

Differences in Wing Morphometrics of *Lymantria dispar* (Lepidoptera: Erebidae) Between Populations That Vary in Female Flight Capability

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ABSTRACT All male gypsy moths, *Lymantria dispar* L., are capable of strong directed flight, but flight in females varies, increasing from west to east geographically across Eurasia. To better understand how the wings differ between female flight capable and flightless strains, a wing morphometric analysis of 821 gypsy moths from eight geographic strains (three *Lymantria dispar dispar* L., four *Lymantria dispar asiatica* Vnukovskij, one *Lymantria dispar japonica* Motschulsky) was performed. Body mass; length and width of both fore- and hindwing; and wing area, aspect, and loads were measured on both sexes from each strain. Gypsy moths were sexually dimorphic; females had a higher wing load, larger aspect ratios, bigger wing area, and heavier body mass than males. Wing loads of females, but not males, differed significantly among geographic strains and were lower in flight capable strains. Wing aspect was less variable within each sex among the strains. Female fore- and hindwing area were both larger in strains with strong directed flight capabilities compared with flightless strains, suggesting both fore- and hindwing areas play significant roles in flight. A logistic regression model using female forewing length and wing load correctly predicted the female flight capability of the source strains >97% of the time and may be a useful tool to use in conjunction with molecular methods for detecting introductions of Asian gypsy moth. None of the male morphometric wing characters were found to reliably predict the female flight capability of the source population.

KEY WORDS gypsy moth, wing morphology, flight capability, Erebidae

Gypsy moth is one of the most serious defoliating forest pests, capable of widespread outbreaks in temperate Holarctic regions (Davidson et al. 2001, Orozumbekov et al. 2009). The gypsy moth, *Lymantria dispar* L., has three recognized subspecies, *Lymantria dispar dispar* L. called the European gypsy moth, *Lymantria dispar asiatica* Vnukovskij called the Asian gypsy moth, and *Lymantria dispar japonica* Motschulsky called the Japanese gypsy moth (Pogue and Schaefer 2007). Asian gypsy moth is distributed in Asia east of the Ural Mountains, widely in Russia, China, Japan, and Korea. Japanese gypsy moth is distributed on all main islands in Japan, but only in a limited area on Hokkaido (Keena et al. 2008). European gypsy moth was originally found in Europe, was accidentally introduced into North America in 1869 (Forbush and Fernald 1896), and has since spread with the leading edge of the infestation reaching Maine, Minnesota, Wisconsin, Illinois,

Indiana, Ohio, West Virginia, Virginia, North Carolina, Ontario, Québec, New Brunswick, and Nova Scotia. The infestation of European gypsy moth in the eastern United States is too well established to eradicate, but measures to slow the spread and control local outbreaks are being taken (Tobin 2008).

Asian gypsy moth is considered to pose a more significant threat globally, owing to its preference for a broader range of host species (Baranchikov 1989), shortened egg chill requirements (Keena 1996, Wei et al. 2014), and the flight capability of females (Keena et al. 2008). However, female gypsy moths capable of strong directed flight were also found outside of Asia and Siberia in the northeastern part of Europe, and Japanese gypsy moth females also are capable of flight (Keena et al. 2008). Flying female gypsy moths are attracted to lights in port areas and their egg masses have been intercepted in North America on ships and their cargo (U.S. Department of Agriculture–Animal and Plant Health Inspection Service–Plant Protection and Quarantine [USDA-APHIS-PPQ] 2014). To prevent introductions of flight capable female gypsy moths from Asia, international collaborations have been set up to monitor port areas and certify ships leaving these ports during moth flights are free of egg masses (USDA-APHIS-PPQ 2014). Pheromone traps are used in North America to monitor around ports for introductions and to delimit established populations so they can be eradicated. For regulatory purposes, the USDA

