World Distribution of Female Flight and Genetic Variation in *Lymantria dispar* (Lepidoptera: Lymantriidae)

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ABSTRACT Female gypsy moths, *Lymantria dispar* L., from 46 geographic strains were evaluated for flight capability and related traits. Males from 31 of the same strains were evaluated for genetic diversity using two polymorphic cytochrome oxidase I mitochondrial DNA restriction sites, the nuclear FS1 marker, and four microsatellite loci. Females capable of strong directed flight were found in strains that originated from Asia, Siberia, and the northeastern parts of Europe, but flight capability was not fixed in most strains. No flight-capable females were found in strains from the United States or southern and western Europe. Wing size and musculature were shown to correlate with flight capability and potentially could be used in predicting female flight capability. The mtDNA haplotypes broadly separated the gypsy moth strains into three groups: North American, European/Siberian, and Asian. Specific microsatellite or FS1 alleles were only fixed in a few strains, and there was a gradual increase in the frequency of alleles dominant in Asia at both the nuclear and microsatellite loci moving geographically from west to east. When all the genetic marker information was used, 94% of the individuals were accurately assigned to their broad geographic group of origin (North American, European, Siberian, and Asian), but female flight capability could not be predicted accurately. This suggests that gene flow or barriers to it are important in determining the current distribution of flight-capable females and shows the need for added markers when trying to predict female flight capability in introduced populations, especially when a European origin is suspected.

KEY WORDS female flight capability, *Lymantria dispar*, gypsy moth, dispersal, genetic variation

The gypsy moth (*Lymantria dispar* L.) is one of the most serious defoliating forest pests, capable of causing widespread outbreaks in temperate Holarctic regions. The gypsy moth range in the Palearctic region is roughly between 60° N and 30° N, but does extend further south (20° N) in the Far East (Giese and Schneider 1979). Moths from Western Europe were introduced into Massachusetts in 1869 (Forbush and Fernald 1896). The gypsy moth has since spread throughout New England and adjacent provinces of Canada, and the leading edge of the infestation has reached Maine, Wisconsin, Illinois, Indiana, Ohio, West Virginia, Virginia, North Carolina, and Ontario, Québec, New Brunswick, and Nova Scotia, Canada. Isolated infestations have been detected in almost every state in the continental United States and are eradicated in states outside the generally infested area. The infestation in the eastern United States is too well established to eradicate, but measures to slow the spread and control local outbreaks are being taken. These are the optimal strategies for dealing with gypsy moth populations based on a bioeconomic model developed by Sharov and Liebhold (1998).

Multiple introductions of gypsy moth strains with flight-capable females have occurred from egg masses on ships and cargo entering ports in western North America from Japan and Far East Russian ports and from pupae on military equipment or trooper belongings entering the east from Germany (Wallner 1996). Most introductions have prompted an eradication program, the largest of which occurred in 1992 and 1994 (Wallner 1996). The biggest concern over these introductions is the presence of flight-capable females in the introduced strains (and possibly in hybrids between them and the existing flightless strain) might increase the potential rate of spread and complicate procedures for detecting and delimiting isolated populations. In addition, there are other concerns because of the wide variation in behavioral, physiological, and genetic characteristics exhibited by the gypsy moth across its geographic range. For example, some strains from Asia possess 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 attractancy to lights that results in egg deposition on vehicles or cargo (Wallner et al. 1995). For regulatory purposes,

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the U.S. Department of Agriculture refers to any biotype of *Lymantria dispar* possessing female flight capability as the Asian gypsy moth (APHIS 2003).

A recent review of *Lymantria* includes two subspecies of *L. dispar* (*asiatica* and *japonica*) and three other species in Japan (*Lymantria albescens* Hori and Umeno, *L. umbrosa* Butler, and *L. postalba* Inoue) in the category of Asian gypsy moth with flight-capable females (Pogue and Schaefer 2007). *L. dispar asiatica* is officially given the common name of Asian gypsy moth and is distributed in Asia, mostly east of the Ural Mountains, and in China and Korea. The Japanese gypsy moth *L. dispar japonica* is distributed on all main islands in Japan, but only in a limited area on Hokkaido.

The distribution of flight-capable females across the gypsy moth's geographical range is somewhat uncertain. It has been suggested that there is a clinal flight polymorphism (Baranchikov 1989), rather than two distinct morphs, 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. There are reports from Japan (Schaefer et al. 1984, Koshio 1996), Lithuania (Zolubas et al. 1999), Russia (Mikkola 1971, Baranchikov 1989, Ponomarev 1994), Korea, and China (Schaefer et al. 1984) of females that 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), and eastern Siberian females have even been reported to cross mountain ranges in large groups during outbreaks (Rozhkov and Vasilyeva 1982), with several of these females observed at lights in Moscow in 1958 (Mikkola 1971). In Western Europe and North America, gypsy moth females do not fly and 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-capable females in Central Europe are conflicting, and a transition zone of occasional female flight has been proposed for Eastern Europe (Baranchikov 1989, Gninenko and Orlinskii 2003). The reports for Central Europe vary from females that are "almost" unable to fly (Heβ 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), to females that exhibit a highly synchronous flight at dusk (Charlton et al. 1999). Reineke and Zebitz (1998) showed that populations with both full female flight and lacking female flight exist in different parts of Germany.

Factors that affect gypsy moth female flight initiation and orientation have been described, including light intensity (Charlton et al. 1999, Keena et al. 2001), ambient and body temperatures (Charlton et al. 1999), mating status (Keena et al. 2001), wavelength of light (Wallner et al. 1995), and larval food source (Keena et al. 1997). Flight capability is reduced in F_1 hybrids (Keena 1994, Reineke and Zebitz 1998) and is polygenic (heritability of 0.60; Keena et al. 2007). Wing size varies continuously with a heritability of 0.70, >90% of the variation in muscle strength is because of environmental causes, and preflight behaviors are inherited through a single gene with two co-dominant alleles, all of which contribute to this flight polymorphism (Keena et al. 2007). There have been no attempts to systematically quantify flight capability of females from populations throughout its entire range.

Individuals from populations with and without flight-capable females cannot reliably be distinguished morphologically, so molecular techniques were developed to determine the origin of males caught in pheromone traps and to better understand the patterns of flight and genetic variation in this species. Several different methods have been used that show that there are detectable molecular differences among populations from Europe, Asia, and North America. Bogdanowicz et al. (1993, 2000) used mitochondrial DNA (mtDNA) to develop a diagnostic assay to trace gypsy moth origins. Pfeifer et al. (1995) analyzed an internal transcribed spacer region (ITS-2) of nuclear ribosomal DNA (rDNA) using polymerase chain reaction (PCR) followed by restriction site polymorphism analysis to develop an assay to distinguish Asian and North American populations. Garner and Slavicek (1996) and Schreiber et al. (1997) developed four strain-specific markers using random amplified polymorphic DNA (RAPD). One of these DNA markers (FS1) targets an autosomal single copy locus that can identify heterozygous individuals (Garner and Slavicek 1996). Genetic variation both within and among gypsy moth populations has been detected using amplified fragment length polymorphisms (AFLP, a multilocus DNA profiling technique) (Reineke et al. 1999) and microsatellite loci (tandemly repeated DNA that has a high mutation rate) (Bogdanowicz et al. 1997). All or some of these marker types are currently used in monitoring programs in both Canada and the United States: two mtDNA restriction fragment length polymorphisms (RFLPs) at two polymorphic nucleotide sites, two locus-specific primers (JS1 and JS2) for the FS1 nuclear DNA locus, and four microsatellite loci. Baseline survey results for North America, Germany, Siberia, and Far East Russian using the mtDNA and FS1 as part of the monitoring program have been published (Prasher and Mastro 1995, Prasher 1996). There has been no attempt to compare the world variation in all of these markers, and they have never been used on the same populations in which female flight capability has been specifically determined. Understanding the relationship between marker results for intercepted males and female flight capability of different world populations would help in assessing the risk that an introduction contains flight-capable females. Here, we document female flight capability and the traits that affect it (wing length, muscle strength, and flight behaviors) from 46 strains of gypsy moth from throughout its range. For 31 of the strains, we determined the

