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MASS FLIGHTS OF *LYMANTRIA DISPAR JAPONICA* AND *LYMANTRIA MATHURA*
(EREBIDAE: LYMANTRIINAE) TO COMMERCIAL LIGHTING, WITH NOTES ON
FEMALE VIABILITY AND FECUNDITY.¹

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ABSTRACT. Adult *Lymantria dispar japonica* (females only) and *Lymantria mathura* (both sexes) flew to commercial lighting during the night in Takizawa Village, Iwate Prefecture, Japan, in large numbers during the first week of August 2008. Males of *L. d. japonica* were conspicuously absent while 93.8% of responding females were mated and subsequently laid an average of 419.2 eggs each post-flight. For *L. mathura* females, only 33.9% were mated during the first half of the night and each mated female carried on average 717.4 eggs (max. 1065). Egg counts were facilitated by a newly developed egg mass matrix digestion process and an approximation of egg count based on egg mass dry weight was calculated. These two moth species have dissimilar behavioral strategies—*L. d. japonica* is diurnal, mated during the afternoon pre-flight and females flew to the lighting after mating. In contrast, *L. mathura* is nocturnal and both sexes responded to the lighting; females generally arrived as virgins (unfertilized) to then mate with males as the night progressed. Lack of a moth flight in 2009 illustrated dramatic interannual population fluctuation prevalent among many lymantriine moths. An illustration of a 2013 mass flight in Ono City, Fukui Prefecture, suggests a more frequent occurrence of such flights in both time and space.

Additional key words: Noctuoidea, proteinase K digestion, female nocturnal flight, dispersal; flight activity; reproduction; Japanese gypsy moth; Asian pink moth

Unexpected events can provide unique opportunities to gain biological insights with both fundamental and applied importance. One such occasion was a chance encounter with massive nocturnal flights of lymantriine moths attracted to commercial lighting in the village of Takizawa (39.75° N, 141.07° E), just north of Morioka, Iwate Prefecture, Honshu, Japan. Takizawa is nestled in a valley surrounded with hills covered with abundant *Quercus* and *Larix* forests. Gerhard Gries (Simon Fraser University) and PWS were on a field trip in the area conducting unrelated field trapping experiments (Gries et al., 2009a; 2009b). During the evening hours of August 1, 2008, while driving through the well-lit commercial center of Takizawa, we observed a massive evening flight of lymantriine moths. That evening and six evenings to follow, we photographed and observed so many flying moths that the quantity of moths was suggestive of snow. Subsequently we became aware that the flight was more regional than at first appearance. Furthermore, a 2013 illustration is added during the review process that shows evidence of a flight of *Lymantria dispar japonica* (Motschulsky) (Japanese gypsy moth) at Ono City, Fukui Prefecture. We suggest that mass flights likely occur over more widespread

areas of Honshu and may occur more frequently than ever imagined.

Flights of *L. d. japonica* and *L. mathura* Moore (Asian pink moth¹) occur infrequently and they must have originated from population outbreaks. Such outbreak events in Honshu are recorded for both species [for *mathura* see Nishitani, 1918; for *japonica* see Inoue and Arisawa, 1984 or Sato and Sotodate, 1975 (these references are in Japanese and are annotated in Schaefer et al. 1988a but are omitted from the Literature Cited section herein)]. The observed female flights and the physiological condition of the females provide insight into the threat of invasion into new environments.

That females of *L. mathura* have the ability to fly has never been in dispute but the flight ability of *L. dispar* females has often been questioned. This is due in part to a fundamental behavioral polymorphism. In the North American and European forms of the gypsy moth, *L. d. dispar*, females are so aerodynamically configured that they are incapable of sustained flight. This is not the case with Asian forms, where flight-capable females demonstrate the capacity of both sustained and ascending flight. In eastern Asia, there are often

¹This name has recently been introduced as a replacement name for “pink gypsy moth”, which is a misnomer and misleading reference to “gypsy moth” to which it should not be associated. *L. mathura* and *L. dispar japonica* are congeners but are not closely related based on behavior, molecular evidence, and on appreciably different sex pheromone communication systems.