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refers to any biotype of *L. dispar* possessing female flight capability as the Asian gypsy moth, but in this article, only the *L. dispar asiatica* subspecies will be called Asian gypsy moth. Molecular methods are used to determine the origin of male gypsy moths caught in pheromone traps, as reliable morphological characters are lacking, but no adequate methods currently exist that can predict the female flight capability of the source population (Keena et al. 2008). Intraspecific polymorphisms in wing length, flight muscles, flight behavior, or all have been shown to exist between dispersing and nondispersing insect individuals (Harrison 1980). In female gypsy moths the two biotypes (flight capable and flightless) have been shown to differ in wing size, size of flight muscles, and preflight behaviors (Shields et al 1997; Keena et al. 2001, 2008). Previous studies of gypsy moths have successfully used discriminant analysis to classify females based on wing size (forewing length and hindwing width) and a segregate for body mass (maximum abdominal width) into three flight capability groups (flightless, flight capable, or F₁) that exhibit a gliding-type flight (Keena et al. 2008). No analysis of wing shape (aspect ratio) or wing loading (body mass divided by total wing area) has been done for gypsy moth females. Wing shape has been shown to have a large impact on the flight performance in other Lepidoptera; longer slender wings have been associated with long-duration flight and short broad wings with slow or more agile flight (Betts and Wootton 1988, DeVries et al. 2010). As wings are complex structures, wing shape and size do not necessarily scale proportionally with body size, so both need to be evaluated independently (Outomuro et al. 2013). In addition, there has been little done to evaluate the male gypsy moths from the three subspecies to determine if there are any male traits that are correlated with the female flight capability of the source population. For example, it might be possible that males from populations with females capable of flight have longer wings than those from populations with flightless females if the inheritance of the trait is purely autosomal.

In this study, a morphometric approach was utilized to assess variation in wing shape and size for both male and female gypsy moths among eight geographic populations (two European gypsy moths, one Japanese gypsy

moths, and five Asian gypsy moths) that vary in female flight capability. This morphometric information was then used to determine if there are female, male, or both characters that could be used to reliably distinguish between populations with and without females capable of sustained flight.

Materials and Methods

Gypsy Moth Strains and Rearing. We analyzed 821 specimens (37–73 individuals of each sex from each strain) of *L. dispar* sourced from eight sites: two sites in China, one site in Japan, two sites in Russia, two sites in Europe, and one site in the United States. Details of each of the eight strains are given in Table 1. Based on the recent review of *Lymantria* (Pogue and Schaefer 2007), the JN strain is the *japonica* subspecies, all Russian and Chinese strains are the *asiatica* subspecies, and all remaining strains should be the *dispar* subspecies. All gypsy moths were transported under permit to the Forest Service quarantine facility in Ansonia, CT. Voucher specimens for each strain were deposited at the Entomology Division, Yale Peabody Museum of Natural History, New Haven, CT.

Larvae from 10 to 14 different egg masses were reared to produce the adults used in this study. Larvae were held in walk-in environmental chambers maintained at 25 ± 1°C, 60 ± 5% relative humidity, and a photoperiod of 16:8 (L:D) h. Larvae were reared in groups of eight (one to two groups per egg mass) in 177-ml clear plastic cups with unwaxed paper lids for 35–47 d. Each cup contained 90 ml of high wheat germ diet (Bell et al. 1981) made with Wesson salt mix without iron, and adding the appropriate amount of amorphous FePO₄ per liter of diet. The following amounts of amorphous FePO₄ per liter of diet were used: 0.21 g for the CJ, CR, JN, and RM strains; 0.17 g for the LJ and RS strains; and 0.13 g for the UC and KG strains. Pupae were harvested, sexed, and stored by sex, egg mass (family), and strain in 473-ml unwaxed squat paper cups with clear plastic lids until adult eclosion. Adults were removed daily, weighed, and measured.

Wing Measurement and Morphometrics Calculations. Moths were allowed to fully harden their expanded wings before measurements were taken.

Table 1. Approximate location (latitude and longitude) of source populations and designations for strains of gypsy moth evaluated in this study, arranged by longitude from east to west

Strain	Country	Collection location	Latitude	Longitude	Sample size	
					Female	Male
JN ^a	Japan	Nagoya, Honshu	35.15N	137.08 E	39	73
RM ^a	Russia	Mineralni, Primorski	44.10 N	133.15 E	57	46
CR	China	Harbin, Heilongjiang	45.78 N	126.61 E	42	44
CJ	China	Yanzikou, Beijing	40.32 N	116.15 E	55	60
RS ^a	Russia	Shira, Khakassi	54.41 N	90.00 E	55	54
LJ ^a	Lithuania	Juodkrante, KuzsinNezijos	55.31 N	21.06 E	37	63
KG ^a	Germany	Knigsberg in Bayer Bavaria	50.10 N	10.34 E	38	45
UC ^a	United States	Bethany, New Haven County, CT	41.25 N	73.00 W	67	46

^a These strains are the same ones that were used in Keena et al. 2008 and more details on the collection date and number of egg masses used to start the strain can be found there.