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	Closest city, region	Latitude	Longitude	Collection date[a]	Egg masses received
JS	Japan	Sapporo, Hokkaido	43.00° N	141.30° E	May 1992 (Aug. 1994)	30 individual
JN	Japan	Nagoya, Honshu	35.15° N	137.08° E	Mar. 1996	4 individual
CL	China	Niuzhuang, Liaoning	41.03° N	122.30° E	Aug. 1992 (Oct. 1994)	30 individual
CS	China	Laixi, Shangdong	35.52° N	120.32° E	Aug. 1992 (Oct. 1994)	30 individual
CH	China	Qianan, Hebei	40.00° N	118.41° E	Aug. 1992 (Oct. 1994)	30 individual
CB	China	Beijing, Beijing	39.53° N	116.23° E	Aug. 1992 (Oct. 1994)	30 individual
RM	Russia	Mineralni, Primorski	44.10° N	133.15° E	Aug. 1992	20 individual
RB	Russia	Bellyk, Krasnoyarsk	54.30° N	91.18° E	Dec. 1992	>30 mixed off rocks
RS	Russia	Shira, Khakassia	54.41° N	90.00° E	Aug. 1994	6 individual
BS	Bulgaria	Sofia, Sofia	42.51° N	23.40° E	Feb. 1995	12 individual
KG	Greece	Kavála, Macedonia	41.00° N	24.25° E	Feb. 1997	58 individual
LJ	Lithuania	Juodkrante, Kuzsin Nezijos	55.31° N	21.06° E	Aug. 1994	47 individual
TO	Croatia	Otok, Slovenia	45.07° N	18.53° E	Mar. 1995	10 individual
TS	Croatia	Strizovojha, Slovenia	45.13° N	18.28° E	Mar. 1995	5 individual
VN	Slovak Republic	Nitra, Bratislava	48.20° N	18.06° E	Jan. 1995	12 individual
WP	Poland	Wroclaw, Piaski Forest, Poznan	51.53° N	16.59° E	Mar. 1995	30 individual
TN	Croatia	Novska, Slovenia	45.20° N	16.59° E	Mar. 1995	7 individual
TL	Croatia	Lirovljani, Slovenia	45.23° N	16.55° E	Mar. 1995	5 individual
AF	Austria	Fichamend, Vienna	48.05° N	16.40° E	Sept. 1993	26 individual
AM	Austria	Mistelbach, Vienna	48.35° N	16.35° E	Sept. 1993	39 individual
AG	Austria	St. Georgen, Burgenland	47.51° N	16.35° E	Sept. 1993	39 individual
TC	Croatia	Losinj, Cres Island	44.32° N	14.28° E	Mar. 1995	11 individual
GK	Germany	Knigsberg in Bayer, Bavaria	50.10° N	10.34° E	Sept. 1993	14 individual off buildings
GV	Germany	Volkach, Bavaria	49.51° N	10.09° E	Sept. 1993	25 individual
GH	Germany	Heilbronn, Baden-Württemberg	49.08° N	09.16° E	Sept. 1993	33 individual
GE	Germany	Heilbronn, Baden-Württemberg	49.08° N	09.14° E	Sept. 1993	5 individual off buildings
GW	Germany	Leingarten, Baden-Württemberg	49.07° N	09.05° E	Sept. 1993	32 individual
SC	Switzerland	Convento, Tessin	46.04° N	08.57° E	Sept. 1993	32 individual
SB	Switzerland	Bedano, Tessin	46.03° N	08.55° E	Sept. 1993	5 individual
SG	Switzerland	Gravezano, Tessin	46.02° N	08.54° E	Sept. 1993	21 individual
GI	Germany	Heidelberg, Baden-Württemberg	49.29° N	08.40° E	Sept. 1993	22 individual off buildings
GU	Germany	Ubstadt, Baden-Württemberg	49.10° N	08.39° E	Sept. 1993	25 individual
GO	Germany	Lorsch, Hessen	49.39° N	08.34° E	Sept. 1993	15 individual off buildings
GL	Germany	Lampertheim, Ofenheim	49.36° N	08.32° E	July 1994	30 individual
GS	Germany	Schutterwald, Baden-Württemberg	48.28° N	07.54° E	Sept. 1993	24 individual
FR	France	Brumath, Touraine	48.44° N	07.43° E	Nov. 1993	25 individual
GG	Germany	Gündlingen, Baden-Württemberg	48.01° N	07.38° E	Sept. 1993	25 individual
FL	France	Loches, Touraine	47.11° N	01.02° E	Sept. 1993	10 individual
FV	France	Verneuil-s-Indre, Touraine	47.03° N	01.02° E	Sept. 1993	29 individual
FP	France	Preuilly-s-Claise, Touraine	46.52.41° N	00.60° E	Sept. 1993	23 individual
FB	France	Benon, Charente-Maritime	46.13° N	00.47° E	Nov. 1994	6 individual
PP	Portugal	Ponte de Sor, Portalegro	39.15° N	08.06° W	Aug. 1994	2 individual
UM	United States	Wrentham, Norfolk County, MA	42.05° N	71.20° W	Feb. 1992 (Nov. 1993)	25 individual
UC	United States	Bethany, New Haven County, CT	41.25° N	73.00° W	Mar. 1994	12 individual
UN	United States	Coinjock, Currituck County,NC	36.18° N	75.57° W	May 1992 (Nov. 1993)	15 individual
UW	United States	Morgantown, Monongalia, WV	39.37° N	79.55° W	Jan. 1994	20 individual

[a] Date in parentheses is when the strain was received at the Ansonia facility.

mtDNA haplotype based on RFLP at two polymorphic nucleotide sites, nuclear DNA genotype at one locus, and microsatellite genotype at four loci for males from the same generation as the flight-tested females. We discuss the relationship between the marker results and female flight capability and implications for management programs. The observed pattern of genetic variation and geographic, environmental, and strain differences are used as a basis for developing scenarios that would explain female flight distribution.

Materials and Methods

Gypsy Moth Strains and Rearing. Information on the source location and collection of the 46 gypsy moths strains are given in Table 1. Based on the recent review of *Lymantria* (Pogue and Schaefer 2007), the JN strain is the *japonica* subspecies, the JS and 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.

All rearings to produce the adults used in this study were 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, and adding 0.12 g of amorphous $FePO_4$ 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, 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. The evaluations were done on individuals from the first laboratory generation after import or transfer from another quarantine (JS, CL, CS, CH, CB, UM, UN) with few exceptions. The flip test was not developed in time for use on strains collected or received before June 1994, and there were too few extra individuals for wing measurements from some strains (GW, GI) so individuals from the second laboratory generation were used.

