

## Inheritance of Female Flight in *Lymantria dispar* (Lepidoptera: Lymantriidae)

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**ABSTRACT** A clinal female flight polymorphism exists in the gypsy moth, *Lymantria dispar* L., where female flight diminishes from east to west across Eurasia. A Russian population where females are capable of sustained ascending flight and a North American population with females incapable of flight were crossed: parentals, reciprocal  $F_1$  hybrids, double reciprocal  $F_2$  hybrids, and all possible backcrosses to both the parental lines were compared. Heritabilities were estimated using a threshold model, female offspring on female parent regressions, and joint-scaling analyses. Heritability of female flight capability measured using a free flight test was at least 0.60, and variation in wing size, muscle strength, and flight behaviors contributed to the flight polymorphism. Relative wing size varied continuously and had a heritability of 0.70. Environmental variation accounted for >90% of the variation in female preflight weight and relative flight muscle strength, as estimated by an inverted female's ability to right herself. Preflight walking behavior and early deposition of eggs were each inherited through a single gene with two co-dominant alleles. There was no evidence for sex-linkage or maternal effects in female flight capability or associated traits. Continued vigilance to exclude and eradicate introductions of strains capable of female flight in North America is warranted even in areas where no females fly, because some of the alleles needed for full flight capability may not be present in the North American populations, and some flight capability is maintained in the hybrids that could increase the rate of spread of *L. dispar*.

**KEY WORDS** female flight propensity, hybrids, *Lymantria dispar*, dispersal, inheritance

Reduction or loss of wings has occurred in most pterygote orders of insects, and many species are polymorphic for flight ability or behavior (Harrison 1980, Zera and Denno 1997). The complete range of wing reduction occurs in females of the genus *Lymantria* L. Males are all fully winged and capable of strong directed flight, which is critical in mate finding. In the Indian species *Lymantria ampla* Walker, females have only lobes for wings, and in the Indian gypsy moth, *Lymantria obfuscata* Walker, the females' wings are about one half of what would be expected for its size (Schaefer 1989). In female gypsy moths, *Lymantria dispar* L., two morphs have been identified, both with apparently fully developed wings that differ in flight capability and associated traits (e.g., size of wings, Wallner 1996; size of flight muscles, Shields et al. 1997; and preflight behaviors, Keena et al. 2001). In Japan (Koshio 1996), Russia (Mikkola 1971, Baranchikov 1989, Ponomarev 1994), and China (Schaefer et al. 1984), *L. dispar* females are capable of ascending flight and are attracted to lights at night (Kenda 1959, Baranchikov 1989, Schaefer 1989, Wallner et al. 1995).

Russian females are reported to fly distances up to 100 km (Rozkhov and Vasilyeva 1982). In Western Europe and North America (where it was accidentally introduced in 1869 from France), gypsy moth females are not capable of sustained or ascending flight (Forbush and Fernald 1896, Schedl 1936, Carter 1984, Keena et al. 2001). However, there have been reports of females gliding (while beating their wings) from trees in the United States (Forbush and Fernald 1896, Sandquist et al. 1973). Reports on flight capability of *L. dispar* in Central Europe are conflicting, and a transition zone of occasional female flight has been proposed for Eastern Europe where the morphs apparently interbreed (Baranchikov 1989). Reports for Central Europe vary from females that are "almost" unable to fly (Heß and Beck 1914), to females that exhibit gliding type flights (Balachowsky and Mesnil 1935, Schwenke 1978), to females that seldom fly and only at night (Bergmann 1953), and to females that exhibit a highly synchronous flight at dusk (Charlton et al. 1999). The result is a clinal flight polymorphism where female flight diminishes from east to west across Eurasia. However, female flight capability is not completely fixed at either end of the observed cline.

Multiple introductions into North America of strains of *L. dispar* with females capable of flight have occurred including egg masses on ships and cargo coming into the Pacific Northwest from Far East Rus-

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sian ports and flying females emerging from pupae on military equipment or troops' belongings coming into the southeast United States from Germany (Wallner 1996). Each introduction has prompted an eradication program, the largest of which occurred in 1992 and 1994 (Wallner 1996). The biggest concern over these new introductions is the capacity for female flight in the introduced strains (and possibly in hybrid offspring of interstrain crosses), which might increase the rate of spread or invalidate detection procedures for the strain already present in North America. Some strains from Asia possess additional traits that make them more threatening to North American forests than the established Western European strain, including a broader host range (Baranchikov 1989), shortened egg chill requirements (Keena 1996), and female attraction to lights that results in egg deposition on vehicles or cargo (Wallner et al. 1995). Determining the inheritance of flight polymorphism in *L. dispar* will distinguish the source of interpopulation variation (genetic or environmental) and will improve our understanding of why the clinal flight polymorphism is maintained. It is also vital to our understanding of the dispersal capability of this forest pest so that new introductions can be accurately delimited and effective management strategies can be developed.

Morphs with different flight capabilities can be encoded by different genotypes, induced by different environments or produced by variation in both genetic and environmental factors (Zera 2004). Insect flight depends on so many different biochemical, physiological, and morphological factors that the trait is most often inherited polygenically (Harrison 1980, Dingle 1984, Han and Gatehouse 1989). Previous work has shown that flight capability in *L. dispar* is reduced in F<sub>1</sub> hybrids (Keena 1994, Reineke and Zebitz 1998), but the mode of inheritance has not been fully determined. Also, there are detectable molecular differences among populations from Europe, Asia, and North America (Bogdanowicz et al. 1993, Garner and Slavicek 1996, Pfeifer et al. 1995, Schreiber et al. 1997, Reineke et al. 1999), but their relationship to behavioral traits is unknown. Russian, Siberian, and European strains are known to hybridize readily in the laboratory with *L. dispar* collected from North America (Keena 1994).

Harrison (1980) has identified three types of polymorphisms that affect flight in insects: variation in wing length, variation in the development of flight muscles, and variation in flight behavior. In this paper, we estimated the proportion of the observed variation caused by genetic and environmental factors, estimate heritability, and test for cytoplasmic effects and sex linkage for *L. dispar* female flight capability and the traits that affect it (wing length, muscle strength, and flight behaviors). To accomplish this, we crossed individuals from a North American population where no females are capable of flight with a Russian population where >90% of the females are capable of sustained ascending flight. We characterized female propensity to initiate flight, capability for flight, muscle strength, morphometric wing and body measurements, and pre-

flight behaviors in the parental, reciprocal F<sub>1</sub> hybrids, reciprocal backcrosses to both parental strains, and double reciprocal F<sub>2</sub> hybrids. Additionally, female and egg mass weights were compared to determine whether there was a detectable negative trade-off between flight and fecundity. We discuss the most likely mode of inheritance for each trait, possible scenarios for the development and maintenance of the clinal polymorphism, and the implications of our findings for management programs.

