Delayed Mating Impacts on the Reproductive Performance of *Ectropis obliqua* (Lepidoptera: Geometridae)[1]

YunQiu Yang[2], LinZhong Zhang[3], YanZuo Zhang[4], LongWa Zhang[5], and YanHua Long[6,7]

Anhui Agricultural University, Hefei, 230036, P.R. China

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Abstract The impact of age at which mating occurs on the mating success, fecundity, fertility, and adult longevity of *Ectropis obliqua* Prout (Lepidoptera: Geometridae) was determined in laboratory testing. When newly emerged males were mated with females that emerged 1, 2, 3, 4, 5, or 6 d earlier, mating success, fecundity, and fertility generally decreased with increased age of the females. Similar responses were observed when newly emerged females were mated with males that had emerged 1, 2, 3, 4, 5, 6, or 7 d earlier and when females and males of the same age were allowed to mate. Adult longevity was inversely related to age at which mating occurred.

Key Words mating delay, fecundity, fertility, adult longevity, reproductive performance

Insect reproduction involves two types of behavior: mating and oviposition (Jimenez-Perez and Wang 2003), which must occur within a limited period or time as delayed mating may reduce fecundity and egg viability, presumably because of physiological changes associated with age (Kawazu et al. 2014). Female insects use a limited amount time after emergence to mate, develop eggs, and find suitable ovipositional hosts; a delay in mating alone can negatively impact reproductive fitness (Fitzpatrick 2006, Huang and Subramanyam 2003, Mori and Evenden 2013, Stelinski and Gut 2009). Increased age of males at mating may also negatively influence number and viability of eggs produced by females (Jimenez-Perez and Wang 2003, Rogers and Marti 1997, Unnithan and Paye 1970). Moreover, studies reporting the effects of delayed mating by both sexes simultaneously are relatively lacking and have yielded variable results with different species (Huang and Subramanyam 2003, Kawazu et al. 2014, Wang et al. 2011).

Our interests in the impacts of delayed mating on the reproductive fitness of *Ectropis obliqua* Prout (Lepidoptera: Geometridae) are based on the potential use

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[2] State Key Laboratory of Tea Plant Biology and Utilization, Anhui Agricultural University, Hefei, 230036, P.R. China.

[3] School of Sciences, Anhui Agricultural University, Hefei, 230036, P.R. China.

[4] University of Georgia, College of Agricultural and Environmental Sciences, Athens, GA.

[5] Provincial Key Laboratory of Microbial Pest Control, Anhui Agricultural University, Hefei, 230036, P.R. China.

[6] School of Life Science, Anhui Agricultural University, 130 Changjiang Road, Hefei, 230036, P.R. China.

[7] Corresponding author (email: yyq_lyh@163.com).

of pheromone-mediated mating disruption of this economically important pest. This management tactic is currently used to control numerous lepidopteran pest species (Peter et al. 2010). And, mating disruption not only prevents mating from occurring through a negative effect on the ability of males to orient toward females, but also delays mating by decreasing female readiness to mate, thereby reducing the number and viability of eggs produced per female (Michereff et al. 2004).

Ectropis obliqua, known locally as the tea geometrid, is a major pest of thousands of hectares of tea (*Camellia sinensis* L.) in China. It is multivoltine, often producing six to seven generations per year (Hazarika et al. 2001, Yang et al. 2015). Adults can mate on the night of emergence from pupation (Yang et al. 2008), and females are monandrous in selection of mates, and infertile females produce only sterile eggs (Yang et al. 2011). The *E. obliqua* sex pheromone was identified as containing a blend of (*Z*,*Z*,*Z*)-3,6,9-octadecatriene and 6,7-epoxy-(*Z*,*Z*)-3,9-octadecadiene (Yang et al. 2015).

Synthetic pheromones may be useful in an integrated pest control program for *E. obliqua,* but nothing is known regarding the effect of the pheromone in delaying mating or on the effect of delayed mating on reproductive performance. In the present study, we examined the effect of mating age of female, male, and both sexes on fecundity, fertility, and adult longevity of *E. obliqua*. This information will be useful in developing mating disruption strategies that use pheromones for suppressing *E. obliqua* populations in tea production.

Materials and Methods

Ectropis obliqua adults were obtained from Shu-Cheng Co. (N 31.47°, E 116.93°), Anhui Province, China, and maintained in the laboratory for approximately five generations. Larvae were reared on fresh tea leaves. Pupae were sexed according to the morphology of the 8th to 10th abdominal segments and were placed separately in moistened sand until emergence. Pupae were checked daily, and healthy adults (aged 0–24 h) that emerged were used in experiments. Adult pupae and larvae were all maintained in controlled conditions at 22 ± 3°C, 60–70% relative humidity, and a light:dark photoperiod of 14:10 h. All experiments were also conducted under these conditions.