The CR strain was started from 15 egg masses collected in 2012 and the CJ strain was started from 15 egg masses collected in 2011.

Body mass was determined by weighing 1–24-h-old moths in pretared glassine envelopes on an electronic balance accurate to 0.0001 g. Moths were then frozen for 24 h before the right fore- and hindwings were carefully removed from each individual at the wing base using fine forceps and scissors. The two wings from an individual were then secured to a foam board using insect pins prior to taking measurements. Using a digital caliper, the following measurements were taken: forewing length, forewing width, hindwing length, and hindwing width. The wing landmarks used for each measurement were as follows: forewing length was from base of the costal vein to the tip of the apical angle (approximately the apical end of the fourth radial branch); forewing width was from the apical end of the third radial branch to the anal margin (a line approximately parallel to the body); hindwing length was from the base of the cubital vein to the apical end of the third medial branch; and hindwing width was from the apical end of the subcostal vein to the anal end of the third anal branch (Fig. 1).

The detached wings were then photographed with a Canon 450D (EF-S18-55 mm, Tokyo, Japan) digital camera while they were held flat by a glass plate. A black background was used for female wings and a white one for male wings to improve the image quality and contrast. A ruler was included in each photo on the same plane as the wings to provide a tool for

calibrating the relationship between pixels and distance. To ensure the same zoom and same height above glass, the camera was set as follows: camera height: 34 cm above glass; zoom: 35 mm; macro-setting: ♂ 1/100 exposure, 5.6 f-stop; ♀ 1/60 exposure, 4.5 f-stop. The digital images were labeled with individual number, sex, and source population.

The wing area was determined using the software IMAGEJ 1.47 (Ferreira and Rasband 2012). The images were first converted to binary and the background color was deleted to leave white space around the black wing image (Fig. 1). The outline of the image was defined to eliminate minor damage to the margin of the wings. With the ruler of known scale, the pixels per mm was calibrated using the scale on the ruler in the image and then forewing and hindwing area was obtained. The total wing area was calculated as twice the measured right forewing and hindwing areas combined. Wing aspect ratios were calculated as the square of forewing length, divided by the forewing area. Wing loading was calculated by dividing body mass by wing area.

Statistical Analysis. The male and female wing data were analyzed separately. The fit of each data set to various distributions was evaluated using PROC UNIVARIATE (SAS Institute 1999, Cary, NC). The Shapiro–Wilk and the Anderson–Darling test were used to assess normality. However, in cases where no distribution met the normality assumption, the distribution that gave the best fit based on the Kolmogorov–Smirnov test was used. The following dependent continuous variables were analyzed in PROC GLIMMIX (SAS Institute 1999): wing load, forewing aspect, hindwing aspect, total wing area, forewing area, hindwing area, body mass, forewing length, forewing width, hindwing length, and hindwing width. A completely randomized design was used with geographical strain as the fixed effect and the maternal family (egg mass) as the random effect. The normal distribution with an identity link was used for the hindwing aspect, forewing area, hindwing area, forewing length, forewing width, hindwing length, and hindwing width. The gamma distribution with a log link function was used for the wing load, wing aspect, total wing area, and body mass. The gamma distribution was chosen because it gave the best fit to these data, which had long right tails because of overdispersion. The body mass measurements were multiplied by 1,000 and the load was multiplied by 100 before analysis to avoid taking the logs of decimal numbers. For each model, residuals were evaluated for normality and the homogeneity of variance was assessed using Levene's test. The group option was used in the random statement to account for unequal variances among geographical strains (all the female parameters except hindwing area and aspect, and all the male parameters except body mass, total wing area and forewing aspect) if they existed. Differences among means were determined by the least-squares means test with $\alpha=0.05$ and a conservative Tukey–Kramer grouping (SAS Institute 1999).

A Pearson correlation analysis (PROC CORR, SAS Institute 1999) was done independently for each sex on