concentrations of egg masses at outdoor light sources – demonstrative evidence of female flight. Historically, there has been a reluctance to acknowledge that females of the Asian forms of gypsy moth are capable of flight. This trait was observed by Schaefer (1978) in Hokkaido where females flew to white birches to deposit their eggs. Since the early 1990's, there has been increasing concern about female Asian gypsy moths being attracted to seaport lighting and subsequent egg deposition on containers or seagoing ships, hence the potential for international invasion of these moth species. This invasion potential has at times impaired commerce (Yokochi, 2007).

Aspects of gypsy moth female flight that have received recent attention include female flight as a genetic trait (Keena et al. 2008); comparisons among various gypsy moth populations (Reineke and Zebitz, 1998); attractiveness to specific light sources or mitigation efforts (Wallner et al. 1995; Iwaizumi and Arakawa, 2010) and details of the female flight behavior including timing of flights and dispersal capabilities (Carlton et al. 1999; Iwaizumi et al. 2010). Although these issues have been addressed, no studies have focused on the importance of viability and fecundity of flight capable females.

The question of female viability and fecundity in both *L. d. japonica* and *L. mathura* prompted collection of females to determine if these flight capable females were viable. Individual females were collected to determine what percent of females arriving at the lights were carrying viable eggs. This finding would help to clarify the relative threat represented by flight capable females as they sought oviposition sites which might have been on shipping containers or seagoing ships in ports that might export egg masses to environments where these moth species do not currently exist.

MATERIALS AND METHODS

Between 21:00 and 01:00 h. on August 1–7, PWS repeatedly observed and photographed massive nightly flights of lymantriine moths attracted to commercial lighting in Takizawa. The abundance of moths represented an unusual opportunity to investigate some important aspects of behavior using methodology described below. During the same week visits to Mizunashi revealed evidence that the flight was more regional in extent as was the subsequent discovery of a video of clouds of moths at a floodlight-lite baseball field in Kuji. All three locations are in Iwate Prefecture, northern Honshu.

Flight Composition. To characterize the species composition of the flight, adult moths were counted (near midnight of August 5–6) and recorded including

the numbers of each sex of both *L. d. japonica* and *L. mathura*, or any other congeners, on walls, buildings, signs, or paved surfaces near a public parking lot and all-night gas station. Species and sex of all specimens were determined on each surface and data summarized. Data were converted to sex ratios.

Female Viability and Fecundity. On August 4, collections of live females of each species were made near the lighting either from pavement surfaces or from walls or lighted signs but all were within reach by hand. Each female was immediately placed inside a folded newsprint triangular envelope (ca. 20 cm hypotenuse) in which the living female was sealed. Confined females were expected to lay their complement of eggs within the envelope before they died. All envelopes were stored in an outdoor insectary at the Forestry and Forest Products Research Institute in Morioka until the following spring. Then they were placed in a deep-freezer to kill the eggs. The collection was then shipped to USDA, ARS, Beneficial Insects Introduction Research, quarantine facility, Newark, Delaware, where the envelopes were refrozen until processing.

The egg masses of the two species were processed differently. Those of *L. d. japonica* were vacuum de-haired, using the technique described in Schaefer et al. (1988b), scored as being embryonated (by the darkened appearance of developed neonate larvae within the egg shell) or not. For a random subsample, eggs were counted on a counting dish made by covering the bottom surface of a petri dish with a plastic surface containing conveniently spaced parallel plastic ridges approximately 1.5 times higher than the diameter of a single egg. This dish allowed eggs to readily align into rows while the eggs were counted dry using a convenient magnification on a dissecting microscope.