Evaluation of Female Flight Capability and Preflight Behaviors. The same room and light controls described by Keena et al. (2001) were used and are briefly described here. 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 strains) was randomly marked with a unique number on its forewing with an indelible pen. Males were kept in the mating containers to be reunited with the females after the tests were completed. 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 was located near the center of the room and 3 m from the shelf that held the bolts. 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, Lincoln, NE) at moth eye level.

The free flight test protocols described by Keena et al. (2007) were used and are briefly described here. 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 ≥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 to one of the following categories based on their exhibited flight ability: capable of directed flight (sustained ascending flight during which the female circled the room), ≥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.

When ≥15 egg masses were available for use from a strain, two females from each egg mass were assessed for flight propensity and capability in the free flight test. When fewer egg masses were available or numbers of females were limited, equal numbers of females were assessed from each egg mass. 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.

Evaluation of Female Muscle Strength. The same females (except for females tested after June 1994—see note in Gypsy Moth Strain and Rearing section) from each of 30 strains used in the free flight test were screened for muscle strength in a flip test, which was done after the flight test and before the full egg mass was laid (some laid a few eggs during the test). The flip test consisted of inverting the female onto its back (wings folded over the back as at rest) on a slick surface and recording whether or not it was able to right itself (Keena et al. 2007). Females were assigned to one of two muscle strength groups: able to right themselves after one or more quick wing beats against the surface or remained inverted after >10 wing beats or no wing beats. Some of the females that could not right themselves vigorously beat their wings and pushed themselves around while raised up on the dorsum of their abdomen, whereas others barely moved. Shields et al. (1997) compared the musculature of females with different flip abilities and showed that those that easily righted themselves with a single wing beat had flight muscle fibers similar in diameter to those of the Russian strain, those that could not right themselves had fibers of similar diameter to the North American strain, and those that flipped with difficulty had fibers of intermediate diameter. Thus, we used this test to approximate the muscle strength of the females.

Evaluation of Female Wing Size. The forewing length (base to tip, FL), maximum hind wing width (HW), and maximum abdominal width (AW) were recorded from a separate set of 20 females from each of 41 strains randomly chosen from females not flight tested. 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. Discriminant analysis (PROC DISCRIM, SAS Institute 1999) was used to classify females using the three morphometric variables (FL, HW, and AW) and strain as the class. The groups used to develop the discriminant function were from a flightless strain (UN) and a strain with strong flight capability (RM), as well as reciprocal F_1s between the two strains that were capable of gliding flight. The 60 F_1 individuals and 30 individuals from

each strain were those used in Keena et al. (2007). These three groups were the base set used to develop the function; the females were cross-validated before all strain females were classified as UN, RM, or F_1.

DNA Extractions. Ten males were evaluated from each of 31 strains from the same generation as the females used in the flight evaluations. Males were used because this is the sex that is caught in pheromone detection traps and used to assess the origin of interceptions. DNA was extracted from one half of the head and thorax of each individual moth as previously described (Bogdanowicz et al. 1993). The material from the other half of the head and thorax was held at −80°C for later use if needed. The mtDNA haplotype based on RFLPs at two polymorphic nucleotide sites, nuclear DNA genotype at one RAPD-based locus, and microsatellite genotypes at four loci were determined.

Mt Marker Analyses (Cytochrome Oxydase I). PCR was used to amplify mtDNA fragments of cytochrome oxidase I (COI) with primers A2191 (5′-CCCGGTAAAATTAAAATATAAACTTC-3′) and S1859 (5′-GGAACIGGATGAACWGTTTAYCCICC-3′) (Bogdanowicz et al. 1993). Approximately 2.0 μl of the DNA extracts was amplified in 20 mM Tris-HCl, pH 8.4, 50 mM KCl, 200 μM of each dNTP, 3.0 mM $MgCl_2$, 0.2 μM of A2191, and 0.7 μM of S1859. Last, 0.6 U of *Taq* polymerase (Invitrogen Life Technologies, Burlington, Ontario, Canada) was added for a total reaction volume of 25 μl. The reactions were carried out in a Perkin Elmer 9600 thermocycler (Applied Biosystems, Streetsville, Ontario, Canada) with an initial denaturation at 95°C for 1 min followed by 35 cycles of 95°C for 15 s, 55°C for 30 s, an extension at 72°C for 90 s, and a final extension at 72°C for 3 min. Five microliters of diluted PCR product (25 μl of PCR reaction mixed with 30 μl of dsH_2O) was digested with 5.0 U of *Bam*HI or 2.5 U of *Nla*III in a total reaction volume of 15 μl. A 12-μl aliquot of the digested PCR product was size-fractioned using electrophoresis on a 1.5% agarose gel in 1× Tris-borate EDTA buffer, stained using ethidium bromide, and visualized under UV light. The *Bam*HI enzyme cut the PCR product if there was a G at the nucleotide site corresponding to nucleotide 2145 in the *Drosophila yakuba* Burla COI sequence and T at the site corresponding to nucleotide 2148 in the *D. yakuba* COI sequence (Bogdanowicz et al. 1993, 2000). If the *Bam*HI enzyme digest produced a band at ≈360 bp, it was reported as B+; the uncut product was represented by a band at ≈400 bp and was reported as B−. The *Nla*III enzyme cut the PCR product if a G was present at the nucleotide site corresponding to nucleotide 1882 in the *D. yakuba* COI sequence (Bogdanowicz et al. 1993, 2000). If the *Nla*III enzyme digest produced a band at ≈350 bp, it was reported as N+; the uncut product was represented by a band at ≈400 bp and was reported as N−.

Nuclear DNA Marker Analyses (FS1). A polymorphic region of the nuclear genome, the FS1 locus was amplified by PCR using locus-specific primers (JS1: 5′-GGATGGTGGGTGTCGTT-3′; JS2: 5′-GGTTGGTTGATGATTAGATG-3′) developed by Garner and Slavicek (1996). The PCR conditions were the same as for the COI marker with the following modifications: 2.0 mM $MgCl_2$ instead of 3.0 mM and 0.4 μM of each JS1 and JS2 primers. The annealing temperature was 50°C instead of 55°C. A 9-μl aliquot of the resulting PCR product was directly size-fractioned using electrophoresis as above. Results are reported for homozygotes as either N or A when a ≈200- or a ≈300-bp band was observed, respectively. Heterozygotes are reported as AN when both the ≈200- and ≈300-bp bands were observed for one specimen. The A stands for Asian because the 300-bp band was originally observed in gypsy moths of Far East Asian origin, and N stands for North American because the 200-bp band was the predominate allele observed in moths of North American origin.

Microsatellites Analyses. Four microsatellite primers (10F1, 49, 198, and 238) identified by Bogdanowicz et al. (1997) were amplified by PCR. Forward primers (2.25 pmol) for 10F1 (5′-CGCACAAAGCTCTCAGATGA-3′), 49 (5′-GAAGCCTACATTCAGCAGTGG-3′), 198 (5′-CGCTTAGTAGATAGTATTATCCATCC-3′), and 238 (5′-ACTGTTCGTTTATTCAATAGTGTTGG-3′) were end-labeled with 0.3 μCi of ^{33}P-ATP (1,000–3,000 Ci/mmol; NEN Life Science Products, Boston, MA) and 0.1 U of T4 polynucleotide kinase. One microliter of gypsy moth DNA was used in a 10-μl PCR reaction containing the end-labeled primer (0.2 μM), 0.225 μM of the corresponding unlabeled reverse primer (10F1: 5′-CGTTACCGCGTGTCTAGATT-3′, 49: 5′-GAAATCCGTCCATCCATTTG-3′, 198: 5′-TAAGTACGAGGTATGCCTGTATTCTT-3′, 238: 5′-ATATCCCTTAGTCGCCTTTTACG-3′) in 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 3.0 mM $MgCl_2$, 200 μM each dNTP, and 0.01 U of AmpliTaqGold polymerase (Applied Biosystems). The reactions were carried out in a Perkin Elmer 9600 or 9700 thermocycler (Applied Biosystems) with an initial denaturation at 95°C for 12 min followed by 40 cycles of 95°C for 15 s, 55°C for 10F1 and 49 or 60°C for 198 and 238, all for 15 s, an extension at 72°C for 30 s, and a final extension at 72°C for 10 min. An aliquot of the PCR product(s) was size-fractioned using electrophoresis on a 6% urea-polyacrylamide gel in 1× Tris-borate EDTA buffer and visualized using autoradiography. Alleles for each specimen analyzed were scored for each locus according to size in number of base pairs relative to a 25-bp ladder (Invitrogen Life Technologies).