Three experiments were conducted in this study. One experiment examined female-delayed mating effects on mating rate, fecundity, fertility, and longevity of adult males and females. In this test, virgin females of different ages (1–6 d after emergence from pupation) were singly paired with individual 0-d-old virgin males in 240-ml plastic containers (15 cm × 5 cm inner diameter [ID]) and provided with a 10% (v/v) honey solution as a food source. In a second experiment, the effects of male-delayed mating on reproductive performance were similarly studied using virgin males of different ages (1–6 d) that were singly paired with individual 0-d-old virgin females. Mating pairs were maintained in 240-ml plastic containers with a 10% honey solution as previously described. A third experiment examined the impacts of delayed mating by both sexes. Virgin females of different ages (1–6 d) were paired with single virgin males of the same age (1–6 d) in 240-ml plastic containers (15 cm × 5 cm ID) and provided with 10% honey solution.

In each experiment, copulation was observed during scotophase at 20-min intervals. After copulation, females were transferred individually to 240-ml containers and allowed to oviposit. Fecundity (total number of eggs produced per female after mating) and fertility (percentage of females producing fertile eggs) were recorded for mated pairs only. Male and female longevity was also recorded. The success of the observed matings was determined by dissecting females after death to determine the presence of a spermatophore in the bursa copulatrix. Each treatment in each experiment was replicated 17 to 35 times, based on availability of adult insects.

Within each experiment, treatment effects were compared using a one-way analysis of variance (ANOVA) (SAS Institute 2004). Statistically different treatment means ($P < 0.05$) were separated by either Chi-square testing (mating success) or Bonferroni's multiple comparison test (fecundity, fertility, longevity) (SAS Institute 2004).

Results

The probability of successful mating, as evidenced by the presence of a spermatophore in dissected females, statistically decreased as the age of the female ($F = 62.88$; df $= 1$; $P < 0.05$) (Table 1), the male ($F = 47.02$; df $= 1$; $P < 0.05$) (Table 2), and both sexes increased ($F = 25.22$; df $= 1$; $P < 0.05$) (Table 3), thus indicating that delayed mating, regardless of sex of the moth, decreases mating success. Moreover, mating success is most severely affected when delayed mating occurs in both sexes when compared with female-only delayed mating ($\chi^2 = 22.59$; df $= 4$; $P < 0.001$) or to male-only delayed mating ($\chi^2 = 45.47$; df $= 5$; $P < 0.001$). Mating success also differed significantly between female-only delayed mating and male-only delayed mating ($\chi^2 = 29.05$; df $= 5$; $P < 0.001$).

In female-only delayed mating, fecundity was statistically the same for females delayed 1, 2, and 3 d before mating (Table 1); however, fecundity of females delayed 4 and 5 d from mating was significantly lower. Fertility decreased with mating delay with females ($F = 84.53$; df $= 4$; $P < 0.001$) (Table 1).

In male-only mating delay, fecundity and fertility of the mated females were significantly decreased with the age of the male beyond emergence (fecundity: $F = 103.89$; df $= 5$; $P < 0.001$; fertility: $F = 30.49$; df $= 5$; $P < 0.001$) (Table 2). Mating delay of both sexes significantly reduced fecundity and fertility (fecundity: $F = 87.64$; df $= 4$; $P < 0.001$; fertility: $F = 102.91$; df $= 4$; $P < 0.001$) (Table 3). Although these experiments did not provide for statistical comparison of female-only, male-only, and both sexes delayed mating, it appears that fecundity and fertility were impacted most by delayed mating of both sexes followed by male-only mating delay and female-only mating delay.

As the period of mating delay increased, adult longevity for females ($F = 69.78$; df $= 4$; $P < 0.001$) and males ($F = 35.21$; df $= 5$; $P < 0.001$) significantly increased (Tables 1, 2). Females ($F = 3.01$; df $= 5$; $P = 0.0156$) (Table 2) and males ($F = 0.85$; df $= 4$; $P = 0.498$) (Table 1) mated without delay demonstrated no significant response in longevity regardless of the age of the mate. Furthermore, as the period of delay before mating increased with both mating pairs, longevity also increased (female: $F = 37.26$; df $= 4$; $P < 0.001$; male: $F = 102.91$; df $= 4$; $P < 0.001$) (Table 3).

Table 1. Effect of female-delayed mating on fecundity, egg fertility, and longevity of *Ectropis obliqua*.*

Age (d)							
Female	Male	Number Moths Paired	Mating Success (%)	Fecundity After Mating (eggs/female)	Egg Fertility (%)	Female Longevity (d)	Male Longevity (d)
1	0	20	100	325 ± 31.4 a	84.3 ± 4.3 a	4.4 ± 0.6 e	5.6 ± 0.5 a
2	0	19	100	308.6 ± 35.5 a	77.6 ± 3.6 b	5.2 ± 0.7 d	5.5 ± 0.7 a
3	0	26	73.1	298.2 ± 26.9 a	69.4 ± 6.7 c	6.2 ± 0.7 c	5.3 ± 0.6 a
4	0	26	61.5	230.8 ± 33.4 b	59.4 ± 6.4 d	7.4 ± 0.6 b	5.4 ± 0.6 a
5	0	26	38.5	174.1 ± 27.9 c	49.4 ± 6.7 e	8.1 ± 0.8 a	5.2 ± 0.7 a
6	0	17	0	—	—	—	—
F		—	62.88	57.54	84.53	69.78	0.85
df		—	1	4	4	4	4
P		—	$P < 0.05$	$P < 0.001$	$P < 0.001$	$P < 0.001$	0.498

* Means (± SE) followed by the same lowercase letter within each column are not significantly different according to Bonferroni test after one-way ANOVA.