## Materials and Methods

**Gypsy Moth Strains.** The gypsy moths used to start the parental strains were collected in 1992 from Mineralni, Russia (20 egg masses, 044.10° N and 133.15° E), and North Carolina (Kill Devil Hill, 10 egg masses, 036.03° N and 075.40° W and Coinjock, 15 egg masses, 036.18° N and 075.57° W); all were transported under permit to the USDA 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.

To create the first laboratory generation, the Mineralni, Russia (R) and the North Carolina (N) strains were reciprocally crossed (R × N and N × R, female parentage always listed first) to produce the initial F<sub>1</sub> hybrids to be used in creating the F<sub>2</sub> hybrids and backcrosses. To produce the second laboratory generation, the parentals were again crossed to create reciprocal F<sub>1</sub> hybrids and the first generation F<sub>1</sub> hybrids were crossed with the parentals to create the eight backcrosses (RN × N, NR × N, N × RN, N × NR, RN × R, NR × R, R × RN, and R × NR) and with each other to create the four double reciprocal F<sub>2</sub> hybrids (RN × RN, RN × NR, NR × NR, and NR × RN). In the third laboratory generation, the parentals and all the crosses were reared for use in these studies. Each egg mass was cut in half longitudinally, and larvae hatching from each egg mass were reared in two of four weekly rearing sets.

**Rearing Methods.** All crosses were reared in the same way and under the same conditions to reduce maternal effects in the generation tested and to expose all genotypes to the same environment. All rearing was conducted in walk-in environmental chambers maintained at 25 ± 1°C, 60 ± 5% RH, and a photoperiod of 16:8 (L:D) h. Larvae were reared in groups of 10 in 237-ml clear plastic cups with unwaxed paper lids for 35–40 d. Each cup contained 90 ml of high wheat germ diet (Bell et al. 1981) made with Wesson salt mix without iron (Purina Mills Test Diet, Richmond, IN) and adding 0.12 g of amorphous FePO<sub>4</sub> per liter of diet. Pupae were harvested, sexed, and stored by sex, egg mass (family), and strain in 473- or 237-ml unwaxed squat paper cups with clear plastic lids until adult eclosion. To facilitate conducting bioassays from 1230 to 1700 hours, all pupae were held a minimum of 2 d in a chamber where the timing of scotophase initiation corresponded to noon; this was 10 h earlier than in the chamber where the larvae were held. Adults were removed daily, weighed, and held in paper cups until

paired individually in 473-ml paper cups for mating. Matings occurred generally the day of emergence (some 1–2 d after) and were random within strain or cross, except that sibling matings were avoided. Virgins not used for flight tests were held individually in the chamber where the pupae were held until mated on subsequent days. Both mated and virgin females were held in constant light on the day they were to be flight tested.

**Evaluation of Female Flight Capability and Pre-flight Behaviors.** In the field, female flight is initiated at dusk, when incident light is  $<3$  lux, and continues for 2–3 h (Wallner et al. 1995, Charlton et al. 1999). We used the same room and light controls described by Keena et al. (2001). The 12 bolts (60 cm high by 10 cm diameter) on which females were placed were far enough apart to prevent adjacent females from interfering with each other. Each of the 12 females to be flight-tested (always individuals from several different crosses) was randomly marked with a unique number on its forewing with an indelible black felt tipped pen. Males were kept in the mating containers to be reunited with the females after the tests were done. Marked females were placed 10 cm from the bottom of each bolt using a twig. The light from a 150-W incandescent floodlight was instantaneously reduced to 0.1 lux, using a rheostat. The light, located near the center of the room and 3 m from the shelf that held the bolts, was mounted vertically in a 1 by 1 by 1-m open wooden box painted black and set on the floor. This lighting system created a relatively even light throughout the room with a faint corona on the white ceiling. Light intensity was measured with a Gossen Luna-Pro meter (Li-Cor Co., Lincoln, NE) at moth eye level.

Two or three observers and one recorder were used for each flight observation. The following behavioral responses were recorded over a 45-min observation period: initiation of wing fanning, walking, egg laying, and flight from the bolts. If a female wing-fanned with or without simultaneous walking for a total of 15 min, or was fanning  $\geq 5$  min at the end of the 45-min period without launching from the bolt, she was placed on a wooden ruler (3 by 31 cm) held at a 20° upward angle pointed toward the light and prodded to obtain an involuntary flight evaluation. Both the voluntary and involuntary flight capabilities were used in the analyses unless otherwise indicated. The females were assigned one of the following phenotypes based on their exhibited flight ability: capable of directed flight (sustained ascending flight during which the female circled the room),  $\geq 2$  m glide (flight lacking upward displacement despite vigorous wing flapping),  $< 2$  m glide (a gentle fall with vigorous wing flapping), or incapable of flight (launched themselves without attempting to fly, or remained stationary, wing-fanned, or walked). Flight bioassays were conducted from 0.5 h before to 5 h after the start of scotophase, averaging three sessions per day.

Eighty-three females from the second laboratory generation (38 parentals and 45 reciprocal  $F_1$ s) were weighed and assessed using the free flight test as already described. These were the mothers of 22 back-

crosses to N, 20 backcrosses to R, 21 double reciprocal  $F_2$ , 13 reciprocal  $F_1$ , and 6 parental families used in the flight study. In the third laboratory generation, 42 females from each strain or cross (2 from each of 21 families) were assessed for flight propensity and capability in the free flight test. After the flight tests, the females were returned to the mating containers and original mates. The pairs were held in the rearing chamber until the female completed oviposition. Egg masses were harvested 22 d after deposition (enough time for embryonation to be completed), weighed, assessed for embryonation, and held in individual glassine envelopes.