Polygenic and Statistical Analyses. Female weight and each of the three wing/body measurements were analyzed by the restricted maximum likelihood estimation method (REML, PROC MIXED, SAS Institute 1999). Strain was the only fixed effect, and family was the random effect. Means of female weights were separated by least squares tests with $\alpha = 0.05$ and a Bonferroni correction (SAS Institute 1999).

The mtDNA results for the two sites were coded as if they were separate alleles at a single locus to allow simultaneous analysis of all the DNA data in a codominant format. A Nei pairwise population matrix of genetic distance for all populations was calculated using GenAlEx 6 (Peakall and Smouse 2006). A principle

Table 2. Percentage of *L. dispar* females from various strains, arranged by longitude from east to west, that exhibited each flight capability classification, flip result, and behavior

Strain	Country	*n*	Female flight			Ability to right self		Laid eggs	Walked while wing fanning	Voluntarily left post	Average female weight (mg)
			None	Glide	Fly	No	Yes				
JS	Japan	51	9.8	11.8	78.4	2.0	98.0	0.0	94.1	78.4	64.9 ± 5.5 f
JN	Japan	53	24.5	11.3	64.2	3.8	96.2	1.9	71.7	56.6	144.0 ± 7.6 ab
CL	China	51	2.0	10.2	87.8	0.0	100.0	0.0	98.0	91.8	123.5 ± 5.1 bcd
CS	China	50	20.0	12.0	68.0	9.0	91.0	0.0	80.0	70.0	112.8 ± 5.2 bcde
CH	China	50	8.0	8.0	84.0	1.0	99.0	0.0	94.0	90.0	117.0 ± 5.3 bcde
CB	China	50	24.0	2.0	74.0	1.0	99.0	0.0	78.0	74.0	114.3 ± 5.2 bcde
RM	Russia	39	17.5	15.0	67.5	1.0	99.0	0.0	82.5	72.5	124.1 ± 6.3 bcde
RB	Russia	36	30.6	52.8	16.7	0.0	100.0	0.0	88.9	55.6	89.6 ± 6.4 ef
RS	Russia	33	0.0	0.0	100.0	0.0	100.0	0.0	100.0	100.0	105.5 ± 7.5 bcde
BS	Bulgaria	37	88.9	11.1	0.0	95.0	5.0	10.8	45.9	0.0	123.4 ± 6.5 bcde
KG	Greece	39	97.4	2.6	0.0	100.0	0.0	5.1	41.0	0.0	165.1 ± 5.9 a
LJ	Lithuania	54	20.4	9.3	70.4	0.0	100.0	7.4	81.1	74.1	100.3 ± 5.0 cde
TO	Croatia	20	90.0	10.0	0.0	100.0	0.0	10.0	55.0	0.0	121.2 ± 8.3 bcde
TS	Croatia	10	88.9	11.1	0.0	90.0	10.0	0.0	60.0	0.0	114.7 ± 12.1 abcdef
VN	Slovak Republic	26	88.5	11.5	0.0	84.0	16.0	11.5	38.5	0.0	121.8 ± 7.2 bcde
WP	Poland	32	21.9	40.6	37.5	3.0	97.0	6.3	81.3	71.9	118.7 ± 6.4 bcde
TN	Croatia	17	82.4	17.6	0.0	71.0	29.0	11.8	58.8	11.8	128.6 ± 9.4 abcde
TL	Croatia	12	90.9	9.1	0.0	41.0	59.0	8.3	58.3	0.0	105.2 ± 10.9 bcdef
AF	Austria	33	90.9	9.1	0.0	100.0	0.0	30.3	51.5	3.0	101.0 ± 6.3 cde
AM	Austria	27	100.0	0.0	0.0	NA	NA	18.5	66.7	0.0	103.6 ± 6.9 bcde
AG	Austria	32	96.9	3.1	0.0	NA	NA	28.1	53.1	3.1	101.4 ± 6.7 cde
TC	Croatia	29	96.6	3.4	0.0	100.0	0.0	0.0	62.1	0.0	131.7 ± 7.4 abcd
GK	Germany	27	45.2	22.6	32.3	NA	NA	19.4	71.0	58.1	92.7 ± 7.0 def
GV	Germany	29	48.3	31.0	20.7	NA	NA	17.2	72.4	51.7	99.5 ± 6.8 bcde
GH	Germany	28	21.4	53.6	25.0	NA	NA	7.1	78.6	82.1	105.6 ± 6.9 bcde
GE	Germany	18	27.8	38.9	33.3	NA	NA	0.0	83.3	72.2	114.9 ± 10.4 bcde
GW	Germany	38	26.3	31.6	42.1	6.0	94.0	10.5	86.8	65.8	92.4 ± 6.1 def
SC	Switzerland	31	100.0	0.0	0.0	93.3	6.7	16.1	61.3	6.5	98.3 ± 6.5 cdef
SB	Switzerland	11	100.0	0.0	0.0	NA	NA	18.2	54.5	0.0	122.1 ± 11.7 abcde
SG	Switzerland	31	100.0	0.0	0.0	NA	NA	12.9	64.5	6.5	101.1 ± 6.8 cde
GI	Germany	32	40.6	34.4	25.0	7.0	93.0	28.1	65.6	59.4	107.0 ± 7.0 bcde
GU	Germany	31	41.9	38.7	19.4	NA	NA	25.8	67.7	38.7	105.3 ± 6.6 bcde
GO	Germany	32	33.3	29.6	37.0	NA	NA	3.7	81.5	66.7	102.6 ± 7.5 bcdef
GL	Germany	31	9.4	21.9	68.8	10.0	90.0	6.3	90.6	78.1	95.4 ± 6.4 bcde
GS	Germany	32	53.1	40.6	6.3	NA	NA	12.5	56.3	46.9	104.7 ± 6.7 bcde
FR	France	32	37.5	25.0	37.5	NA	NA	21.9	71.9	59.4	101.8 ± 6.4 cde
GG	Germany	29	62.1	34.5	3.4	NA	NA	10.3	51.7	27.6	102.3 ± 6.9 bcde
FL	France	27	96.3	3.7	0.0	NA	NA	33.3	44.4	3.7	102.0 ± 7.3 bcdef
FV	France	33	97.0	3.0	0.0	90.0	10.0	24.2	51.5	6.1	101.7 ± 6.3 cde
FP	France	30	96.7	3.3	0.0	NA	NA	36.7	10.0	6.7	97.6 ± 6.5 cdef
FB	France	31	93.5	6.5	0.0	92.0	8.0	19.4	45.2	9.7	102.5 ± 7.6 bcdef
PP	Portugal	15	100.0	0.0	0.0	89.0	11.0	13.3	13.3	20.0	137.5 ± 12.0 abcde
UM	United States	38	97.4	2.6	0.0	NA	NA	42.1	28.9	2.6	120.8 ± 5.8 bcde
UC	United States	19	100.0	0.0	0.0	97.0	3.0	21.1	31.6	0.0	103.1 ± 8.5 bcdef
UN	United States	46	97.8	2.2	0.0	98.0	2.0	43.5	6.5	4.3	129.1 ± 5.3 bc
UW	United States	20	100.0	0.0	0.0	99.0	1.0	45.0	20.0	5.0	120.7 ± 9.1 abcde

Average weights of females evaluated in flight test.