Table 2. Effect of male-delayed mating on fecundity, egg fertility, and longevity of *Ectropis obliqua.**

Age (d)							
Female	Male	Number Moths Paired	Mating Success (%)	Fecundity After Mating (eggs/female)	Egg Fertility (%)	Female Longevity (d)	Male Longevity (d)
0	1	31	96.8	346.9 ± 26.1 ab	86 ± 7.8 a	4.8 ± 0.7 a	5.6 ± 0.6 d
0	2	28	83.9	363.3 ± 40.0 a	87.5 ± 7.8 a	4.50.8 ab	6.2 ± 0.6 cd
0	3	30	71.4	311.4 ± 30.3 bc	80.1 ± 7.2 ab	4.6 ± 0.6 ab	6.6 ± 0.8 c
0	4	31	70	283.8 ± 39.5 c	74.9 ± 7.0 bc	4.4 ± 0.5 ab	7.5 ± 0.7 b
0	5	35	60	174.1 ± 38.0 d	71.2 ± 6.5 c	4.1 ± 0.5 ab	7.9 ± 0.7 ab
0	6	32	25	126.6 ± 34.0 e	59.2 ± 6.5 d	4.0 ± 0.6 b	8.5 ± 0.6 a
0	7	32	0	—	—	—	—
F		—	47.02	103.89	30.49	3.01	35.21
df		—	1	5	5	5	5
P		—	$P < 0.05$	$P < 0.001$	$P < 0.001$	0.0156	$P < 0.001$

* Means (± SE) followed by the same lowercase letter within a column are not significantly different according to Bonferroni test after one-way ANOVA.

Table 3. Effect of both male and female delayed mating on fecundity, egg fertility, and longevity of *Ectropis obliqua*.*

Age (d)							
Female	Male	Number Moths Paired	Mating Success (%)	Fecundity After Mating (eggs/female)	Egg Fertility (%)	Female Longevity (d)	Male Longevity (d)
1	1	32	87.5	301.7 ± 31.7 a	73.2 ± 6.3 a	4.4 ± 0.5 d	5.1 ± 0.5 c
2	2	32	43.8	271.0 ± 39.9 a	53.9 ± 6.7 b	5.4 ± 0.6 c	7.1 ± 0.7 b
3	3	32	40.6	209.4 ± 37.4 b	43.3 ± 6.2 c	5.5 ± 0.8 bc	7.3 ± 0.6 b
4	4	32	12.5	142.9 ± 33.2 c	33.1 ± 6.3 d	6.1 ± 0.7 b	8.3 ± 0.7 a
5	5	33	18.2	98.2 ± 26.3 d	24.0 ± 9.7 e	7.3 ± 0.7 a	8.4 ± 0.9 a
6	6	33	0	—	—	—	—
F		—	25.22	87.64	102.91	37.26	49.63
df		—	1	4	4	4	4
P		—	$P < 0.05$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$

* Means (± SE) followed by the same lowercase letter within a column are not significantly different according to Bonferroni test after one-way ANOVA.

Discussion

Increased female and male age at mating negatively affects the reproductive potential of female *E. obliqua*, including mating success, fecundity, fertility, and adult longevity. The overall effect of age on reproductive performance significantly varies depending on whether the delayed mating occurs in females only, males only, or both sexes simultaneously. Mating delay involving both sexes simultaneously appears to have the greatest potential for imposing a potent negative impact on the reproductive performance of *E. obliqua*. Female-only mating delays also negatively impact reproductive performance, more than that observed with male-only mating delay. Similar responses have been observed in several other insect species (Jimenez-Perez and Wang 2003, Kawazu et al. 2014, Stelinski and Gut 2009).

Our results further show that longevity is longer as the age at which *E. obliqua* males and females are mated increases. Increased adult longevity associated with delayed mating has been reported with other lepidopteran species (Ellis and Steele 1982, Jiao et al. 2006, Lingren et al. 1988). Increased longevity with the delay of mating is widely postulated to be a result of reduced energy expenditure associated with reproductive activities.

The sexual maturation period of *E. obliqua* is approximately 1 d after emergence from pupation. Our data show that peak reproductive performance can be achieved if females mate at 1 or 2 d after emergence. If mating disruption delayed female mating until 4 d after emergence, and we further assume that the female mates with a male of the same age, fecundity after mating would decrease from a mean of 301.7 to 142 eggs per female and that fertility would decrease from 73% to 33%. These findings may prove useful for developing programs on population suppression of *E. obliqua* at regional scales, including mass trapping and mating disruption techniques. Specifically, pheromone-mediated mating disruption may not only inhibit mating, but also delay mating. Our results further suggest that female mating should be delayed for a minimum of 3–4 d after emergence to achieve control.

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