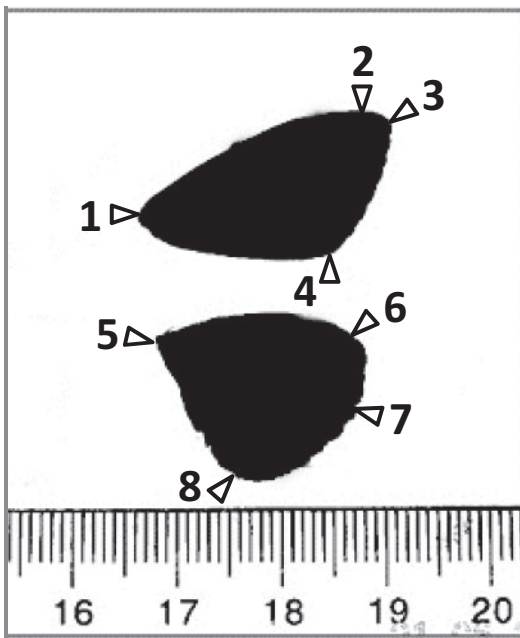


Fig. 1. The landmarks used for each wing measurement were as follows: forewing length was from base of the costal vein (1) to the tip of apical angle, approximately the apical end of the fourth radial branch (3); forewing width was from the apical end of the third radial branch (2) to the anal margin (4); hindwing length was from the base of the cubital vein (5) to the apical end of the third medial branch (7); and hindwing width was from the apical end of the subcostal vein (6) to the end of the third anal branch (8).

the wing morphometric characters (wing load, forewing aspect, hindwing aspect, total wing area, forewing length, forewing width, hindwing length, and hindwing width) and body mass to determine which traits were statistically independent enough (had absolute *r* values of <0.4) to be included in a model. The wing size characters were all strongly correlated and they were also correlated with body mass so forewing length was selected to represent this group. Fore- and hindwing aspect ratios were correlated with each other but not with wing load or the size characters so both wing load and forewing aspect were chosen to be included. The geographical strains were grouped by female flight capability: flightless (KG and UC), mixed flight (30% not strong fliers, LJ), and strong flight (CJ, CR, RM, RS, and JN). The flight capability classifications were either based on what was reported by Keena et al.

(2008) or on MAK unpublished data. Half of the individuals from each strain within the flightless and the strong flight groups were selected for use in creating a model to distinguish between the two groups and the other half of the data were held to evaluate the model. The mixed flight group was also retained for use in model evaluation. Separate logistical regression analyses (PROC LOGISTIC, SAS Institute 1999) were run for each sex with flight group as the response variable and all possible combinations of forewing length, wing load, and forewing aspect ratio as the continuous variables to assess their predictive power. The results of the best model and the one for forewing length alone are presented because forewing length is easy to measure, is often mentioned as a distinguishing trait for females, and wing loading cannot be calculated for the dead dry male moths trapped in pheromone traps, which may be the only material available for use in identifying the subspecies of an introduction. Akaike's information criteria (AIC; Akaike 1973) were used to evaluate the fit of the models: the smaller the AIC value the better the fit. The maximum likelihood parameter estimates in each model were used to calculate the probability of female flight (positive values indicating flight) for each male and female moth from the reserved set and the percentages of correct and incorrect classifications were determined.

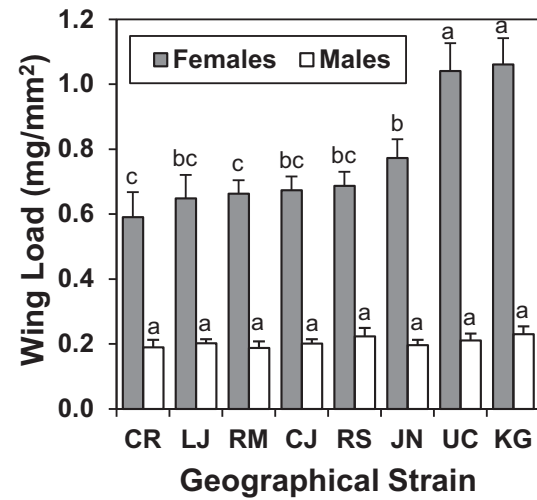


Fig. 2. Comparison of wing load among eight gypsy moth strains (geometric mean ± 95% CI error bars), which are ordered by increasing wing load. Different letters indicate significant differences in wing load among gypsy moth strains within each sex based on Tukey–Kramer's multiple comparison tests at the 5% significance level.

