (5 small squares- 4 at its corner and 1 at the center of the big central square). Sperms are then counted in the above squares.

Statistical Analysis

The data collected were subjected to one way ANOVA, correlation and regression analysis (Sokal and Rohlf, 1995) to find out the significant differences.

Results and Discussion

Ejaculate allocation

Virgin males contribute more ejaculate to females than did once-, twice- or thrice mated males. The ejaculation size

Table 1. Effect of male mating history on fecundity, hatching and ejaculation size in *Antheraea mylitta* (Values are mean \pm SD) [Female moth age 0 day (fresh 2-4 hrs old), fresh wt-6.4-6.5 g]

Mating type	Mated on day	Mating No.	Lifetime fecundity	Hatching %	Total eupyrene sperm count ($\times 10^5$)
Repeated (same male is used successively after a rest of 16 hrs) A combine effect of repeated mating and aging	0	1	334 \pm 31 ^a	91.97 \pm 1.91 ^a	8.553 \pm 0.392 ^a
	1	2	242 \pm 16 ^b (- 27.5%)	74.73 \pm 4.23 ^b (- 18.7%)	5.521 \pm 0.311 ^b (-35.4%)
	2	3	211 \pm 14 ^c (- 36.8%)	48.71 \pm 3.55 ^c (- 47.0%)	3.416 \pm 0.446 ^c (-60.1%)
	3	4	139 \pm 12 ^d (- 58.4%)	7.43 \pm 1.99 ^d (- 91.9%)	1.584 \pm 0.089 ^d (-81.5%)
r			-0.9840*	-0.9821*	-0.9931*
Alternate (same male was used after a rest of 40 hrs) A combine effect of repeated mating and delayed aging	0	1	334 \pm 31 ^a	91.97 \pm 1.91 ^a	8.553 \pm 0.392 ^a
	2	2	198 \pm 14 ^c (- 40.7%)	45.43 \pm 1.19 ^c (- 50.6%)	3.294 \pm 0.350 ^c (-59.2%)
	4	3	140 \pm 15 ^d (- 58.1%)	8.43 \pm 1.27 ^d (- 90.8%)	1.750 \pm 0.385 ^d (-79.5%)
r			-0.9741*	-0.9978*	-0.9539*
Single mating (separate male was used) Effect of Aging	0	1	334 \pm 31 ^a	91.97 \pm 1.91 ^a	8.553 \pm 0.392 ^a
	1	1	263 \pm 24 ^c (- 21.2%)	79.39 \pm 3.37 ^c (- 13.7%)	6.656 \pm 0.349 ^c (-22.2%)
	2	1	189 \pm 18 ^c (- 43.4%)	60.31 \pm 3.05 ^f (- 34.4%)	5.456 \pm 0.387 ^b (-36.2%)
	3	1	132 \pm 15 ^d (- 60.5%)	45.56 \pm 5.16 ^c (- 50.5%)	3.631 \pm 0.456 ^c (-57.5%)
	4	1	98 \pm 14 ^f (- 70.6%)	7.86 \pm 1.68 ^d (- 91.4%)	1.969 \pm 0.304 ^d (-77.0%)
r			-0.9910*	-0.9766*	-0.9982*

Significant differences are indicated by different superscripts (one-way ANOVA at 5% level)

* $P < 0.001$

amounts to 8.553×10^5 eupyrene sperms during its first mating which declined drastically by 35.4%, 60.1% and 81.5% during subsequent matings. Thus, out of a total allocation of 19.074×10^5 eupyrene sperms, a major share of 44.8% were allocated during first mating; and then 28.9% during 2nd, 17.9% during 3rd and only 8.3% during 4th mating ($r = -0.9931$, $P < 0.001$). When the males used alternatively, the ejaculation size declined to an extent of 59.2% upon second mating and 79.5% upon third mating ($r = -0.9539$, $P < 0.001$). The single mating (age-related changes) revealed 22.2% decline in ejaculation size on day 1 and further declined on day 2 (36.2%), day 3 (57.5%) and day 4 (77.0%). ($r = -0.9982$, $P < 0.001$) (Table 1, Fig. 1). This reduction in ejaculation size might be due to constrains in production rate (Arnqvist and Danielsson, 1999; Moore *et al.*, 2004), or, the variation in female fecundity may affect the evolution of male ejaculate allocation (Reinhold *et al.*, 2002). Ejaculation size