**Evaluation of Female Muscle Strength.** In the third laboratory generation, the same 42 females from each strain or cross used in the free flight test were screened for muscle strength in a flip test, which was done after the other test and before the full egg mass was laid. The flip test consisted of inverting the female onto her back, with wings folded over the back as at rest, on a slick surface. A female was assigned to one of the following muscle strength phenotypic groups: able to right itself after one quick wing beat against the surface (they generally flip their abdomen up over their head), able to right itself after at least two wing beats, remained inverted after  $>10$  wing beats, or remained inverted with no wing beats but substantial leg movement. Individuals that remained stationary were excluded from the analysis. Some of the females that could not right themselves vigorously beat their wings while raised on the dorsum of their abdomen, whereas others barely moved. This test was inspired by a similar test using genetically dystrophic chickens, where ability to rise from a supine position was part of a pre-clinical muscular dystrophy drug evaluation program (Entrikin et al. 1977). A comparison of the musculature of females with different flip abilities showed that those that easily right themselves with a single wing beat have flight muscle fibers similar in diameter to those of the Russian strain, those that cannot right themselves have fibers of similar diameter to the North American strain, and those that flip with difficulty have fibers of intermediate diameter (Shields et al. 1997).

**Evaluation of Female Wing Size.** The weight (WT), forewing length (base to tip [FL]), maximum forewing width (FW), maximum hind wing width (HW), prothoracic width (TH), maximum abdominal width (AW), and abdominal length (AL) of a separate set of females from each strain and cross were recorded. One female was randomly chosen from each of the 25 families reared for potential use in the flight tests. Separate females were used, because measuring the wings before flight could have affected the free flight results, and after the flight tests, females had to be flip tested and returned to the mating cups quickly because many were ready to lay eggs. A stepwise discriminant analysis (PROC STEPDISC; SAS Institute 1999) was used to determine which of the four variables that showed significant variation between crosses (based on the statistical analyses described below; FL, FW, HW, and AW) had potential discrim-

inatory power for classifying the crosses. A discriminant analysis (PROC DISCRIM; SAS Institute 1999) was used to classify females using the three morphometric variables with discriminating power (FL, HW, and AW) and type of cross as the class. All females from the two parental strains (N and R) and the reciprocal  $F_1$ s constituted the base set used to develop the function; these females were cross-validated before all  $F_2$  and backcross females were classified as N, R, or  $F_1$ . Phenotypes of large, medium, and small wings were assigned to the R,  $F_1$ , and N groups, respectively.

**Statistical Analyses.** The following variables were analyzed by restricted maximum likelihood estimation method (REML, PROC MIXED; SAS Institute 1999): time to initiate wing fanning, duration of wing fanning before flight, female weight, and each of the six wing/body measurements. The model for flight capability used cross-type and bolt number as fixed effects and family as a random effect. The model used for the fanning measurements treated cross or cross type, days from adult emergence to mating, time of day the bioassay was run, and temperature as fixed effects, whereas family was treated as a random effect. For the wing/body measurements, cross type was the only fixed effect and family was the random effect. In each case, means were separated with least squares tests with  $\alpha = 0.05$  and a Bonferroni correction (SAS Institute 1999). A one-way analysis of variance (ANOVA) followed by a *t*-test with a Bonferroni correction (Statistix 2003) was used to determine if there were between cross differences in the percent of the female's body weight that the egg mass represented. Only egg masses that were embryonated and normal (not loose eggs or several small clumps) were used in this analysis. A least squares linear regression (Statistix 2003) of egg mass weight on female body weight also was conducted to determine the relationship between the two.

**Cytoplasmic Effects and Sex-Linked Genes.** Differences between reciprocal  $F_1$ s and between backcross populations may be caused either by maternal effects or by sex-linked genes or both. Taking into account the fact that female Lepidoptera are the heterogametic sex (ZW) and males are the homogametic sex (ZZ), effects of loci on the W-chromosome are confounded with maternal effects, so both will be referred to as "cytoplasmic effects." Z-linkage would show up as a difference between reciprocal crosses in  $F_1$  females and a resemblance to the paternal line. Two of the backcrosses provide an opportunity to assess cytoplasmic effects in females. The females of the RN  $\times$  N and NR  $\times$  N backcrosses both received their W-chromosome from the N father, but they received different cytoplasmic and Z-chromosomes. The NR  $\times$  R and RN  $\times$  R females both received their W-chromosome from the R father but different cytoplasmic and Z-chromosomes. Data for each trait evaluated were tested for normality, and the appropriate nonparametric or parametric tests (Kruskal-Wallis one-way ANOVA, two-sample *t*-test, or Yate's corrected  $\chi^2$  for percentage data; Statistix 2003) were performed to compare the means or percentages.

**Estimating Heritability.** The degree of genetic determination for a trait exhibiting continuous variation (broad sense heritability, Falconer 1989) is the relative contribution of genetic factors ( $V_G$  = additive variance [ $V_A$ ] + dominance variance + interaction deviations) to the total phenotypic variation ( $V_P$  =  $V_G$  + environmental variation [ $V_E$ ]) or  $V_G/V_P$ . The "narrow sense" heritability ( $h^2$ ) is defined as  $h^2 = V_A/V_P$  (Falconer 1989) and is a measure of the degree to which the trait is transmitted from parent to offspring.

In all the following analyses, reciprocal crosses are pooled within each cross type ( $F_1$ ,  $F_2$ , BN, and BR). Heritabilities were estimated in three ways: using a three class with two threshold model, female offspring on female parent regressions, and joint scaling tests (Falconer 1989; Lynch and Walsh 1998). Data sets appropriate for use in each method were not available for all traits, so the heritability of only a few traits was assessed in multiple ways.

The broad sense heritabilities for female flight capability, muscle strength, and wing size were estimated by fitting the data to a three class with two thresholds method proposed by Falconer (1989). The percentage of females ( $p_1$  and  $p_2$ ) above two thresholds,  $T_1$  and  $T_2$ , are used in the calculations. The thresholds for each trait were as follows: (1) female flight capability,  $T_1$  = the point at which females can glide and  $T_2$  = the point at which females can fly; (2) female muscle strength,  $T_1$  = the point at which females right themselves and  $T_2$  = the point at which females can right themselves with one wing flap; and (3) female wing size,  $T_1$  = the point at which wings are of  $F_1$  size and  $T_2$  = the point at which wings are of R size. The population mean ( $m$ ) as deviations from  $T_1$  in threshold units, the expected mean ( $Em$ ), the SD ( $\sigma$ ), and the variance ( $V$ ) were all estimated. When the observed  $p_1$  or  $p_2$  were 0.0 or 100.0, 0.01 and 99.99, respectively, were used in the calculations, and when both  $p_1$  and  $p_2$  were 0.0,  $p_1$  was 0.1 and  $p_2$  was 0.01. Broad base heritability,  $V_G/(V_G + V_E)$ , was estimated from the difference of the variance between the  $F_2$  ( $V_G + V_E$ ) and the  $F_1$  ( $V_E$ ).