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.

NA, data not available.

coordinates analysis was run using the NEI matrix in GenAlEx 6 (Peakall and Smouse 2006) to look for major patterns in relatedness and to compare with female flight. Data for only the first two coordinates are used.

Number of alleles per locus, mean observed heterozygosity (Ho), and pairwise fixation indexes (F_{ST}) were calculated and tests for deviations from Hardy-Weinberg and linkage disequilibrium were assessed using Arlequin ver. 3.1 (Excoffier et al. 2005). A modified false discovery rate (FDR) method (Benjamin and Hochberg 1995) was used to determine the experiment-wide significance level to limit type 1 error caused by multiple tests being performed. Data from all seven DNA marker sites were analyzed using the admixture model in Structure version 2.2 (Pritchard et al. 2000). This Bayesian model-based method described by Pritchard et al. (2000) assumes there are a certain number of ancestral clusters (K) that are characterized by different allele frequencies and then sorts genotypes into groups by minimizing deviations from Hardy-Weinberg and linkage equilibrium within groups. Three independent runs (100,000 burn-in steps and 100,000 post burn-in steps) for each K from 1 to 15 were conducted. The method described by Evanno et al. (2005), which uses the rate of change in

Table 3. Cross-validation (RM, UN, and F_1) and classification results for *L. dispar* individualss from each strain, arranged by longitude from east to west, using three morphometric variables (mean ± SE)

Strain	*n*	Percentage classified			Forewing length (mm)	Hindwing width (mm)	Abdominal width (mm)
		UN	F_1	RM			
UN	50	96.0	4.0	0.0	27.9 ± 0.3	14.8 ± 0.2	9.7 ± 0.2
F_1	60	6.7	86.6	6.7	32.1 ± 0.5	17.1 ± 0.3	8.9 ± 0.2
RM	50	0.0	12.0	88.0	36.7 ± 0.5	19.3 ± 0.3	8.8 ± 0.2
JS	20	0.0	20.0	80.0	36.5 ± 1.0	20.5 ± 0.7	9.1 ± 0.3
JN	20	0.0	25.0	75.0	38.4 ± 0.8	19.6 ± 0.5	9.7 ± 0.2
CL	20	0.0	25.0	75.0	37.3 ± 0.9	23.2 ± 0.8	9.6 ± 0.2
CS	20	5.0	55.0	40.0	37.0 ± 0.8	22.8 ± 0.6	10.1 ± 0.2
CH	20	0.0	31.6	68.4	36.3 ± 0.7	21.2 ± 0.6	9.5 ± 0.2
CB	20	0.0	45.0	55.0	37.0 ± 0.9	22.4 ± 0.6	9.9 ± 0.2
RB	20	0.0	20.0	80.0	36.6 ± 0.8	20.0 ± 0.7	9.1 ± 0.3
RS	20	0.0	5.0	95.0	37.0 ± 0.9	19.5 ± 0.3	9.1 ± 0.2
BS	20	85.0	15.0	0.0	29.0 ± 0.7	16.3 ± 0.4	9.7 ± 0.3
KG	20	95.0	5.0	0.0	33.4 ± 0.5	17.5 ± 0.3	11.8 ± 0.4
LJ	20	5.0	95.0	0.0	31.9 ± 0.7	17.4 ± 0.5	8.8 ± 0.3
TO	20	80.0	20.0	0.0	27.6 ± 0.7	16.6 ± 0.6	9.7 ± 0.3
TS	20	85.0	15.0	0.0	29.3 ± 0.7	15.4 ± 0.3	9.7 ± 0.3
VN	20	100.0	0.0	0.0	29.3 ± 0.5	16.9 ± 0.6	10.4 ± 0.3
WP	20	15.0	80.0	5.0	32.7 ± 1.3	19.4 ± 1.0	9.6 ± 0.4
TN	20	90.0	10.0	0.0	28.2 ± 0.7	15.0 ± 0.3	9.4 ± 0.3
TL	20	80.0	20.0	0.0	27.9 ± 0.7	14.7 ± 0.4	9.1 ± 0.2
AF	20	60.0	40.0	0.0	27.5 ± 0.8	13.9 ± 0.4	8.7 ± 0.3
AM	20	65.0	35.0	0.0	28.4 ± 0.8	14.8 ± 0.4	8.9 ± 0.3
AG	20	65.0	35.0	0.0	26.5 ± 0.8	13.7 ± 0.4	8.4 ± 0.4
TC	20	100.0	0.0	0.0	27.5 ± 0.7	15.7 ± 0.7	10.2 ± 0.3
GV	20	5.0	70.0	25.0	32.5 ± 0.7	17.4 ± 0.3	8.6 ± 0.2
GH	20	0.0	85.0	15.0	32.9 ± 0.6	17.5 ± 0.4	8.8 ± 0.2
GW	20	10.0	80.0	10.0	31.7 ± 0.7	16.7 ± 0.8	9.1 ± 0.3
SC	20	60.0	40.0	0.0	27.2 ± 0.8	14.6 ± 0.4	8.5 ± 0.3
SG	20	55.0	45.0	0.0	28.2 ± 0.8	14.5 ± 0.4	8.8 ± 0.3
GI	20	15.0	85.0	0.0	30.7 ± 1.7	18.0 ± 0.7	9.6 ± 0.2
GU	18	0.0	66.67	33.3	32.5 ± 0.5	17.3 ± 0.4	8.5 ± 0.2
GL	20	5.0	90.0	5.0	32.0 ± 0.7	17.4 ± 0.4	8.7 ± 0.2
GS	20	0.0	75.0	25.0	31.8 ± 0.8	17.7 ± 0.5	8.5 ± 0.3
FR	15	0.0	86.7	13.3	31.6 ± 0.9	16.3 ± 0.6	8.8 ± 0.3
GG	20	5.0	95.0	0.0	31.7 ± 0.6	17.0 ± 0.4	9.0 ± 0.2
FV	20	65.0	30.0	5.0	27.9 ± 0.9	14.0 ± 0.3	8.8 ± 0.3
FP	20	55.0	45.0	0.0	27.4 ± 0.7	14.5 ± 0.3	8.7 ± 0.2
FB	20	60.0	40.0	0.0	27.9 ± 0.5	14.8 ± 0.3	8.7 ± 0.2
PP	9	100.0	0.0	0.0	26.9 ± 1.0	14.7 ± 0.4	10.0 ± 0.5
UM	20	70.0	30.0	0.0	30.9 ± 0.7	16.3 ± 0.7	10.5 ± 0.3
UC	20	95.0	5.0	0.0	28.3 ± 0.3	14.9 ± 0.3	9.9 ± 0.2

The UN, RM, and F_1 data were used to create the discriminant function (PROC DISCRIM, SAS Institute) and then were cross-validated. After cross-validation, the world strain data were classified using the function.