Eggs of *L. mathura* were first removed (with some difficulty) from the newsprint of the envelopes in which they were laid and then dry weighed using an Ohaus-C, Pioneer Electronic balance (Ohaus Corp., Parsippany, NJ). Because *L. mathura* females insert their eggs en mass under bark scales while secreting fluids from the accessory glands that firmly cement individual eggs into a hardened matrix, direct egg counts were impossible without first processing these egg clusters in a digestion solution. The solution was obtained by dissolving 25 mg of proteinase K (Sigma Chemical Co., St. Louis, MO) in 5 mL of buffer (50 mM TRIS, plus 5 mM CaCl₂ at pH 8.0). This digestion process was devised by KGS. Digestion was allowed to proceed at room temperature for 2 to 5 days after which the solution was removed with a disposable plastic pipette. Any remaining clumps were teased apart with forceps and dissecting scalpel. Loosened eggs, setal hairs and matrix debris were all

washed onto the same plastic counting dish described above and counted under a shallow film of tap water. Fertile (embryonated) eggs, infertile eggs, and a few enclosed larvae were tallied.

Return Visit in 2009. Our 2008 data did not fully explain the time of female *L. mathura* mating, PWS returned to Takizawa in August 2009 expecting to capture additional female *L. mathura*. The intent was to segregate collections before and after 2200 h and to record numbers of pairs in copulo throughout the night.

RESULTS

Scenes of the moths at commercial lighting in Takizawa convey some of the abundance of moths that week (Figs. 1, 2) and accumulated gypsy moth egg masses (Fig. 3) at Mizunashi (39.88° N, 141.30° E, about 50 km from Takizawa) suggest that the flight was more widespread than just in Takizawa. See discussion for reference to a video that dramatically illustrates the same event and further suggests a more regional occurrence of our witnessed mass moth flight. During the manuscript submission and review process, another similar mass flight (Fig. 4) occurred at Ono City, Fukui Prefecture, Honshu (35.94° N, 141.49° E) further suggesting a re-occurring nature of these mass flights.

Counts made at Takizawa totaled 918 moths of four lymantriine species (Table 1). Among 65 live-isolated *L. d. japonica* females, 93.8% laid apparently full compliments of eggs that in every case were embryonated. The remaining 6.2% of females failed to produce any eggs. A random subsample (N = 16) of eggs per egg mass laid within the envelopes averaged 419.3 eggs per female (=mass) (S.D. = 62.2, range 311–523). Infertile eggs numbering <1% were disregarded.

Based on 56 confined *L. mathura* females, 53.6% of them laid some eggs but all were infertile (i.e. non-embryonated). An additional 12.5% laid no eggs at all, while only 33.9% of females laid fertile eggs that later embryonated indicating that these females had mated before being collected. Among this latter group were four females observed in copulo and so noted at the time of collection. This observation suggests that active mating occurred during the nocturnal hours and maximum egg viability would increase through the night. The subset of embryonated *L. mathura* egg masses averaged 717.4 eggs per female (N = 19, S.D. = 227.2, range 296–1065). Total eggs produced was the sum of a mean of 8.0 (S.D. = 4.2) enclosed larvae; 38.8 (S.D. = 36.0) infertile eggs; and 670.5 (S.D. = 227.2) fertile (embryonated) eggs per female.

Dry weights of the individual *L. mathura* egg masses (averaged 0.1642 gm (S.D. = 0.0518)) obtained prior to

digestion permitted an estimate of the egg count using a regression line expressed by $Y = 4203.64 X + 27.29$ ($R^2 = 0.92$; $P < 0.05$) where $X =$ Dry weight of *L. mathura* egg mass and $Y =$ Number of eggs contained in that egg mass.

A single *L. fumida* Butler female, another nocturnal species, failed to oviposit and was therefore likely unmated. No females of *L. monacha* (Linnaeus) appeared in the count but a single male was registered.

DISCUSSION

Four species of sympatric *Lymantria* (*dispar*, *mathura*, *monacha*, *fumida*) with coincident adult flights responded to the same commercial lighting, and were recorded in the count. Only one other species (*bantaizana*) is known to be present in the general area (Gotoh et al. 2004).