The heritabilities of female flight capability, time to initiate wing fanning, and female weight were estimated using regressions (linear regression; Statistix 2003) of female offspring on female parents where the values are the distance from the overall mean (origin). The slope value  $b$  ( $\pm 95\%$  CI) is an estimate for  $h^2/2$  (Falconer 1989). If  $b$  significantly exceeds zero, there is a significant genetic contribution to the trait. Both this and the preceding approaches used to estimate heritability are simplified because the interactions between loci (epistasis), between alleles at a locus (dominance), and between genotype and environment have been excluded.

In a joint scaling test (Lynch and Walsh 1998), weighted least-square regression is used to estimate the parameters of an additive and dominance model (i.e., the expected mean phenotype,  $m$ , the composite additive effect,  $a$ , and the composite dominance effect,  $d$ ). If the assumption of normality is met, a  $\chi^2$  statistic for goodness-of-fit can be used to compare the esti-

Table 1. Percentage females in crosses between R and N strains of *L. dispar* that exhibited each behavior

Cross	Stationary	Walked <10 cm	Laid eggs	Wing fanned	Walked while wing fanning	Voluntarily attempted flight
N × RN	16.7	11.9	23.8	71.4	47.6	14.3
N × NR	14.3	21.4	19.0	64.3	33.3	9.5
RN × N	16.7	7.1	21.4	76.2	38.1	7.1
NR × N	14.3	11.9	19.0	73.8	47.6	7.1
R × RN	2.4	0.0	0.0	97.6	90.5	73.8
R × NR	2.4	0.0	2.4	97.6	88.1	81.0
RN × R	0.0	0.0	4.8	100.0	88.1	78.6
NR × R	2.4	0.0	0.0	97.6	88.1	76.2
RN × RN	7.1	0.0	9.5	92.9	76.2	38.1
NR × NR	2.4	2.4	7.1	95.2	78.6	47.6
RN × NR	7.1	0.0	7.1	92.9	73.8	47.6
NR × RN	4.8	0.0	16.7	95.2	59.5	26.2
N × R	7.1	0.0	14.3	92.9	78.6	33.3
R × N	4.8	0.0	7.1	95.2	73.8	16.7
R × R	2.4	0.0	2.4	97.6	92.9	92.9
N × N	47.6	9.5	35.7	42.9	7.1	0.0

The strain or cross of the female parent is listed first. Not all behaviors are mutually exclusive. Females that did not fly characteristically exhibited some or all of the first three behaviors, whereas those that flew exhibited the last three behaviors in sequence.

mates with the observed means. A similar procedure was used to estimate additive, dominance, and environmental variance components using the maximum likelihood method with the observed variance of the six basic pooled crosses being used as the initial weights ( $df/2 \times [\sigma^2 + 2]$ ) until the  $\chi^2$  test values reached a minimum (Lynch and Walsh 1998). The estimated variance components were used to calculate heritabilities. The traits FL, HW, AW, and WT were evaluated using this approach. Allometric equations were first developed using the whole data sets for each wing measurement trait ( $\ln y = \ln \alpha + \beta \times \ln WT$ ), and the character values were replaced by the derivation from the fitted allometric equation (observed - predicted) to eliminate the effects of size on the phenotypic variation (Lynch and Walsh 1998). The  $\ln$  of WT was used in the calculations. We applied the (Zeng 1992) modification of the Castle-Wright estimator to estimate the minimum number of genes contributing to the difference between the two lines for these four traits.

The fit of a single gene (no dominance) model for inheritance of the walking while fanning preflight behavior and laying eggs instead of flying trait were also evaluated using the pooled cross type means for each behavior. To determine how well the models fit the data, the expected and observed frequencies for each cross and phenotype combination were compared using PROC FREQ with the TESTF = () option (SAS Institute 1999). Yate's corrected  $\chi^2$  (Statistix 2003) was used when the expected and observed values for a single cross and phenotype combination were compared.

## Results

**Female Phenotype Summary.** R females generally remained motionless for several minutes before initiating wing fanning. They fanned in place for a few minutes before walking, while wing-fanning, to the top of the bolt, and initiating flight. About one half of

the N females remained where they were placed or walked a short distance, and then most initiated egg laying. The other half of the N females wing fanned with or without simultaneous walking, but none voluntarily launched themselves from the bolts. Almost all of the females from the reciprocal  $F_1$ ,  $F_2$ , and backcross to the R parent wing fanned, and the majority walked while wing fanning. However, several of these females walked to the top, lifted their front legs off the substrate, and turned and walked around or up and down the post still wing fanning. Some launched themselves from the top either on the initial or subsequent ascent, with many only able to glide while beating their wings. The backcross to the N parent females exhibited either the N parent or hybrid behavioral sequence. Propensity to fly, as measured by the percentage of females that voluntarily left their post in a flight attempt, was low in the N parent and backcross to the N, high in the R parent and backcross to the R, and intermediate in the  $F_1$  and  $F_2$ . Females from the  $N \times R F_1$  had a higher propensity to fly than those from the  $R \times N F_1$ . Table 1 summarizes the behaviors exhibited by all the females in each cross type.

Cross type had a significant effect on flight capability ( $F = 32.76$ ;  $df = 15,484$ ;  $P < 0.0001$ ), but the bolt on which the female was placed did not ( $F = 0.41$ ;  $df = 11,484$ ;  $P = 0.9532$ ). All N parental strain females were incapable of flying or gliding, whereas almost all females from the R parental strain were strong fliers (Fig. 1). More than one half of  $F_1$  females were capable of gliding, with only a few  $N \times R$  cross females capable of strong directed flight. The full range of flight capability was seen in the  $F_2$ s, whereas the majority of backcross females tended to have flight capabilities similar to the parental strain (Fig. 1).

