

Report on Female Flight Activity of the Asian Gypsy Moth, *Lymantria dispar* (Lepidoptera: Lymantriidae) and Flight Suppression with a Yellow Light Source in Japan

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Abstract: The flight duration of the female *Lymantria dispar* in Japan was observed in a net cage (H 1.9 m, W 1.3 m, D 2.9 m) under seminatural conditions at 17:00–21:00 in May–July in 2007–2009. Flight distance was estimated from the duration data and flight speed data obtained from the former test (21.3 m/min.). Virgin and mated females of 4 different localities were examined for the tests. As a result, the duration and distance at 3 localities (Chiba, Kobe, and Hachinohe, N=108) were estimated as about 10 min. and 200 m. In contrast, those of Tomakomai (N=29) were about 30 min. and 600 m. The mean durations were not significantly different between virgin and mated female in the same localities. Next, the attractiveness and suppressive effect of yellow fluorescent light (Y) on nocturnal flight compared with black light (B) were examined in the same cage at 19:00–21:00 in May–July in 2007–2009. Both sexes were attracted by B, whereas Y showed a little attraction. In addition, female flight was strongly suppressed under Y lighting, and the effect was maintained under Y+B lighting.

Key words: *Lymantria dispar*, flight, nocturnal behavior, physical control, phototaxis

Introduction

Recently, KEENA *et al.* (2008) evaluated world distribution of female flight and genetic variation in *Lymantria dispar*. They showed that females capable of strong directed flight were found particularly in strains originating from Asia and Siberia, whereas it was never observed in strains from the United States and southwestern Europe. They also stated that 94% of the individuals were accurately assigned to their broad geographic group of origin (North America, Europe, Siberia, and Asia) by DNA analyses (mtDNA, nuclear DNA, and microsatellites).

Until now, female flight activity of the Asian gypsy moth (AGM) of the Japanese population is known to have a peak period, 1 to 2 hours after sunset, and they change behavior to oviposition afterward (KOSHIO, 1996; IWAIZUMI *et al.*, 2010). The flight speed of the AGM was estimated as 21.3 m/min. by measurement of time and flight tract of a female, using the video function of a digital camera (IWAIZUMI *et al.*, 2010). However, only a little data has been obtained on flight duration. Thus we investigated female flight duration of the AGM in a net cage in several locality samples in Japan, and estimated flight distance to determine the suitable control area to be covered for the suppression of the AGM.

In Japan, it was well documented that AGM males and females had positive phototaxis and were attracted by light sources (KENDA, 1959). To avoid the attachment of AGM egg masses to vessels, control measures to suppress female flight behavior to light are expected. WALLNER *et al.* (1995) showed that illumination with UV (ca. 480 nm) cut filter reduced AGM attraction in the Russian Far East. In addition, a physical control method using yellow fluorescent light to suppress the behavior of nocturnal insects has been developed and utilized in the cultivation of flowers and vegetables (KONO and YASE, 1996). Thus, whether yellow light can suppress AGM flight was examined in a net cage.

Materials and Methods

1. The experiment on the flight activity of the female AGM

(1) Insects

The moths were prepared by collecting egg masses from four localities (Chiba, Kobe, Hachinohe, and Tomakomai) in 2006–2008. The larvae hatched from the egg masses were reared by providing them with leaves of cherry, chestnut,

and *Myrica rubra*. In 2007, we reared larvae and pupae in our department (room temperature 27–30°C and natural photoperiod). In 2008 and 2009, they were reared outdoors in a plastic case with a screen net, or in the insect rearing room (Koito Co., PCSH-3, 20–25°C, 40–80% relative humidity (RH), 16L:8D) from egg masses laid by adults in 2007 and 2008. After emergence, they were kept in the department until the start of experiments.

(2) Methods

Virgin and mated female adults 0–2 days after emergence were used for the test. The mated females were prepared by releasing individuals of both sexes in a small net cage (mating cage, W 30 cm, H 30 cm, D 45 cm) in the daytime to let them copulate freely.

At around 17:00, the female moths were transferred to a net cage (experimental cage, W 1.3 m, H 1.9 m, D 2.9 m) installed in a greenhouse. Then, the behaviors of individuals (1 to 6) were observed until 21:00. The forewings of the females were marked with oil marker to identify individuals, and the start and end of flights were recorded. The test was done May–July in 2007–2009. In order to check reflight after the end of each test, the position of each female was recorded and checked again the next morning, 6:00–9:00. The temperature was kept at 22–27°C with an air conditioner in the greenhouse.