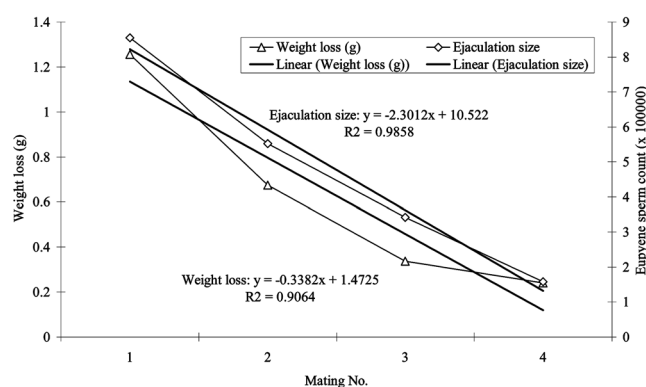


Fig. 1. Relationship of weight loss and total eupyrene sperms ejaculated by male during repeated mating in *Antheraea mylitta*.

and the number of sperms it contain vary among males (Eady, 1995; Savalli and Fox, 1998) and, within individ-

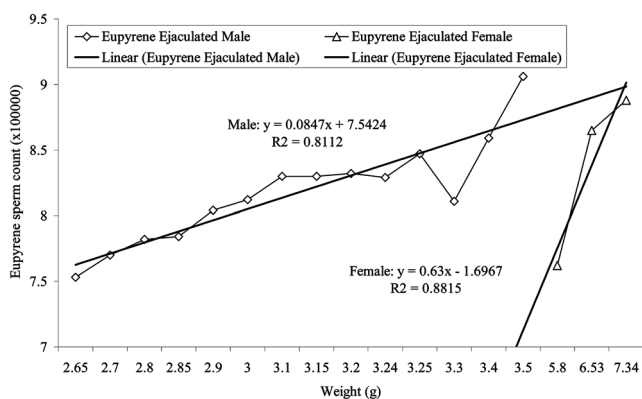
Table 2. Female mating preference and its effect on fecundity and fertility in *A. mylitta*. (Values are mean \pm SD)

Details of mating preference	Mating success (%)	Realized fecundity (nos.)	Hatching %
FF x FM (Fresh females x fresh males)	84.1 \pm 6.47 ^a	291 \pm 13 ^a	92.56 \pm 1.13 ^a
FF x VM1 (Fresh females x Virgin males 1 day old)	13.71 \pm 5.71 ^b	258 \pm 12 ^b	86.60 \pm 3.31 ^b
FF x MM1 (Fresh females x Mated males 1 day old)	2.19 \pm 1.79 ^c	194 \pm 13 ^c	77.30 \pm 2.27 ^c

Significant differences are indicated by different superscripts (one-way ANOVA at 5% level)

uals, vary with age (Fox *et al.*, 1995a, b) and male mated status as observed in the present investigation. The empirical evidence suggests that male indeed allocate ejaculates according to variation in female fecundity and thus the female body size affects the ejaculation size (Mallard and Barnard, 2003; Simmons *et al.*, 1993; Wedell, 1992). Our results further corroborate the prediction that males are investing more ejaculate in early copulations than the later ones, assuming the probability of achieving additional matings decreases over time (Reinhold *et al.*, 2002). Alternate mating revealed that *A. mylitta* fails to replenish its sperm quantity following a mating where upon 2nd mating the quantity of eupyrene sperms delivered was *at par* with the quantity of that the male ejaculated during its third repeated mating (Table 1).