Females from both parental strains and all the crosses exhibited varying abilities to right themselves from the inverted position (Fig. 2). Most N females could not right themselves, although over one half vigorously tried (Fig. 2); >90% of R females righted

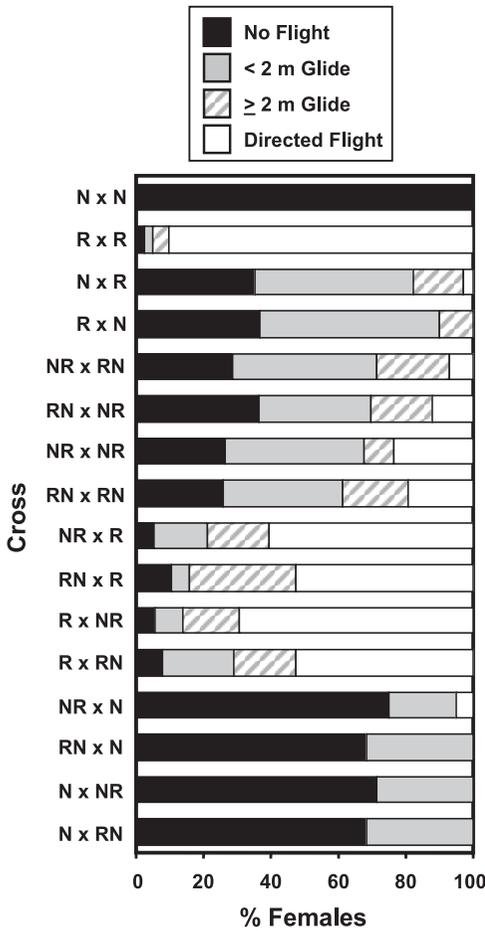


Fig. 1. Percentage of *L. dispar* females from various crosses between a Russian strain (R) and a North American strain (N) that exhibited each flight capability classification. The female parent is given first.

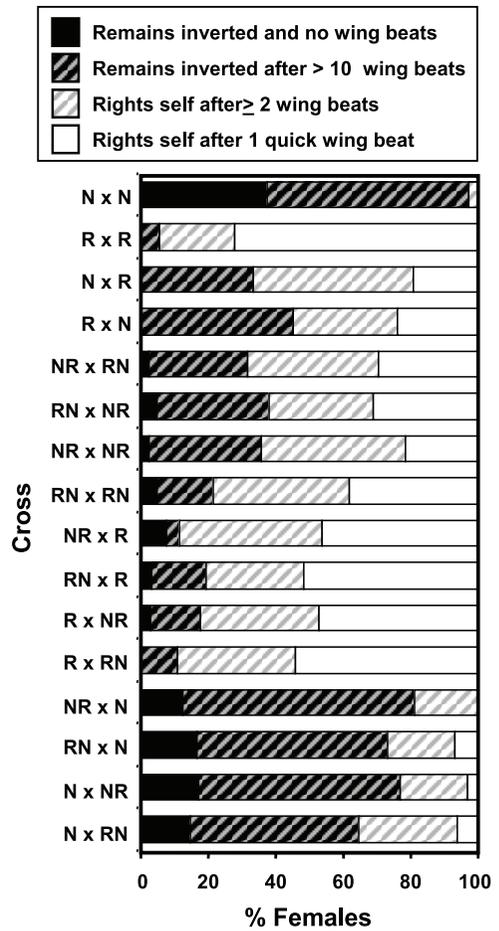


Fig. 2. Percentage of *L. dispar* females from various crosses between a Russian strain (R) and a North American strain (N) that exhibited the indicated flip-test result. The female parent is given first.

themselves, most with one or two quick wing beats (Fig. 2). With the exception of the backcross to the N, >50% of all hybrid females righted themselves, although many with difficulty (Fig. 2).

Prefanning time was shortest for R and R x RN females and longest for N females, with other crosses being intermediate (Fig. 3). Cross ( $F = 2.46$ ;  $df = 15,554$ ;  $P = 0.0017$ ), female age ( $F = 10.95$ ;  $df = 2,554$ ;  $P < 0.0001$ ), and temperature ( $F = 7.83$ ;  $df = 1,554$ ;  $P = 0.0053$ ) all had significant effects on the prefanning time. Females flight tested on the day of emergence ( $16.6 \pm 1.0$  min) took significantly longer to initiate fanning than did those that were 1 ( $12.5 \pm 0.7$  min) or 2 d old ( $11.5 \pm 1.0$  min). Females fanned significantly sooner at 20 ( $14.8 \pm 0.9$  min) than 21°C ( $12.3 \pm 0.6$  min). Because the number of females initiating flight was low for some reciprocal crosses, fanning time was analyzed only by cross type, which had a significant effect on wing fanning duration ( $F = 13.91$ ;  $df = 4,247$ ;  $P < 0.0001$ ). R females fanned for the shortest time before flying, backcross to the N parent fanned for the longest time, and the other cross types had interme-

diante fanning times (Fig. 4). Female age ( $F = 2.57$ ;  $df = 2,257$ ;  $P = 0.0781$ ), the number of hours after the start of scotophase ( $F = 0.83$ ;  $df = 3,257$ ;  $P = 0.4773$ ), and temperature ( $F = 3.01$ ;  $df = 1,257$ ;  $P = 0.0841$ ) had no significant effect on fanning duration.

The mean weights of females that were flight tested within each cross ranged from 1,020 to 1,342 mg and varied significantly by cross ( $F = 3.12$ ;  $df = 15,593$ ;  $P = 0.0001$ ). Females from the NR x N ( $1,342 \pm 62$  mg) and RN x R ( $1,310 \pm 49$  mg) backcrosses were significantly heavier than those from the N x RN ( $1,020 \pm 45$  mg) and RN x RN ( $1,034 \pm 49$  mg) crosses (Bonferroni *t*-test,  $\alpha = 0.05$ , critical value of  $t = 3.549$ ). The mean percent of the female's body weight that became the egg mass ranged from 48 to 53% for the crosses but did not significantly vary between crosses ( $F = 1.57$ ;  $df = 15,593$ ;  $P = 0.0774$ ). The linear relationship between these two variables was egg mass weight =  $0.5056 \pm 0.0032 \times$  female weight in milligrams ( $r^2 = 0.77$ ,  $t = 160.34$ ,  $P < 0.0001$ ).