The magnitude of the 2008 moth flight can be further appreciated by viewing an on-line video at <https://www.youtube.com/watch?v=RvS-PPZ1w3w> (First viewed April 2013) which shows moths attracted to lights at a baseball field in Kuji, Iwate Prefecture. This video was uploaded by Toshiro Komatsu on the same date that Schaefer and Gries first encountered the flight in Takizawa village. Kuji is on the east coast of Iwate Prefecture at a distance of ca. 50 km NE of Takizawa. The video was taken 7–9 PM a few days before being uploaded (Toshiro Komatsu, per. comm. to Y. Higashiura) and it shows the “resemblance of snow” mentioned previously and further suggests both the overall magnitude and the regional extent of this tremendous moth flight. Although it is difficult to identify both species in the video, we believe that both *L. dispar japonica* and *L. mathura* were present, just as we documented a few days later in Takizawa.

The two most numerous species responded differently to the commercial lighting qualifying the potential threat of dispersal and possible establishment of new populations of these two important Asian moths (Pogue and Schaefer, 2007). For *L. d. japonica*, in which males are diurnally active, the males were conspicuously absent in the evening flight and must have remained inactive in the darkened forested hills surrounding Takizawa. Data showed that the overwhelming majority of females had successfully mated, with nearly 94% of all females of *L. d. japonica* fertilized and carrying an average of 420 eggs per female. Field observations by PWS over many seasons beginning in 1975, indicates that mating occurs during afternoon hours preceding any possible evening flight by females. This strategy suggests a very high dispersal potential with a corresponding probability of establishment in a new environment, either on a local or global scale.

TABLE 1. Numbers and sex ratios of lymantriine moths at commercial lighting in Takizawa Village, Iwate Prefecture, Honshu, Japan, during the night of August 5, 2008.

<i>Lymantria</i> spp.	Male	Female	Sex Ratio (M:F)
<i>mathura</i>	620	97	86.5 : 13.5 (6.4 : 1)
<i>dispar japonica</i>	5	194	2.5 : 97.5 (1 : 39)
<i>monacha</i>	1	0	—
<i>fumida</i>	0	1	—

In contrast, for *L. mathura*, in which males are nocturnal, both sexes responded to the commercial lighting with a much higher percentage of males compared to females (Table 1). Progression of the normal flight season might come into play in these species as in both cases, males generally eclose before females. To what extent earlier male emergence might change with the progression of the flight season remains unknown. For *L. mathura*, there was no evidence of behavioral dimorphism in response to lighting as there was in *L. d. japonica*.

It is interesting to note that in a somewhat analogous situation in the Russian Far East (involving a different subspecies of *L. dispar* and different lighting sources), the responses to commercial lighting sources were dissimilar (Wallner et al. 1995). For *L. mathura* the male:female ratio was similar to that in Japan but for *L. dispar asiatica* Vnukovskij in Russia the ratio was reversed. Wallner et al. (1995) reported the male:female ratio to be 11:1 while in Takizawa it was 1:39. It may also have resulted from the light source being positioned reasonably close to the moth infested forest and the artificial lighting may have simulated daylight conditions and stimulated males to become active and induced flight to the light sources. It remains unclear why these differences resulted, but it may also be due to subspecific behavioral differences or reactions to different lighting qualities, particularly since in Russia, the greatest capture of moths was at fluorescent blacklight lamps.

Regarding female viability at the time of flight, specimens examined indicated that only 34% of *L. mathura* females but nearly 94% of *L. d. japonica* females were viable at the time of capture. Since female *L. d. japonica* call diurnally and the males respond and mate, it is understandable that by evening most of the females will have mated. Mated females then are likely to take flight during the evening of the day they eclose (Charlton et al. 1999). With *L. mathura*, a strictly nocturnal species, eclosure of adults occurs during the afternoon (based on numerous observations of recently eclosed females on tree boles over many years) but mating does not occur in daylight hours. Flight follows

within a few hours after sunset, which may help to explain why so many of the collected females were infertile. All evidence suggests that the percentage of fertilized females will likely increase as night progresses. This was further suggested by a field notebook entry on the night of August 4–5 that in Takizawa more *L. mathura* pairs of moths were observed in copulo with each passing hour. As for *L. mathura* fecundity, our estimate of 717.4 eggs/female is appreciably more than the 258 eggs/egg mass recorded in Korea (Lee and Lee, 1996). We surmise, based on these two different estimates, that once mated, females may distribute their full egg complement among 2 or 3 separate egg masses.