2. The experiment on yellow fluorescent light to suppress AGM flight behavior

(1) Insects

Moths were prepared by the collection of egg masses or larvae from three localities (Chiba, Yokohama, and Tomakomai) in 2006–2009. Rearing conditions were the same as those of the flight activity test described above. Males and virgin females 0–2 days after emergence were used for the tests. The progenies of Chiba and Tomakomai strains were used for the experiment in 2008 and 2009.

(2) Methods

Yellow fluorescent light (Y, wavelength 500–700 nm) and black light (B, wavelength 300–400 nm) (National Co., 20 W) were tested on AGM adults. Observations of their behavior with light sources were conducted in a net cage (W 1.3 m, H 1.9 m, D 2.9 m) installed in a greenhouse. The test moths were released from a corner of the cage. After releasing one male or several virgin females, Y and/or B were turned on, and the numbers (times) attracted to the light sources were counted. In addition, the numbers of females that showed flight behavior were counted simultaneously to evaluate the suppressive effect of the light sources on flight. The tests were done from 19:00 to 21:00 under natural photoperiod conditions (May–July 2007–2009). The temperature was kept at 22–27°C by an air conditioner in the greenhouse.

Experiment 1: Male response to the light sources

The behavior of males was observed under the following three lighting conditions (positions) of Y and B. The attraction of males to Y or B was counted as one time when the male rested close to the lights. Trials were done repeatedly on the same male 10 or 20 times. Chiba and Yokohama strains were used for this experiment.

- (1) Separate setting of Y and B: Y and B were set separately at opposite corners of the cage.
- (2) Close setting of Y and B: Y and B were set close together (50 cm from each other) on the lateral side of the cage.
- (3) Independent setting of Y or B: Y or B were set at the center of the cage suspended on a cherry branch.

Experiment 2: Virgin female response to the light sources

Y and B were set 50 cm apart, near the middle of the lateral side (H 1.9 m, W 1.3 m) of the cage. These lights were turned on and off in the following manner. Then, the attraction of virgin females to the lights was counted. In addition, the numbers of females that showed flight behavior were also counted simultaneously. Chiba and Tomakomai strains were used for this experiment.

- (1) Y→B: At first, Y was turned on at 19:00; then the numbers of females attracted by Y and those dispersed from releasing sites by flight were counted at 20:00. After that, Y was turned off and B was turned on; then the numbers of females attracted by B and showing flight activity were counted at 21:00.
- (2) B→Y: The opposite situation of (1).
- (3) Y: At 19:00, Y was turned on; then the numbers of females attracted by Y and showing flight activity were counted at 21:00.
- (4) B: The same as in (3), except B was used.

- (5) Y+B: At 19:00, both Y and B were turned on; then the numbers of females attracted by Y+B and showing flight activity were counted at 21:00.

Results

1. The experiment on the flight activity of female AGM

The frequency distributions of flight duration (min.) of the Chiba strain are shown in Fig. 1. In the case of virgin females, a duration within 15 min. was most frequent (23), and the longest record was 35 min. In the case of mated females, a duration within 15 min. was most frequent (18), and the longest record was 24 min.

Those of the Kobe strain are shown in Fig. 2. In the case of virgin females, a duration within 15 min. was most frequent (12), and the longest record was 30 min. In the case of mated females, the duration within 15 min. was most frequent (6), and the longest record was 60 min.

Those of the Hachinohe strain are shown in Fig. 3. In the case of virgin females, a duration within 15 min. was most frequent (13), and the longest record was 25 min. In the case of mated females, a duration within 15 min. was most frequent (14), and the longest record was 38 min.

Those of the Tomakomai strain are shown in Fig. 4. In the case of virgin females, a duration of within 15 min. was relatively frequent (6), and the longest record was 74 min. In the case of mated females, a duration of 31–45 min. was relatively frequent (4), and the longest record was 60 min.

Most of the mated females of all strains laid egg masses that night after their flight (usually oviposition started within one hour after flight), and never flew again. Most of the virgin females of all strains also never flew again until the next morning.