The weights of females for which morphometric measurements were taken did not significantly vary by

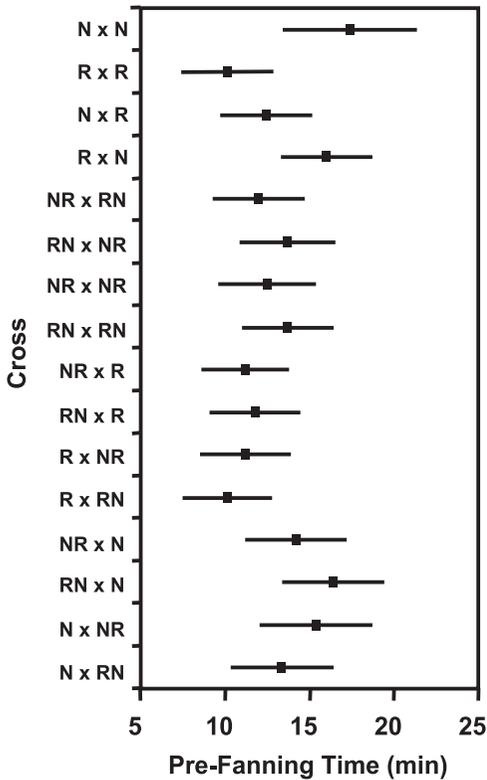


Fig. 3. Mean time ( $\pm$ SE) to initiation of wing fanning in *L. dispar* females from various crosses between a Russian strain (R) and a North American strain (N). The female parent is given first.

cross type (Table 2) but were lower on average than those that were flight tested. Female wing measurements and abdominal width varied significantly by cross type: R and backcross to the R > F<sub>1</sub> and F<sub>2</sub> > backcross to the N > N (Table 2). There were no significant differences between the cross types in thoracic width or abdominal length measurements, so these were excluded from the discriminate analysis (Table 2). The results of the discriminate analysis using these variables are given in Table 3. Both the N and R parentals had some variation in wing size; with  $\leq 14\%$  of the females classified as F<sub>1</sub>s (Table 3). Less than 10% of F<sub>1</sub> and F<sub>2</sub> females were classified as either N or R, the rest being classified as F<sub>1</sub>. Backcrosses were classified as approximately one half F<sub>1</sub> and one half the parental strain involved in the backcross (Table 3).

**Cytoplasmic Effects and Sex Linkage.** No significant Z-linkage was found for flight capability, ability of the female to right herself, morphometric wing measurements, female weights, or behaviors associated with flight. However, a significant Z-linkage was found for flight propensity as measured by a female leaving the post voluntarily ( $\chi^2 = 0.0143$ ). No significant cytoplasmic effects were found for all the traits except for female weight when NR  $\times$  R and RN  $\times$  R backcrosses were compared ( $t = 4.27$ ,  $df = 58$ ,  $P = 0.0001$ ). These findings suggest that the traits where no significant

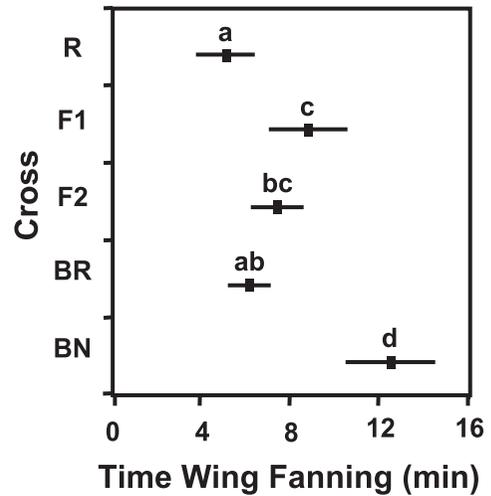


Fig. 4. Mean duration ( $\pm$ SE) of wing fanning before attempting flight for *L. dispar* females from various crosses between a Russian strain (R) and a North American strain (N). Means with different letters over them are significantly different based on a least squares separation test with a Bonferroni adjustment for  $\alpha = 0.05$  (SAS Institute 1999). BR, backcross to the R parent; BN, backcross to the N parent.

Z-linkage or cytoplasmic effects were found are autosomally inherited.

**Heritability Estimates and Mode of Inheritance.** Broad sense heritability, estimated using the threshold model (Falconer 1989) and the differences in variances between the F<sub>2</sub> and F<sub>1</sub>, were at least 0.61 for female flight capability, 0.01 for female muscle strength, and 0.70 for female wing size (Table 4). The mean of the F<sub>1</sub> was intermediate between the values of the R and N parentals, and the mean of the F<sub>2</sub> was near that of the F<sub>1</sub>, but had a larger variance. Backcross means fell between the F<sub>1</sub> and the parent used in the cross and had variances similar to that of the F<sub>1</sub> and F<sub>2</sub>. The results for female flight capability generally agreed with the expectations for a polygenic character and suggest that it is a continuously varying trait in *L. dispar*. The heritabilities estimated for the female muscle strength indicate that environmental factors have a strong influence on this trait.

Heritability estimates, using a mean female offspring on female parent regression (Falconer 1989), for female flight capability, preflight body weight, and time to initiation of wing fanning were  $0.600 \pm 0.162$ ,  $0.069 \pm 0.107$ , and  $0.658 \pm 0.219$ , respectively (Table 4). Both flight capability and time to initiation of wing fanning showed a significant ( $P < 0.01$ ) and relatively high heritability. However, there was no evidence for a significant genetic basis for the inheritance of female weight.

In the joint-scaling means comparison (Lynch and Walsh 1998), there was a significant additive component for FL ( $4.91 \pm 0.97$ ,  $\chi^2 = 0.038$ ,  $df = 3$ ,  $P = 0.998$ ) and HW ( $2.55 \pm 0.44$ ,  $\chi^2 = 0.034$ ,  $df = 3$ ,  $P = 0.998$ ), but not for AW or WT. There was no significant dominance component in all four of the means models. The

**Table 2.** Mean ( $\pm$ SE) weight (mg) and measurements (mm) of *L. dispar* females from crosses between R and N strains