Results

The male and female *L. dispar* were sexually dimorphic both in wing morphometrics and colors (females white and males brown; Tables 2 and 3). Males had smaller wings, weighed less, and had smaller wing loads than females. The wing load of females was significantly different among geographical strains ($F = 31.02$; $df = 7, 82$; $P < 0.0001$; Fig. 2). CR and KG females' wing load was significantly greater than that of females from the other strains. JN females' wing load was significantly greater than CR and RM females' wing loads. There were no significant differences between strains in wing loads of males ($F = 2.08$; $df = 7, 82$;

Table 2. Differences in female gypsy moth wing morphometrics among geographic strains

Strain	Sex	BM (g)	FWL (mm)	FWW (mm)	HWL (mm)	HWW (mm)	FWA (cm ²)	HWA (cm ²)
CR	Female	0.88 ± 0.12bc	34.78 ± 1.00bc	20.06 ± 0.53ab	25.36 ± 0.72bc	20.05 ± 0.82ab	4.46 ± 0.22b	3.32 ± 0.12c
LJ	Female	0.55 ± 0.08d	27.65 ± 0.80de	14.39 ± 0.31c	19.57 ± 0.53d	14.04 ± 0.38c	2.68 ± 0.13cd	1.82 ± 0.12d
RM	Female	1.09 ± 0.05bc	35.89 ± 0.62bc	20.44 ± 0.44a	25.42 ± 0.31c	20.99 ± 0.32ab	4.74 ± 0.16b	3.55 ± 0.12bc
CJ	Female	1.31 ± 0.05ab	38.48 ± 0.54a	21.60 ± 0.31a	28.14 ± 0.30a	21.91 ± 0.42a	5.75 ± 0.16a	3.97 ± 0.11ab
RS	Female	1.04 ± 0.05c	33.72 ± 0.52c	18.48 ± 0.25b	24.65 ± 0.30c	19.81 ± 0.31b	4.40 ± 0.10b	3.26 ± 0.12c
JN	Female	1.47 ± 0.06a	38.57 ± 0.92ab	21.21 ± 0.35a	28.07 ± 0.64ab	22.05 ± 0.72ab	5.90 ± 0.26a	4.08 ± 0.13a
UC	Female	1.04 ± 0.05c	27.87 ± 0.35d	14.44 ± 0.23c	19.75 ± 0.27d	14.97 ± 0.28c	2.97 ± 0.08c	2.09 ± 0.11d
KG	Female	0.84 ± 0.08c	25.49 ± 0.56e	14.26 ± 0.44c	18.47 ± 0.48d	14.37 ± 0.44c	2.42 ± 0.11d	1.75 ± 0.12d
Statistics	Female	$F = 19.19$ $df = (7, 82)$ $P < 0.0001$	$F = 78.87$ $df = (7, 82)$ $P < 0.0001$	$F = 100.66$ $df = (7, 82)$ $P < 0.0001$	$F = 100.94$ $df = (7, 82)$ $P < 0.0001$	$F = 76.65$ $df = (7, 82)$ $P < 0.0001$	$F = 87.86$ $df = (7, 82)$ $P < 0.0001$	$F = 64.46$ $df = (7, 300)$ $P < 0.0001$

BM, body mass; FWL, forewing length; FWW, forewing width; HWL, hindwing length; HWW, hindwing width; FWA, forewing area; HWA, hindwing area. For each trait the mean ± SE is given, different letters in each column indicate significant differences between strains within each sex based on Tukey–Kramer's multiple comparison tests at the 5% significance level. The differences in the denominator degrees of freedom are due to the use of the group by strain that had to be used when variances were not equal between strains.

Table 3. Differences in male gypsy moth wing morphometrics among geographic strains