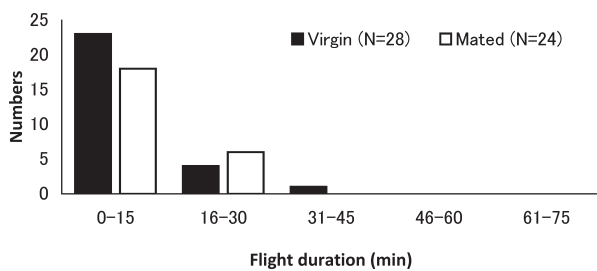


Fig. 1. Frequency distributions of the flight duration of *Lymantria dispar* (Chiba strain, 2007–2009).

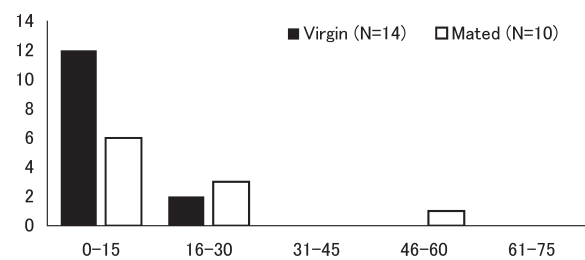


Fig. 2. Frequency distributions of the flight duration of *Lymantria dispar* (Kobe strain, 2008–2009).

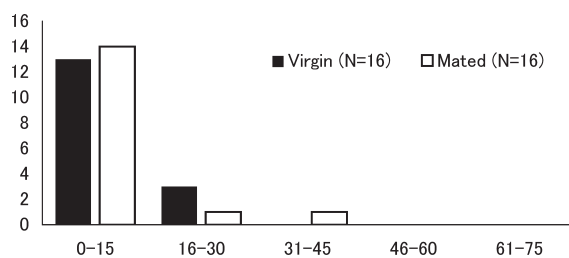


Fig. 3. Frequency distributions of the flight duration of *Lymantria dispar* (Hachinohe strain, 2008–2009).

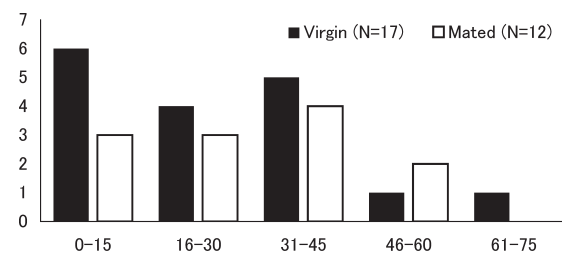


Fig. 4. Frequency distributions of the flight duration of *Lymantria dispar* (Tomakomai strain, 2008–2009).

Table 1 shows the mean flight durations (min.) of the females of four localities and the estimated flight distance (flight speed [21.3m/min.]×flight duration [min.]). In case of the three localities other than Tomakomai, both virgin and mated females flew about 10 min.; thus flight distance was estimated at about 200m. In contrast, both virgin and mated females of the Tomakomai strain flew a longer duration and distance, about 30 min. and 600m. The mean flight durations of both virgin and mated females of the Tomakomai strain were significantly different from those of the other three strains ($p<0.01$, ANOVA; $p<0.05$, Bonferroni test). Mean durations were not different significantly between virgin

and mated females of the same localities ($p>0.05$, t -test).

The female flight period in a day is shown in Table 2, based on the flight records of individuals of the four strains. The results revealed that most of them flew in the limited period of 19:00–20:00, which coincided with one hour after sunset.

Table 1. Nocturnal flight duration and estimated flight distance of female *Lymantria dispar* collected at four localities in Japan (2007–2009)

Locality	Virgin/Mated	N	Flight duration (min)	Flight distance* (m)
			(Mean $\pm$ SD)	(Mean $\pm$ SD)
Chiba	V	28	10.11 $\pm$ 8.77a	215.34 $\pm$ 186.80
	M	24	11.96 $\pm$ 5.45a	254.75 $\pm$ 116.09
Kobe	V	14	8.79 $\pm$ 7.16a	187.23 $\pm$ 152.51
	M	10	16.70 $\pm$ 16.46ab	355.71 $\pm$ 350.60
Hachinohe	V	16	7.69 $\pm$ 8.02a	163.80 $\pm$ 170.83
	M	16	8.81 $\pm$ 9.49a	187.65 $\pm$ 202.14
Tomakomai	V	17	27.47 $\pm$ 17.33b	585.11 $\pm$ 369.13
	M	12	30.92 $\pm$ 15.74bc	658.60 $\pm$ 335.26

*Flight speed (21.3 m/min) $\times$ Flight duration (min)

Values with the different letter (a, b, c) are significantly different ($p<0.05$, Bonferroni test).