Male loses its weight during repeated mating from 3.01 g to 1.754 g during its first mating; which further declined to 1.08 g, 0.743 g and 0.502 g in subsequent mating. Thus, male contributions constituted a substantial proportion of their pre-mating body mass (first mating mean \pm SD = 41.53 \pm 3.35%; second mating, 22.38 \pm 2.07%; third mating, 11.34 \pm 3.99% and fourth mating, 7.99 \pm 3.21%, $r = -0.9560$, $P < 0.001$) and the changes between any two mating treatment were found to be significant. There was a close association of weight loss and ejaculation size during repeated mating which revealed that weight loss ($y = -0.3382x + 1.4725$, $R^2 = 0.9064$) and decline in ejacu-

**Fig. 2.** Effect of body weight on ejaculation size in *Antheraea mylitta*.

lation size ($y = -2.3012x + 10.522$, $R^2 = 0.9858$) was due to repeated mating in *A. mylitta* (Fig. 1). Earlier findings by Savali and Fox (1999a) and Fischer *et al.* (2009) reported the loss as a measure of ejaculation size are also in close agreement with our findings.

Male size and sperm number

There was a positive and significant correlation between male weight and ejaculation size ($y = 0.0847x + 7.5424$, $R^2 = 0.8112$), which indicate that male weight contribute 81% to the ejaculation size (Fig. 2). A positive association between a male size and spermatophore mass that had been reportedly found in butterflies and other insects (Bissoondath and Wiklund, 1996; Karlsson, 1998; Wedell and Cook, 1999; Wiklund and Kaitala, 1995) confirms our findings. Further, this finding corroborates previous finding that spermatophore mass and fertile sperm numbers increased with male size, while non fertile sperm numbers were unrelated to male size (Fischer *et al.*, 2009; Lewis and Wedell, 2007).

Female fitness

Mating preference

Fresh females prefer to mate with fresh virgin males (84%), than virgin males of one day old (13.7%), and one day old males with mating experience (2.3%). The realized fecundity and hatching of eggs were also significantly more when fresh females mated with fresh virgin males. Significant differences were observed among the treatment groups in mating success (df 2, 18; $F = 531.7729$, $P < 0.0001$), realized fecundity (df 2, 18; $F = 103.0297$, $P < 0.0001$) and hatching of eggs (df 2, 18; $F = 71.5481$, $P < 0.0001$ (Table 2).

Lifetime fecundity

The life time fecundity in *Antheraea mylitta* was recorded to be 334 \pm 31 and decreased as the male's number of previous matings increased ($r = -0.9840$, $P < 0.001$). When males were mated repeatedly with virgin females during aging, their lifetime fecundity was declined by 27.5%, 36.8% and 58.4% upon 2nd, 3rd and 4th mating respectively. In alternate mating, the lifetime fecundity was also declined significantly (by 40.7% upon 2nd mating and

Table 3. Relationship between female wt and ejaculation size received. (Values are mean \pm SD)

Moth size (wt. in g)		Total eupyrene sperms ($\times 10^5$) received	Realized fecundity	Hatching % (Larvae hatched out)	Sperm : egg ratio
Male	Female				
3.33 \pm 0.134 ^a	5.80 \pm 0.158 ^a	7.62 \pm 0.455 ^a	194 \pm 12 ^a	82.8 \pm 3.3 ^a (161)	3927:1
3.30 \pm 0.141 ^a	6.53 \pm 0.156 ^b	8.65 \pm 0.425 ^b	282 \pm 9 ^b	93.2 \pm 2.2 ^b (263)	3067:1
3.33 \pm 0.134 ^a	7.34 \pm 0.102 ^c	8.88 \pm 0.450 ^b	330 \pm 7 ^c	92.2 \pm 4.3 ^c (304)	2692:1

Significant differences are indicated by different superscripts (one-way ANOVA at 5% level)

58.1% during 3rd mating). Single mating revealed that aging alone was responsible for a decline in lifetime fecundity of 21.2%, 43.4%, 60.5% and 70.6% on day 1, day 2, day 3 and day 4 respectively ($y = -60.3x + 323.8$, $F = 164.802$, $R^2 = 0.9821$, $P < 0.001$) (Table 1).