These measurements were made on a different set of females than the flight bioassay females (some were the next generation) and represented the complete size range.

the log probability of data between successive K values (ΔK), was used to estimate K. A single run of Structure version 2.2 (Pritchard et al. 2000) from the estimated K was used to assess the proportion membership of each strain in the inferred clusters and assign individuals to clusters.

Results

Female Flight Summary. World variation in female gypsy moth flight and related traits are given in Table 2. Females capable of strong directed flight originated from Asia, Siberia, and the northeastern parts of Europe. The RS strain was the only one where 100% of the females flew. Far Eastern strains exhibited >64% female flight, whereas lower percentages of female flight were exhibited by European strains. No flight-capable females were found in strains from the United States or Europe south of the Carpathian Mountains and Alps, most of France, or further west in Northern Europe. Weights of females that were flight tested varied significantly by strain ($F = 6.36$; df = 45,813; $P < 0.0001$). Female flight was observed in both of the strains with the heaviest and lightest weight females. When flight-capable females were present in the strain, >90% of the females could right themselves. When no females with flight were present in the strain, <16% could right themselves, with one exception (TL), and <20% left their post voluntarily. Females from 11 strains laid no eggs during the flight trials; all but 3 of these strains were from the Far East or Siberia.

Variation in female wing measurements is given in Table 3. The majority of females from the Asian and Siberian strains had wings classified as RM, and <5% were classified as UN. The majority of females from European strains, where some females were capable of

Table 4. Mean ± SD observed heterogeneity (H_O), no. of microsatellite alleles per locus, and percentage of individualss with each DNA haplotype or genotype for *L. dispar* strains, arranged by longitude from east to west

Strain	Country	Female flight	Mean H_O	Number of microsatellite alleles				*n*	MtDNA COI gene haplotypes			Autosomal DNA genotypes (FS1)			
				10F1	49	198	238		B− N−	N+ B−	N+ B+	AA	AN	NN	Null
JS	Japan	Yes	0.392 ± 0.201	3	2	3	2	10		100		100			
JN	Japan	Yes	0.525 ± 0.377	3	4	2	4	10			100	100			
CL	China	Yes	0.550 ± 0.173	4	6	4	7	10			100	100			
CS	China	Yes	0.520 ± 0.164	7	5	5	5	10			100	60	40		
CH	China	Yes	0.650 ± 0.238	4	5	5	8	10			100	100			
CB	China	Yes	0.700 ± 0.200	7	5	4	8	10			100	100			
RM	Russia	Yes	0.689 ± 0.121	5	6	4	9	10			100	100			
RB	Russia	Yes	0.498 ± 0.252	3	4	4	4	10		100		70	30		
RS	Russia	Yes	0.693 ± 0.262	4	3	4	3	10		100		100			
BS	Bulgaria	No	0.550 ± 0.058	3	1	4	5	10		100		30	60	10	
KG	Greece	No	0.542 ± 0.292	3	2	4	7	10		100		40	50	10	
LJ	Lithuania	Yes	0.524 ± 0.365	5	3	4	6	10		100		60	20	10	10
TO	Croatia	No	0.420 ± 0.179	2	4	2	6	10		100		30	50	20	
TS	Croatia	No	0.620 ± 0.334	4	2	4	6	10		100		30	70		
VN	Slovak Republic	No	0.478 ± 0.341	3	4	4	6	10		100		70	10	10	10
WP	Poland	Yes	0.511 ± 0.335	4	2	4	7	10		100		50	40	10	
TN	Croatia	No	0.542 ± 0.183	4	1	2	6	10		100		50	30	20	
TL	Croatia	No	0.600 ± 0.339	3	2	6	5	10		100		10	80	10	
AF	Austria	No	0.580 ± 0.277	3	3	4	7	9		100		10	80	10	
TC	Croatia	No	0.362 ± 0.287	2	2	4	4	10		100		40	10	50	
GW	Germany	Yes	0.440 ± 0.305	2	3	5	4	10		100		30	20	50	
SC	Switzerland	No	0.390 ± 0.297	5	2	2	6	10		100		20	40	40	
GI	Germany	Yes	0.570 ± 0.268	3	3	5	5	10		100			60	40	
GL	Germany	Yes	0.496 ± 0.294	2	3	4	5	10		100			50	50	
GG	Germany	Yes	0.480 ± 0.217	3	4	4	4	10		100		20	40	40	
FP	France	No	0.500 ± 0.224	2	4	5	9	10		100			50	50	
FB	France	No	0.470 ± 0.273	2	3	3	5	10		100			30	70	
PP	Portugal	No	0.300 ± 0.141	1	1	2	2	10		100				100	
UC	United States	No	0.440 ± 0.167	2	2	3	2	10	100				20	80	
UN	United States	No	0.400 ± 0.163	2	2	2	2	10	100					100	
UW	United States	No	0.525 ± 0.275	2	2	3	3	10	100					100	

B, *Bam*H1 site; N, *NI*a site in the COI gene.

flight, had wings classified as F_1. In all strains with no flight-capable females, more than one half of the individuals had wings classified as UN and only one (FV) had wings classified as RM.

DNA Variation Summary. Mitochondrial haplotypes for all 310 males and FS1 genotypes for 308 males were determined (Table 4). All Far East strains (except for JS) had the N+B+ mtDNA haplotype, the strains from Siberia and Europe all had the N+B− haplotype, and the strains from the United States had the N−B− haplotype. The AA autosomal DNA genotype occurred in 100% of the males sampled from the Asian and Siberian strains, with the exception of the CS and RB strains. Males from three of the four U.S. strains and the PP strain all had the NN genotype. Across Europe, both alleles were present, and the percentage of the A allele tended to decrease from East to West. Up to 70% of the males from strains with no flight-capable females had the AA genotype.

The number of microsatellite alleles per locus for each strain are given in Table 4, and the percentages of each allele are summarized for strains lumped by geographical regions (Asia, Europe/Siberia, and North America) are given in Fig. 1. Only three significant (FDR-corrected $\alpha = 0.009$) deviations from Hardy-Weinberg equilibrium were found at locus 49 for strains WP ($\chi^2 = 10$, df = 1, $P = 0.002$), AF ($\chi^2 = 15.2$, df = 3, $P = 0.002$), and SC ($\chi^2 = 10$, df = 1, $P = 0.002$). There was only one instance of significant linkage disequilibrium (FRR corrected $\alpha = 0.009$) for the GI strain between loci 10F1 and I98 ($\chi^2 = 21.8$, df = 8, $P = 0.0058$). Diagnostic fragments could not be

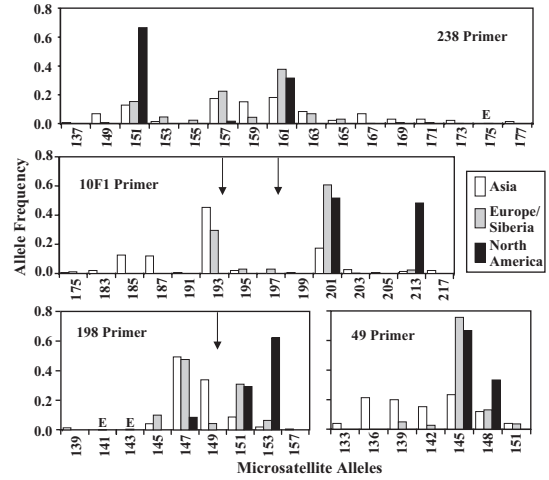

Fig. 1. Microsatellite allele frequencies for strains grouped into three broad geographic regions (Asia, Europe and Siberia, North America). The letter E indicates that the European/Siberian region had a very low frequency of the given allele, and the arrows indicate other alleles found in North American but not observed in this study.