Cross	Weight (mg)	Forewing length (mm)	Forewing width (mm)	Hindwing width (mm)	Prothoracic width (mm)	Abdominal width (mm)	Abdominal length (mm)
R Parent	761.6 $\pm$ 68.9a	36.5 $\pm$ 0.6a	21.0 $\pm$ 0.4a	19.4 $\pm$ 0.3a	6.2 $\pm$ 0.1a	8.7 $\pm$ 0.2b	22.9 $\pm$ 0.5a
N Parent	895.4 $\pm$ 66.9a	27.8 $\pm$ 0.4d	16.2 $\pm$ 0.2d	15.0 $\pm$ 0.2d	6.6 $\pm$ 0.1a	9.6 $\pm$ 0.2a	23.1 $\pm$ 0.5a
F <sub>1</sub>	761.8 $\pm$ 44.8a	32.1 $\pm$ 0.5b	18.3 $\pm$ 0.3b	17.1 $\pm$ 0.3bc	6.2 $\pm$ 0.1a	8.9 $\pm$ 0.2ab	22.4 $\pm$ 0.4a
F <sub>2</sub>	743.0 $\pm$ 31.7a	32.1 $\pm$ 0.3b	18.2 $\pm$ 0.2b	17.1 $\pm$ 0.2b	6.4 $\pm$ 0.1a	9.0 $\pm$ 0.1ab	22.7 $\pm$ 0.3a
BR	871.9 $\pm$ 31.9a	35.3 $\pm$ 0.3a	20.2 $\pm$ 0.2a	18.8 $\pm$ 0.2a	6.5 $\pm$ 0.1a	9.2 $\pm$ 0.1ab	23.4 $\pm$ 0.2a
BN	865.3 $\pm$ 26.9a	30.7 $\pm$ 0.2c	17.5 $\pm$ 0.1c	16.3 $\pm$ 0.1c	6.5 $\pm$ 0.1a	9.4 $\pm$ 0.1ab	23.1 $\pm$ 0.2a

Values within the same column followed by the same letter are not significantly different based on a least squares mean separation test ( $\alpha = 0.05$ ) with a Bonferroni adjustment.

BR, backcross to the R parent; BN, backcross to the N parent.

heritability estimates, from the joint-scaling variance models (Lynch and Walsh 1998), for FL, HW, AW, and WT are shown in Table 5. Both FL and HW had relatively high heritability, but there was no evidence for a significant genetic basis for the inheritance of female weight. The estimates of the number of significant factors involved in the inheritance of FL and HW suggest a polygenic mode of inheritance.

Inheritance of the walking while fanning preflight behavior fit a single gene with no dominance model fairly well ( $\chi^2 = 32.15, P = 0.0007$ ; without the F<sub>1</sub>:  $\chi^2 = 4.69, P = 0.8603$ ). The only significant deviation was that the percentage of F<sub>1</sub> females exhibiting this behavior (76.2%) was higher than the mid-parent value (52.5%) that would be expected (Yate's corrected  $\chi^2 = 13.41, P = 0.0003$ ). The proportion of females that laid eggs instead of exhibiting preflight behaviors fit the single gene with no dominance model very well ( $\chi^2 = 10.11, P = 0.5204$ ).

**Discussion**

Flight polymorphism in female *L. dispar* is the result of variation in the combination of wing size, flight musculature, and flight behavior and apparently under the control of multiple genes. There was no evidence of Z-linkage or cytoplasmic effects in the inheritance of wing size or flight musculature and very limited evidence of Z-linkage for flight propensity. A single gene with no dominance was found to control the preflight behaviors of walking while fanning and early deposition of eggs. There was substantial continuous

variation and the occurrence of intermediate phenotypes for both wing size and muscle strength. Heritability estimates under these laboratory conditions indicated that a large portion of the variation in wing size was caused by additive genetic effects but that there was a strong environmental or other factor responsible for most of the phenotypic variation in muscle strength.

The lack of a measurable genetic basis for flight muscle strength and female weight under the environmental conditions used in this study does not mean that the gene expression and heritability estimates would be the same in all environmental conditions. Environmental factors such as density, temperature, and food quality are known to affect expression of flight morphs (Zera 2004) and flight muscles size (Marden 2000) in other insects, and they affect adult size and weight in *L. dispar*. An indication that the test environment could have reduced the phenotypic variation in female weight is that in previous studies the R strain was significantly heavier than the N strain (Keena 1994) but they did not significantly differ in weight in this study. There are two possible environmental factors that could have significantly impacted the weight of the R strain more than the N strain. First, the R strain requires more dietary iron than was provided to attain its maximum weight. Second, group rearings tend to result in smaller individuals, particularly in cups where there are more females than males (M.K., unpublished data). Flight muscles and female flight capability could be affected by similar environmental factors as the female weight. For example, adults from larvae reared on artificial diet, as was done here, exhibit less female flight capability than adults from larvae that had fed on foliage (Keena et al. 1997). Thus, heritability estimates and retention of flight in

**Table 3.** Cross-validation (R, N, and F<sub>1</sub>) and classification (F<sub>2</sub>, BR, and BN) results for *L. dispar* individuals from each cross using morphometric variables: forewing length, hindwing width, and abdominal width

From cross	Percentage classified into cross		
	N	R	F <sub>1</sub>
N	90.0	0.0	10.0
R	0.0	86.7	13.3
F <sub>1</sub>	5.0	0.0	95.0
F <sub>2</sub>	8.3	5.8	85.8
BN	37.8	0.0	62.2
BR	0.0	42.5	57.5

The N, R, and F<sub>1</sub> data were used to create the discriminant function (PROC DISCRIM; SAS Institute), and were cross-validated. F<sub>2</sub>, BR (backcross to the R parent), and BN (backcross to the N parent) data were classified using the function.

**Table 4.** Estimation of variance components and heritability for some *L. dispar* traits obtained by applying a three class, two threshold model (Falconer 1989) to phenotype percentage data

Trait	V <sub>P</sub>	V <sub>E</sub>	V <sub>G</sub>	H
Female flight capability	0.3937	0.1534	0.2403	0.6104
Female muscle strength	0.9237	0.9133	0.0104	0.0112
Female wing size	0.1157	0.0348	0.0810	0.6997

Variance components calculated as follows: V<sub>P</sub> = V<sub>F2</sub>, V<sub>E</sub> = V<sub>F1</sub>, V<sub>G</sub> = V<sub>P</sub> - V<sub>E</sub>, and V<sub>A</sub> = 2V<sub>F2</sub> - (V<sub>BR</sub> + V<sub>BN</sub>). Heritabilities calculated as follows: H = (V<sub>G</sub>)/V<sub>P</sub>.