Strain	Sex	BM (g)	FWL (mm)	FWW (mm)	HWL (mm)	HWW (mm)	FWA (cm ²)	HWA (cm ²)
CR	Male	0.17 ± 0.05b	25.00 ± 0.43b	15.60 ± 0.27b	19.35 ± 0.28b	15.20 ± 0.24bc	2.44 ± 0.08bc	2.03 ± 0.06bc
LJ	Male	0.13 ± 0.05c	21.69 ± 0.22d	13.18 ± 0.14c	16.32 ± 0.18d	13.09 ± 0.16c	1.71 ± 0.04e	1.45 ± 0.03e
RM	Male	0.17 ± 0.05b	24.38 ± 0.26b	15.37 ± 0.29b	18.29 ± 0.22b	15.54 ± 0.17b	2.42 ± 0.06b	2.05 ± 0.05b
CJ	Male	0.25 ± 0.05a	28.63 ± 0.36a	17.69 ± 0.33a	21.69 ± 0.33a	17.75 ± 0.38a	3.41 ± 0.07a	2.88 ± 0.09a
RS	Male	0.18 ± 0.05b	23.14 ± 0.24c	13.79 ± 0.17c	17.27 ± 0.16c	14.53 ± 0.17cd	2.19 ± 0.04c	1.83 ± 0.04cd
JN	Male	0.25 ± 0.05a	29.08 ± 0.36a	17.89 ± 0.25a	21.78 ± 0.22a	18.27 ± 0.24a	3.52 ± 0.07a	2.99 ± 0.07a
UC	Male	0.16 ± 0.05b	21.69 ± 0.26d	13.15 ± 0.20c	16.54 ± 0.25cd	13.91 ± 0.24de	1.99 ± 0.05d	1.77 ± 0.05d
KG	Male	0.15 ± 0.05bc	21.16 ± 0.21d	13.15 ± 0.16c	16.12 ± 0.17d	13.71 ± 0.17e	1.80 ± 0.04de	1.50 ± 0.04e
Statistics	Male	$F = 26.54$ $df = (7, 341)$ $P < 0.0001$	$F = 101.97$ $df = (7, 82)$ $P < 0.0001$	$F = 73.01$ $df = (7, 82)$ $P < 0.0001$	$F = 98.80$ $df = (7, 82)$ $P < 0.0001$	$F = 67.41$ $df = (7, 82)$ $P < 0.0001$	$F = 159.05$ $df = (7, 341)$ $P < 0.0001$	$F = 90.22$ $df = (7, 82)$ $P < 0.0001$

BM, body mass; FWL, forewing length; FWW, forewing width; HWL, hindwing length; HWW, hindwing width; FWA, forewing area; HWA, hindwing area. For each trait the mean ± standard error is given, different letters in each column indicate significant differences between strains within each sex based on Tukey–Kramer’s multiple comparison tests at the 5% significance level. The differences in the denominator degrees of freedom are due to the use of the group by strain that had to be used when variances were not equal between strains.

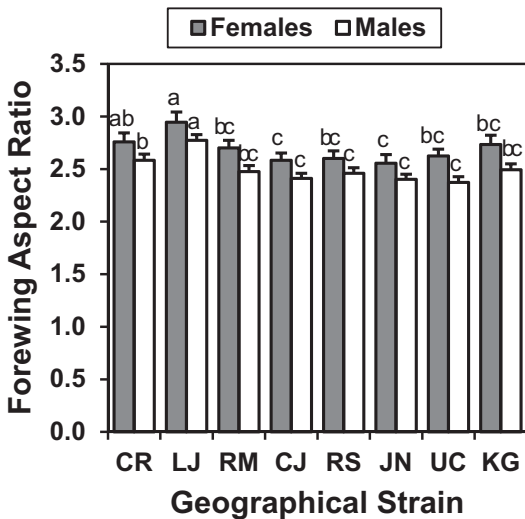


Fig. 3. Comparison of forewing aspect ratio among eight gypsy moth strains (geometric mean ± 95% CI error bars). Different letters indicate significant differences in wing aspect ratio among gypsy moth strains within each sex based on Tukey–Kramer’s multiple comparison tests at the 5% significance level.

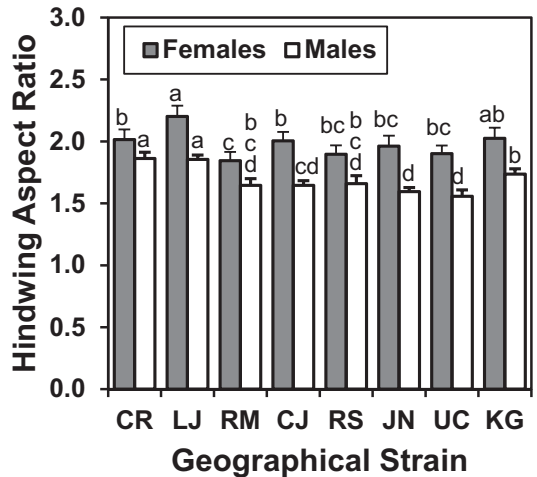


Fig. 4. Comparison of hindwing aspect ratio among eight gypsy moth strains (geometric mean ± 95% CI error bars). Different letters indicate significant differences in wing aspect ratio among gypsy moth strains within each sex based on Tukey–Kramer’s multiple comparison tests at the 5% significance level.

$P = 0.0553$), but biologically significant differences may exist.