Table 2. Numbers of female *L. dispar* flying in each 30-minute period after sunset, in a net cage, May–July 2007–2009, Yokohama City.

Period	No. flights observed			
	Chiba st.	Kobe st.	Hachinohe st.	Tomakomai st.
18:30–19:00	0	1	1	0
19:00–19:30	34	21	14	15
19:30–20:00	11	1	11	14
20:00–20:30	0	1	0	0
20:30–21:00	0	0	0	0
Total	45	24	26	29

2. The experiment on yellow fluorescent light to suppress AGM flight behavior

Experiment 1 (males)

The results are shown in Table 3. In all tests, males were exclusively attracted to black light except in one case. Thus, it was considered that Y did not have attractiveness for AGM males.

Experiment 2 (females)

The results are shown in Table 4. In both tests 1 and 2, which were conducted by changing the light sources, Y and B indicated that B has attractiveness for females (Test 1: 5/28, Test 2: 6/37) whereas Y has none. In addition, under Y lighting, only one female showed flight, whereas most females flew under B lighting (Test 1: 25/28, Test 2: 27/37). This result indicated that Y had suppressive effect on the flight behavior of the female AGM.

The above effects of Y and B were also shown in tests 3 and 4, which examined Y and B independently. However, some females were attracted by Y (Chiba: 3/26, Tomakomai: 1/21) or flew under Y lighting (Chiba: 3/26, Tomakomai: 6/21), indicating the suppressive effects of Y were not always perfect.

Next, Test 5, which was conducted by setting Y and B close together, indicated that the suppressive effect of Y on the flight of female AGM was also maintained under the coexistence of B.

Table 3. Comparison of the attraction of male AGM *Lymantria dispar* to yellow fluorescent light (Y) and black light (B) light sources in a net cage.

Test	Position of Y and B	No. of trials (No. of individuals)	Number of attractions	
			Y	B
1	separate	30 (3)	0	30
2	close	40 (4)	0	40
3	independent	30 (3)	1	30

Test 1: Y and B were set separately at opposite corners of the cage.

Test 2: Y and B were set closely (50 cm apart) on a lateral side of the cage.

Test 3: Y or B were set in the center of the cage.

All tests were done from 19:00 to 21:00 in June–July 2007 and 2009.

Table 4. Comparison of the attraction of female AGM, *Lymantria dispar* to yellow fluorescent light (Y) and black light (B) light sources in a net cage.

Test	Situation	Strain	No. trials	Number of attractions (%)			Number of flights (%)		
				Y	B	Y+B	Y	B	Y+B
1	Y → B	Chiba	28	0 (0)	5 (17.9)		1 (3.6)	25 (89.3)	
2	B → Y	Chiba	37	0 (0)	6 (16.2)		0 (0)	27 (73.0)	
3	Y	Chiba	26	3 (11.5)			3 (11.5)		
		Tomakomai	21	1 (4.8)			6 (28.6)		
4	B	Chiba	26	8 (30.8)			23 (88.5)		
		Tomakomai	9	7 (77.8)			9 (100)		
5	Y+B	Chiba	60	1 (1.7)			5 (8.3)		
		Tomakomai	17	3 (17.6)			3 (17.6)		

All tests were done from 19:00 to 21:00 in May–July 2008 and 2009.

Discussion

1. Experiment on the flight activity of the female AGM

From the results, most females from 3 localities, other than Tomakomai, flew within 30 minutes, thus the distance was 600 m at most. There was no significant difference between virgin and mated females.

In contrast, about half of the females of the Tomakomai strain flew more than 30 minutes, and the longest record was 75 min. The mean flight distance and the longest record were estimated about 600 m and 1600 m, respectively. The Tomakomai strain had high flight performance compared to other strains, and thus we have to consider wide-area survey and control against them. INOUE (1982) stated that *L. dispar* in Hokkaido had the characteristics of small body size and pale wing color compared to individuals in Honshu, Shikoku, and Kyushu. It is interesting that a possible difference in flight activity between Hokkaido and other regions is shown in this study.