Recent experiments demonstrate antagonistic effects of *L. dispar* pheromone on attraction of male *L. mathura* (Gries et al. 2009a) but temporal differences in behavior between these two species tend to minimize any antagonism or interference with their respective pheromone communication systems even though they share the same habitat and compete for the same food sources (Nishio, 2000; Schaefer 2012). The important differences in behavioral events then result in females of the two sympatric species arriving at commercial lighting in different physiological conditions, mated for *L. d. japonica* and unmated for *L. mathura*. The importance of oviposition as the next behavioral event has a significant bearing on egg mass placement and the possible risk of invasion to new habitats.

The field trip in 2009 was expected to reveal some of the nocturnal flight activity observed in 2008, although the visit to Takizawa occurred slightly later in the season (August 10–20), there was no evidence of a 2009 moth flight. Walls, signs, storefronts etc. showed no evidence of new egg masses as they had the previous August. The outbreak had apparently collapsed during the 2009 spring season. Katsunori Nakamura, (Forestry and Forest Products Research Institute, Morioka), a local forest entomologist, indicated that early in the season there had been numerous larvae of both species present, but that diseases (likely both fungus and virus) had dramatically decimated existing populations resulting in too few adults to produce a 2009 moth flight.



FIGS. 1–4. (1, 2) Nocturnal illustrations of illuminated commercial surfaces on which moths had settled in Takizawa, Iwate Prefecture, Japan, August 1–6, 2008. Identifiable are both sexes of *Lymantria mathura* and lesser numbers of females only of *Lymantria dispar japonica*. (3) Accumulated *L. d. japonica* egg masses from females that had flown to Mizunashi school building lights during the same week. Note variability in individual egg mass color. (4) Morning after results of a 2013 *L. d. japonica* moth flight at Motomachi, Ono City, Fukui Prefecture, Japan. Photo taken July 27, 2013 by Mizuki Mizutani and used with permission. (Photos 1–3 by Paul Schaefer).

Evidence of a 2013 mass flight (even though apparently only *L. d. japonica*) (Fig. 4) suggests that similar mass flights may occur more frequently than first thought. It would be helpful to know just what factors are responsible for driving population outbreaks that result in the observed mass flights of females, and more particularly, do all females participate or is it restricted to only a subset of dispersal capable females.

The conspicuous lack of an adult moth flight in 2009 also illustrates the dramatic interannual fluctuations in the population dynamics of lymantriine moths, in this case, both *L. d. japonica* and *L. mathura*. In nearly 40 years of experience partly described in Schaefer (1989),

PWS has observed defoliating population levels of other lymantriine moths including *L. d. asiatica* Vnukovskij in Mongolia and Korea; *L. d. dispar* (L.) in New England; *Orgyia cana* Henry Edwards in California; *Gynaephora rossii* (Curtis) in the alpine zone of Mt. Daisetsu (elev. 2290 m) and *Ivela auripes* (Butler) both in Hokkaido, Japan; *Leucoma salicis* (L.) in Maine; *Lymantria fumida* Butler and *L. lucescens* (Butler) both in Honshu, Japan; *Euproctis chrysorrhoea* (L.) in Maine and Massachusetts; *Lymantria xyliina* Swinhoe in Taiwan; and *Orgyia antiqua badia* Henry Edwards locally in British Columbia, Canada. Such widely distributed outbreak events tend to reinforce a contention that

population outbreaks are an integral part of the population dynamics of lymantriine moths. To understand why and how such outbreaks appear and then often abruptly vanish, as experienced in Takizawa, is clearly a subject requiring further long-term study.

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