**Table 5.** Estimation of variance components ( $\pm$ SE if available), heritability ( $h^2$ ), and no. of effective factors ( $n_e$ ) for some *L. dispar* traits using predicted values in an additive + dominance model

Trait	$V_P$	$V_E$	$V_A$	$h^2$	$n_e$	$\chi^2$	df	<i>P</i>
Forewing length	4.735 $\pm$ 0.482	3.170 $\pm$ 0.368	1.565	0.331	7.26 $\pm$ 5.34	3.1174	3	0.3739
Hindwing width	1.429 $\pm$ 0.126	0.704 $\pm$ 0.089	0.724	0.507	4.17 $\pm$ 3.06	0.0328	3	0.9984
Abdominal width	0.387 $\pm$ 0.041	0.331 $\pm$ 0.037	0.056	0.145	-0.98 $\pm$ 5.55	0.0002	3	1.0000
Female weight	0.227 $\pm$ 0.025	0.214 $\pm$ 0.024	0.013	0.059	-3.57 $\pm$ 57.1	0.0000	3	1.0000

Deviations from fitted allometric equations (using the WT as the scaling factor) were used in place of the data to eliminate the effects of size on the phenotypic variation (Lynch and Walsh 1998). The ln of WT was used.

Variance components calculated as follows: phenotypic variance  $V_P = V_{F2}$ , environmental variance ( $V_E$ ) =  $V_{F1}$ , genetic variance ( $V_G$ ) =  $V_P - V_E$ , and additive genetic variance ( $V_A$ ) =  $2V_{F2} - (V_{BR} + V_{EN})$ . Heritabilities were calculated as  $h^2 = V_A/V_P$ , and  $H = V_G/V_P$ . Because there was no dominance variance  $V_A = V_G$  and  $h^2 = H$ , so only  $V_A$  and  $h^2$  were presented.

hybrids could be higher if foliage-fed larvae were used.

Polygenic inheritance has previously been suggested, but not confirmed, for female flight in *L. dispar* (Reineke and Zebitz 1998, Keena et al. 2001). At least 60% of the observed variation in female flight capability was attributable to additive genetic effects. Similar heritability estimates have been found for other insects with flight polymorphisms (Roff 1986). Neither of the two parental populations used in these crossing experiments was completely homozygous for all traits involved in female flight capability. This indicates that selective pressures are likely responsible for maintaining the cline in flight polymorphism and that it is a case of continuous variation rather than just changing percentages of two morphs over the range. Continuous variation that results from polygenic inheritance ensures that genetic variation is maintained, which has fitness benefits in an uncertain environment, and genotype-environment interactions can modulate the phenotype to ensure this variation is maintained.

In *L. dispar*, the female is the heterogametic sex, and sex chromosomes may function as switch genes that canalize development to determine sex (Robinson 1971). Despite the flight polymorphism being exhibited only in the female, all males fly, and we found no evidence for the involvement of a W-linked gene. Alternatively, it has been shown that the expression of brachyptery and associated reproductive differences in *Gryllus* sp. are associated with juvenile hormone esterase (JHE) activity in the last stadium and that a daily rise in juvenile hormone in the flighted morph activates flight behavior (Zera 2004). A similar endocrine-based mechanism may determine the expression of *L. dispar* flight capability in females and maintain flight in males, but this has not been evaluated. If hormonal concentrations or enzyme activity are involved in the regulation of this polymorphism, female flight capability would be expected to fit a two threshold, three class inheritance model as it did.

The costs of flight likely are the main factor that results in the predominance of flightless females in the absence of strong selection for flight. Rankin and Burchsted (1992) suggested that the costs of flight include the following: energetic and developmental cost of the flight muscles and longer wings, metabolic cost in fuel for flight, risks of increased predation and

not finding a suitable habitat, and potential reproductive cost. The first three costs clearly could play a role in selection for flightless females in *L. dispar*, but the fourth is less likely. The potential reproductive costs documented or suggested for other insects are increased time to first oviposition, decreased available energy for egg development, decreased overall fecundity, and decreased lifespan (Rankin and Burchsted 1992). Use of fuel for flight should not impact egg development, because *L. dispar* eggs are already fully developed at female eclosion. This also would make histolysis of the flight muscles to free more energy of little advantage, and there was no evidence that this was the cause for the observed differences in flight muscles (Shields et al. 1997). We have already shown that fecundity relative to body mass does not vary between females with different flight capabilities. This is consistent with there being no significant additive genetic component to phenotypic variation in female weight under our experimental conditions. *L. dispar* adults live for only about a week and lay all their eggs in one mass soon after mating, so reduced life span is less likely to be a factor. Flightless females do lay their egg masses sooner than flighted females, but the difference of 1 or 2 d should be of little advantage. Further documentation of potential fitness trade-offs with female flight are needed to help predict population-level outcomes of introductions of flight-capable female strains into areas with flightless females.

Some female flight capability is retained after hybridization, but the proportion of the population with strong directed flight is reduced in the laboratory. In a freely hybridizing population, the amount of flight capability maintained would depend on several factors: initial ratio of flight capable to flightless females, costs versus fitness of flight in the particular environment, propensity of different hybrids to mate, etc. Should females with full flight capability be introduced into North America in an area where the flightless females are already established, the populations would hybridize, and the ability of *L. dispar* to spread could be increased. Increased flight capability could occur because there are likely some alleles that confer greater flight capability that apparently are not present in the North American population. Differences in larval growth rates exist (Keena et al. 1995) that would increase the probability of individuals with similar genotypes mating. Flight capable females orient toward

light (Wallner et al. 1995), and their egg masses tend to be concentrated in particular locations. These egg laying habits could produce a patchy distribution of flight capable females in subsequent generations. Additionally, greater female flight capability is expected when larvae are reared on foliage (Keena et al. 1997). The variation in female flight found in parts of Europe where all genotypes are presumably present seems to be further evidence that female flight capability is not completely lost through hybridization.

A bigger concern would be the introduction of females capable of strong directed flight into an area where *L. dispar* currently is not present. Even if the costs of flight are large, females capable of strong directed flight should remain in the population for many generations because of the polygenic inheritance. If the size of the introduced population is large enough, the tendency of flighted individuals to concentrate in particular locations (i.e., near lights) would allow them to overcome the usual difficulty in finding mates, thus increasing the probability of establishment. This would result in the rapid spread of the population making delimitation and eradication of infestations more difficult. This underscores the need for effective exclusion methods and the continued monitoring of *L. dispar* population densities where females capable of flight are present in the country of origin (USDA 1996) to predict when and where the risk of inadvertent introduction is most severe.

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