amplified from 1 male for locus 49, 2 males for locus 10F1, 12 males for locus 198, and 15 males for locus 238, with the majority of problems in the JS and Russian strains for the latter locus. The U.S. strains had only two to three alleles per locus, and in general, these were the most common worldwide (Fig. 1). Allele 193 at the 10F1 locus was more common in Europe than the second one (213) found in U.S. strains. There were only nine private (i.e., occurred in only one strain) alleles. These were found at low frequencies in the four loci, with seven found only in Far East Asian strains and none were found in United States strains. There were an additional seven alleles (three for 10F1, two for 49, one each for 198 and 238) that were found only in Asian strains. The average number of total alleles was highest in the Asian strains (18.3), intermediate in the European/Siberian strains (14.8), and lowest in the U.S. strains (9.0).

Comparisons of the Distribution of DNA and Female Flight Variation. Pairwise F_{STs} between strains and the significance of the population differentiation at the 0.0001 level (FDR modification for $\alpha = 0.05$) are given in Table 5. None of the Siberian, 26% of the European, 33% of the North American, and 76% of the Asian strain compositions within each geographical area indicated significant population differentiation. The North American strains were significantly differentiated from strains in all other geographic areas, but European strains with and without flight-capable females were only distinct in 27% of the pairings.

Principle coordinate analysis based on the calculated Nei distances between all the strains showed seven groups of strains (Fig. 2). The strains with the mtDNA haplotype N−B− were all in one group, the strains with haplotype N+B+ were in two groups, and the strains with the N+B− were spread over four groups. The strains within the central diagonal formed by the N+B− populations are roughly geographically distributed with the western most strain (PP) at the top and eastern most strain at the bottom (JS). Within the central group, the European strains with and without flight-capable females are mixed, whereas all the other groups could be classified as either with or without flight-capable females.

When the DNA genotypes and haplotypes were used to assess population structure, three ancestral clusters were predicted with an estimated Ln probability of −3,625.2, mean α of 0.0451, and ΔK of 91.6 (the ΔKs for all other K values tested were ≥19 points lower). A population structure analysis run using only the nuclear loci resulted in a prediction of only two ancestral clusters, basically combining two and three in the assessment using all DNA markers. Figure 3 gives the percentage membership in each cluster for each strain. All strains had at least a low probability of membership in each cluster. All but one individual from the Asian strains was assigned to cluster 1, 75% of the individuals from Siberian strains were assigned to cluster 1, and all individuals from the United States were assigned to cluster 3. Ninety-four percent of the individuals from the European strains were assigned to cluster 2 and the remaining 6% were assigned to cluster 1. There was no apparent genetic distinction between European strains with and without females that were capable of flight.

Discussion

Females capable of strong directed flight were found in strains that originated from Asia, Siberia, and the northeastern parts of Europe. In only the RS strain from central Siberia were all females tested capable of flight; most strains that had flight-capable females also had individuals capable of only gliding and/or no flight. No flight-capable females were found in strains from the United States or southern and western European strains. Thus, female flight in *L. dispar* is not simply an Asian strain trait, and the trait is not fixed in most populations.

Flying females were reared both from egg masses collected in European forests settings and from egg masses scraped from buildings. Eight of the 12 strains from Germany were from forests, and the percentage of flight-capable females ranged from 3 to 69%, whereas, consistently, about one third of the females from egg masses collected off buildings near lights were capable of flight. Percentages of female flight >30% in the progeny would be expected if a fully flight-capable female was to mate with a male that carried genes for intermediate flight capability (Keena et al. 2007). Thus, flight-capable females seem to mate randomly, but because of their attraction to light, several females will deposit their eggs at the same location. This occurs in the forest as well; at German forest locations, large numbers of egg masses were deposited on trees with light colored bark. This may have been the reason for similar percentages of female flight found in GE and GH, which were from buildings and a nearby forest, respectively.

Two traits that are part of the flight polymorphism, wing musculature and size, may be useful in predicting female flight capability at the population level. In populations with some flight-capable females, >90% of the females could right themselves, and generally when no females were capable of flight, <16% could right themselves. The possibility of female flight being present in the population existed when the majority of the females in the population had wings that scored as F_1 or RM. This trait is also readily observable when the females have their wings at rest over their backs, in that the last few segments of the female's abdomen are visible when the wings are too short for flight. The forewing lengths for most of the strains fell within the ranges for their respective subspecies based on specified distribution (Pogue and Schaefer 2007). Exceptions were for the European strains with flight-capable females and the KG strain with no female flight, which both fit the *asiatica* rather than the *dispar* wing length range. Wing color and markings of the females also could not accurately predict subspecies. Female wing color was consistent with the subspecies based on the distribution given by Pogue and Schaefer (2007), except that the JS strain from Hokkaido was generally white and did not have a brown cast, characteristic of

Table 5. Pairwise F_{ST} values between *L. dispar* strains below diagonal and significance of F_{ST} *P* values at the 0.0001 level above diagonal