Correlation between female weight and ejaculation size

Heavier females were ejaculated with significantly more sperms by males than the less weighed females, so also the fecundity and fertility were higher in heavier females. Low weighed females (5.80 \pm 0.158 g) were ejaculated with 7.62×10^5 eupyrene sperms, while the middle weight females (6.53 \pm 0.156 g) were ejaculated with 13.5% more (8.65×10^5 eupyrene sperms) and heavier female (7.34 \pm 0.102 g) with 16.5% more (8.88×10^5 eupyrene sperms). The regression equation ($y = 0.63x - 1.6967$, $R^2 = 0.8815$, $P < 0.001$) revealed female weight contribute 88% to the ejaculated mass. The realized fecundity was tends to increase significantly in middle weight (by 45.4%) and heavier females (by 70.1%) from 194 in low weighed females. Similarly, the hatching in middle weight and heavier females were significantly higher than the low weight females (Table 3, Fig. 2). The sperm: egg ratio also tends to decline from 3927:1 (in low weighed females) to 3067:1 (in middle weighed group) and 2692:1 in heavy weight group. Such a huge quantity of sperm ejaculation by male in to the body of female might be the reason behind monoandrous nature which ceases the receptivity of the female further in this wild species. Larger ejaculate volumes associated with low degree of polyandry that are reported in bushcricket texa, agrees with our findings (Vahed, 2006).

Earlier reports also revealed that male provided larger females with ejaculates frequently contain more sperm that have increased fecundity in many insects (Engqvist and Suer, 2003; Gage, 1998; Gage and Barnard, 1996; Wedell and Cook, 1999). That at least some male Lepidoptera seem capable of assessing female quality and

reproductive potential, and adjust their ejaculate accordingly. Such strategic investment of reproductive sources e.g. by tailoring ejaculates in relation to female size that have been repeatedly documented (Wedell *et al.*, 2002) corroborates our present findings.

Females mated to virgin males had significant higher fecundity than those mated to previously mated males. The lifetime fecundity, hatching and total eupyrene count declines ($P < 0.001$) as the number of mating increases in repeated mating (where the combine effect of both repeated mating and aging of male moth was observed), so also in alternate mating (where the effect of delayed aging along with repeated mating was seen) and in single mating where the effect of aging of male moth was only observed (Table 1).

Size of the ejaculate plays a significant role in female fitness which generally decreases with the number of times that its mate has mated previously. Decreasing size of the ejaculate has a deep effect on female fitness (Rönn *et al.*, 2008). Female fitness components that were affected by male mating history brings about the changes in ejaculation size and composition over successive matings is the key along with effects on hatching rate of eggs (Rönn *et al.*, 2008) corroborates our findings of the present study.

Our results show that male continue to mate with virgin females even beyond the point (4th in repeated and 3rd in alternate matings) where male ejaculate depletion results in female fitness being compromised. The fecundity of *C. maculata* females was significantly dependent on the male mating history, shown by Ofuya (1995) by mating with males suffering from severe ejaculate depletion, agree with our findings.

The size, quality and number of spermatophore delivered by males have been shown to be highly sensitive to factors like male age at mating, body weight, larval and adult feeding regime, mating order and the time that elapse between consecutive matings (Torres-Vila and Jennions, 2005). Male mating history strongly affects female

reproductive output in Lepidoptera. Females mated to virgin males had higher fecundities than those mated to previously mated, experienced males (Torres-Vila and Jennions, 2005) further confirms our findings.

The most obvious proximate explanation for the effect of male mating history on female fecundity is that mating history affects spermatophore size as in *A. mylitta* where the virgin males produced larger number of eupyrene sperms than experienced males. This suggests that lepidopteran males suffer a depletion of spermatophore precursors after each mating.

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