Within each geographical strain the female moths tended to have larger wing aspect ratios than males both for fore- and hindwings. Aspect ratios differed significantly by strain for females (forewing: $F = 8.79$; $df = 7, 300$; $P < 0.0001$; hindwing: $F = 7.36$; $df = 7, 300$; $P < 0.0001$). The LJ strain had a larger forewing aspect ratio than all but the CR strain (Fig. 3) and larger hindwing aspect ratio than all but the KG strain (Fig 4). The LJ strain males also had the largest forewing aspect ratios and strain had a significant impact on the ratio ($F = 24.43$; $df = 7, 341$; $P < 0.0001$). The LJ and CR strain males had the largest hindwing aspect ratios and there was a significant strain effect on the ratio ($F = 28.19$; $df = 7, 82$; $P < 0.0001$).

The total wing area of male gypsy moths was smaller than that of female moths across all eight geographical strains (Fig. 5). Total wing area of both females ($F = 76.57$; $df = 7, 300$; $P < 0.0001$) and males ($F = 159.05$; $df = 7, 341$; $P < 0.0001$) varied significantly among strains. Individuals from the CJ and JN strains had significantly larger total wing area than all other strains in males and larger than all but the RM strain in the females. Individuals from the KG and LJ strains had significantly smaller total wing area than all other strains in males and smaller than all but the UC strain in the females. Geographical strain had a significant effect on body mass in both the female ($F = 19.19$; $df = 7, 82$; $P < 0.0001$) and male ($F = 26.54$; $df = 7, 341$; $P < 0.0001$). The body mass of JN females was significantly heavier than that of all but the CJ strain (Table 2).

LJ females weighed less than the females of all the other strains. The body mass of CJ and JN males was significantly heavier than that of males of the other strains (Table 3).

Table 4 provides the parameter estimates, odds ratios, and model fit statistics for the two best models for predicting the female flight capability of a strain based on male or female wing morphometric characters. Both female forewing length and wing loading were significant predictors of female flight capability and a model containing both had the lowest AIC fit statistic. This model correctly predicted female flight for 96.7% of the reserved females from flight capable strains and lack of flight for 98.1% of the females from the flightless strains. When the model was evaluated using the LJ females, it predicted that 97.3% would be capable of flight. When the other model that only used female forewing length was

evaluated using the reserved females, 90.2% of flight capable and 94.3% of flightless females were correctly predicted, while only 40.5% of the LJ females were predicted to be flight capable.

Of the characters evaluated, only male forewing length was found to be a good predictor of the female flight capability of the source strain (Table 4). When the model that only used male forewing length was evaluated, it correctly predicted female flight for 90.6% of the reserved males from strains with flight capable females. The model only predicted no flight for 65.3% of the reserved males from strains with flightless females. When the model was evaluated using the LJ males, it only predicted that 33.3% of the males were from a strain that the females had flight capability.

Discussion

Females from geographical strains that were known to be capable of strong directed flight had significantly smaller wing loads than the strains with flightless females. There were no significant differences between the geographical strains in the wing loads of males, although the differences may be large enough to be biologically significant. Males with lower wing loads may be capable of stronger or longer distance flight than those with higher wing loads. Within each geographical strain, the female moths tended to have a larger wing aspect ratio than males. Although wing aspect ratios did differ between strains for both males and females, the differences did not correspond with the known female flight capability of the strains. Wing load and aspect ratio are predicted to be linked to maneuverability; other Lepidoptera with low wing load and rounder wings (lower aspect ratio) have been shown to have greater maneuverability (Betts and Wootton 1988, DeVries et al. 2010). As gypsy moth males all fly in a zigzag pattern to stay within the pheromone plume to find females to mate with (Cardé and Haganan 1979), low wing loads and aspect ratios would be expected. Also, wing size (total wing area) varies considerably more than shape (wing aspect ratios) between strains for females, which would be expected if the heritability (proportion of the variation that is attributable to genetics) of these two traits

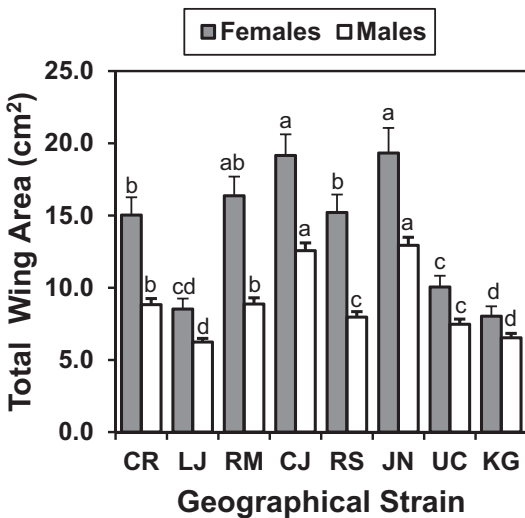


Fig. 5. Comparison of total wing area among eight gypsy moth strains (geometric mean ± 95% CI error bars). Different letters indicate significant differences in total wing area among gypsy moth strains within each sex based on Tukey–Kramer’s multiple comparison tests at the 5% significance level.