However, we never observed females that could fly long distances up to 100 km (KEENA *et al.*, 2008) in this experiment. Recently, LIEBHOLD *et al.* (2008) investigated the distribution of AGM egg masses in Kanazawa City, Japan, and clarified that the egg masses were very abundant within 1 km from the edge of forest land. From this evidence, we consider that AGM populations in Japan do not have such traits of long distance flight.

The flight data of *L. dispar* is compared with those of *Spodoptera litura* (NODA and KAMANO, 1988) and *Carpocapsa sasakii* (ISHIGURI and SHIRAI, 2004) in flight mill experiments. In flight speed, *S. litura* is the fastest (50–60 m/min.), *L. dispar* (20 m/min.) and *C. sasakii* (25 m/min.) are considered to be nearly equal, although the methods are different among the three species. As for flight duration, *C. sasakii* (600 min.) is the longest, followed by *S. litura* (200 min.). That of *L. dispar* (10–30 min.) is considered to be much shorter than for the other two species. In flight distance, *C. sasakii* (25 m/min. × 600 min. = 15 km) is the longest, followed by *S. litura* (50 m/min. × 200 min. = 10 km). That of *L. dispar* (0.2–0.6 km) is considered to be much shorter than the other two species. In addition, *L. dispar* females lay eggs within three days after emergence, and never fly again until their death (IWAIZUMI *et al.*, 2010), while females of *S. litura* and *C. sasakii* are known to maintain their flight activity for more than five days after emergence (NODA and KAMANO, 1988; ISHIGURI and SHIRAI, 2004). Therefore, the flight period of the *L. dispar* female in her life is also shorter than

those of *S. litura* and *C. sasakii*. From these comparisons, it is apparent that the flight ability of the *L. dispar* female is inferior to that of the other two species.

From the viewpoint of female flight control, the flight period in a day is critical. In this experiment, it is shown that most of the female moths fly 19:00–20:00. At present, how to control AGM in port areas, especially protecting vessels from oviposition of egg masses by females, has become an important issue for domestic port authorities (YOKOCHI, 2007). We suggest light sources of vessels and/or port areas should be checked in the period 1 to 2 hours after sunset, and any control measures be taken if the AGM is discovered.

2. The experiment on yellow fluorescent light to suppress flight behavior of the AGM

From the results and former reports, it was clarified that the AGM has the habit of being attracted by light sources, as other nocturnal insects are. The yellow fluorescent light that cut out ultraviolet radiation was shown to have a suppressive effect on the flight of the female AGM.

In the tests on females, they were kept 3m away from the yellow light source where the light intensity was estimated as 10 lux (National Co.). As for the light intensity to suppress the nocturnal behavior of Noctuidae, *Spodoptera litura*, *S. exigua*, and *Helicoverpa armigera*, KONO and YASE (1996) described that a minimum of 1 lux was necessary for effective control. According to the illumination standards of the Japanese Industrial Standard (JIS), illumination in a yard in a port area must be kept at a minimum of 20 lux. From these facts, the yellow fluorescent light and other light sources that cut UV radiation (e.g. sodium lamps) may be useful for behavioral control of the AGM in port areas. In the future, we think it is worthwhile to test these light sources for the practical control of the AGM in the field.

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和 文 摘 要

日本産アジア型マイマイガ（AGM）雌成虫の飛翔能力
および黄色光源が飛翔活動に及ぼす影響

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日本産 AGM 雌成虫の日没後飛翔時間を網室内（高さ 1.9m、幅 1.3m、奥行き 2.9m）で測定し、以前の調査で得られている飛翔速度データ（分速 21.3m）をもとに飛翔距離を推定した。供試虫は 4 産地から卵塊を採集し飼育・羽化した成虫を用いた。その結果、千葉、神戸、八戸産雌成虫（合計 108 個体）の飛翔時間は平均約 10 分、飛翔距離は約 200m と推定された。一方、苫小牧産雌成虫（29 個体）の飛翔時間・距離は平均約 30 分、600m と推定された。い

ずれの産地でも処女雌、既交尾雌の飛翔時間に有意差はなかった。次に黄色蛍光灯による AGM の誘引抑制と飛翔抑制効果を同一網室内で調査した。黄色蛍光灯（Y）とブラックライト（B）を単独または併用して点灯し、雌雄成虫の誘引と飛翔活動を観察した結果、雌雄とも Y に比べて B に多く誘引された。また、雌については Y 点灯時に飛翔活動が強く抑制され、Y と B を同時点灯した場合でもその効果は維持された。