Table 4. Logistic regression parameter estimates (±SE), odds ratios (95% Wald CL), and model fit statistics for predicting the female flight capability of the strain using male or female wing forewing length (FWL) and wing loading (WL)

		Logistic models			
		Female		Male	
Parameter Estimates	Intercept	-25.57 ± 4.34	-18.54 ± 6.57	-26.88 ± 4.72	-27.01 ± 4.84
	FWL	0.85 ± 0.14	1.00 ± 0.28	1.20 ± 0.21	1.21 ± 0.21
		Wald χ^2 34.86	Wald χ^2 12.60	Wald χ^2 33.61	Wald χ^2 33.53
		df 1, <i>P</i> < 0.0001	df 1, <i>P</i> = 0.0004	df 1, <i>P</i> < 0.0001	df 1, <i>P</i> < 0.0001
Odds Ratios	WL		-0.12 ± 0.04		0.004 ± 0.03
			Wald χ^2 11.80		Wald χ^2 0.01
			df 1, <i>P</i> = 0.0006		df 1, <i>P</i> = 0.9044
AIC ^a statistic	FWL	2.34 (1.76–3.09)	2.71 (1.56–4.69)	3.34 (2.22–5.01)	3.34 (2.22–5.02)
	WL		0.88 (0.83–0.95)		1.00 (0.94–1.07)
		61.19	26.95	92.63	94.62

^aAIC, Akaike’s information criteria (Akaike 1973) used to evaluate the fit of the models, lower values are better fits.

differs, which could lead to different patterns of evolution, as was found for *Drosophila mediopunctata* (Bitner-Mathé and Klaczko 1999a,b). Further work would be needed to estimate heritability for these traits.

Average forewing length within a strain was longer in females than in males, and longer in females from *L. d. asiatica* (34–39 mm) strains than *L. d. dispar* strains (25–28 mm). This is consistent with the diagnostic female forewing lengths for the two subspecies, *L. d. asiatica* 28–41 mm and *L. d. dispar* 20–30 mm (Pogue and Schaefer 2007). Additionally, the females from the strains within the *asiatica* subspecies have longer hindwings than the females from the *dispar* subspecies, which suggests that both wings play significant roles in flight capability. This is different from the finding that only forewings are related to dispersal in *Melitaea cinxia* (L.), another lepidopteran (Breuker et al. 2007).

The logistic regression model using female forewing length and wing loading correctly predicted the female flight capability of the source strains >97% of the time, which was better than just using female forewing length alone. In fact, some females from some of the Asian strains should not be predicted to be capable of flight because flight capability is not fixed (some individuals with only gliding-type flight) in any of the populations that were evaluated (Keena et al. 2008). This model could be a useful tool (if live females are available so fresh weights can be obtained) to use in conjunction with the molecular methods to predict the flight capability of females in the source population. This would be especially useful if the molecular methods suggest the source population is from Europe because many European populations have only flightless females, while others, like the LJ strain, can have >70% of the females that can fly.

Even though all males fly, males from the Far East Asian populations (females fly) tended to have larger wings than those from Europe (some females can fly) or North America (females don't fly) when reared in a common environment. However, the logistic regression model based on male forewing length (a significant predictor of female flight) incorrectly predicted the female flight capability of the source strain frequently (up to 67% in the LJ strain) and so can not be reliably used.

Previous studies demonstrated that flight capability of female gypsy moth has a heritability of 0.60 and forewing length has a heritability of 0.70, both with no evidence of sex linkage or maternal effects (Keena et al. 2007). This would suggest that the genes for critical flight characteristics such as wing length are most likely located on the autosomes rather than mitochondrial DNA or the sex chromosomes. Male moths, all of which can fly, did not separate reliably into groups based on the female flight capability of the strains, suggesting that there has to be one or more genes on the W chromosome (females are the heterogametic sex in Lepidoptera) that directly regulate flight genes in females or indirectly regulate it by coding for sexually dimorphic body masses. Further research to identify the genes involved in flight is needed to determine exactly how flight is regulated in the two sexes.

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