Strain	JS	JN	CL	CS	CH	CB	RM	RB	RS	BS	KG	LJ	TO	VN	WP	TN	TL	AF	TC	GW	SC	GI	GL	GG	FP	FB	PP	UC	UN	UW
JS	0.00	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
JN	0.16	0.00	+	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
CL	0.20	0.09	0.00	+	−	+	+	+	−	−	+	−	−	−	−	−	−	+	−	+	−	−	+	−	+	+	+	+	+	+
CS	0.22	0.02	0.09	0.00	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+
CH	0.24	0.05	0.04	0.03	0.00	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+
CB	0.21	0.05	0.08	0.09	0.08	0.00	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
RM	0.41	0.17	0.19	0.21	0.17	0.21	0.00	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
RB	0.12	0.15	0.11	0.18	0.16	0.21	0.41	0.00	−	+	+	−	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+
RS	0.22	0.17	0.08	0.16	0.14	0.19	0.37	0.08	0.00	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
BS	0.38	0.30	0.11	0.27	0.24	0.31	0.49	0.28	0.27	0.00	−	−	−	−	+	−	−	−	−	−	−	+	+	−	+	−	+	+	+	+
KG	0.25	0.21	0.07	0.19	0.19	0.22	0.40	0.18	0.16	0.03	0.00	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	+	+	+	+
LJ	0.19	0.18	0.07	0.17	0.15	0.17	0.36	0.05	0.06	0.12	0.05	0.00	+	−	+	−	−	+	−	+	−	−	+	+	+	+	+	+	+	+
TO	0.29	0.22	0.09	0.23	0.21	0.26	0.46	0.22	0.25	0.02	0.05	0.13	0.00	−	−	−	−	+	−	−	−	−	−	−	−	−	+	+	+	+
TS	0.22	0.18	0.05	0.19	0.17	0.20	0.40	0.14	0.14	0.02	−0.02	0.04	0.00	0.00	−	−	−	−	−	+	−	−	+	−	+	+	+	+	+	+
VN	0.31	0.17	0.04	0.15	0.12	0.21	0.38	0.25	0.17	0.07	0.02	0.15	0.07	0.03	0.00	−	+	−	−	−	−	+	−	−	+	+	+	+	+	+
WP	0.23	0.17	0.02	0.17	0.13	0.16	0.34	0.12	0.11	0.04	−0.01	0.01	0.05	0.00	0.05	0.00	−	−	−	−	−	+	+	−	+	+	+	+	+	+
TN	0.33	0.27	0.09	0.26	0.23	0.26	0.46	0.23	0.21	−0.01	0.01	0.06	0.03	−0.01	0.07	0.02	0.00	−	−	−	−	+	−	−	−	+	+	+	+	+
TL	0.37	0.28	0.10	0.26	0.24	0.30	0.45	0.27	0.23	0.03	0.00	0.13	0.08	0.02	0.03	0.03	0.05	0.00	−	−	−	−	−	−	−	−	+	+	+	+
AF	0.25	0.18	0.05	0.16	0.15	0.21	0.37	0.14	0.17	0.04	0.00	0.04	0.02	0.01	0.06	−0.01	0.04	0.03	0.00	−	−	−	−	−	−	−	+	+	+	+
TC	0.41	0.33	0.15	0.30	0.26	0.35	0.50	0.31	0.29	0.06	0.04	0.16	0.12	0.08	0.09	0.07	0.09	−0.01	0.05	0.00	−	−	−	−	−	−	+	+	+	+
GW	0.29	0.21	0.08	0.18	0.16	0.25	0.37	0.15	0.17	0.13	0.07	0.11	0.12	0.08	0.10	0.06	0.14	0.05	−0.01	0.06	0.00	−	−	−	−	−	+	+	+	+
SC	0.37	0.30	0.11	0.29	0.27	0.31	0.49	0.29	0.26	0.02	0.01	0.14	0.02	0.00	0.06	0.05	0.02	−0.02	0.03	0.04	0.09	−	−	−	−	−	+	+	+	+
GI	0.33	0.22	0.11	0.21	0.19	0.27	0.41	0.22	0.25	0.14	0.11	0.15	0.08	0.08	0.13	0.10	0.15	0.09	0.02	0.09	0.02	0.00	−	−	−	−	+	+	+	+
GL	0.43	0.32	0.15	0.29	0.28	0.35	0.49	0.34	0.31	0.09	0.08	0.21	0.10	0.09	0.09	0.12	0.13	0.02	0.06	0.04	0.05	0.07	0.00	−	−	−	+	+	+	+
GG	0.30	0.21	0.06	0.20	0.19	0.23	0.39	0.19	0.20	0.07	0.05	0.11	0.02	0.04	0.07	0.03	0.08	0.04	−0.02	0.08	0.01	0.03	0.04	0.00	−	−	+	+	+	+
FP	0.37	0.26	0.12	0.24	0.24	0.28	0.42	0.27	0.26	0.09	0.06	0.17	0.07	0.07	0.08	0.08	0.10	0.02	0.03	0.03	0.03	0.02	−0.02	0.00	0.00	−	−	+	+	+
FB	0.42	0.33	0.18	0.31	0.30	0.37	0.51	0.32	0.33	0.11	0.08	0.18	0.10	0.10	0.16	0.11	0.14	0.04	0.02	0.04	0.02	0.05	0.01	0.02	−0.01	0.00	−	+	+	+
PP	0.66	0.56	0.38	0.53	0.51	0.58	0.71	0.59	0.57	0.25	0.27	0.41	0.31	0.31	0.35	0.31	0.31	0.17	0.22	0.18	0.24	0.29	0.13	0.21	0.15	0.09	0.00	+	+	+
UC	0.54	0.45	0.26	0.43	0.40	0.45	0.57	0.40	0.38	0.33	0.29	0.28	0.35	0.28	0.35	0.26	0.31	0.22	0.20	0.27	0.17	0.23	0.25	0.19	0.21	0.22	0.39	0.00	−	−
UN	0.62	0.51	0.33	0.49	0.45	0.52	0.64	0.50	0.50	0.34	0.37	0.38	0.37	0.34	0.40	0.34	0.36	0.27	0.27	0.25	0.23	0.21	0.27	0.25	0.22	0.24	0.39	0.14	0.00	+
UW	0.61	0.50	0.34	0.48	0.46	0.52	0.63	0.48	0.46	0.44	0.38	0.40	0.43	0.37	0.42	0.37	0.43	0.30	0.30	0.35	0.22	0.26	0.31	0.27	0.25	0.31	0.50	0.06	0.21	0.00

Pairwise F_{ST} and *P* values calculated using all the DNA marker results and the no. of different alleles distance method in Arlequin ver. 3.1 (Excoffier et al. 2005).

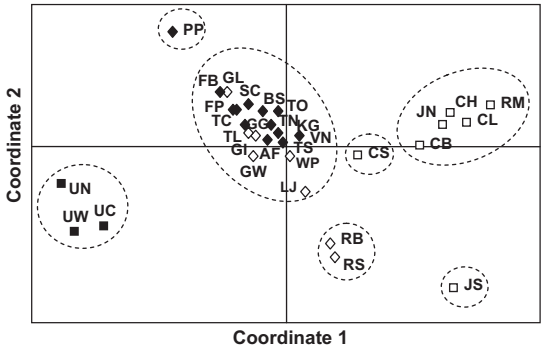

Fig. 2. Principle coordinate analysis based on Nei-pairwise population genetic distances. Flight capability of the females from the population is indicated (no flight, closed markers; some female flight, open markers), and general geographic groupings are shown (dotted ovals).

japonica. In addition, the presence of a prominent post medial band characteristic of subspecies *asiatica* and *japonica* was found in females from strains without flight capable females; all the females from PP, which had the shortest wings, had the prominent band. The alternate was also true—some females from JS and RS, which should be the *asiatica* subspecies, lacked the prominent band. Thus, the subspecies of the European strains with flight-capable females seems to be *asiatica*, but Europe is not listed as part of its distribution. This emphasizes that both wing and muscle traits are highly variable, and several females from a population should be evaluated for all traits in combination before making any predictions of flight capability or subspecies.

Female weight is not a good predictor of flight capability because both the heaviest and lightest females were capable of flight. This is consistent with the 0.069 heritability estimated for female weight (Keena et al. 2007), which suggests that environmental factors have a very strong influence on female weight. In fact, female weight seems to be tied in part to the number of instars the female goes through, which varies among

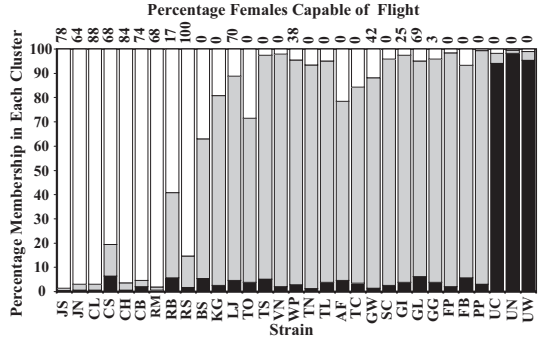

Fig. 3. Proportion of membership of each strain in each of the three predicted ancestral clusters (1, no fill; 2, gray fill; 3, black fill) and percentage of flight-capable females in each strain. Clusters were determined by population assignment using the admixture model in Structure (Pritchard et al. 2000) and all seven DNA markers.

strains. Many females from the RS and LJ strains go through less and females from the JN go through more than the standard six instars (M.A.K., unpublished data). Fecundity, which is positively correlated with female weight, does not vary significantly between flight-capable and flightless females (Keena et al. 2007), so at least this aspect of fitness is not affected.

The mtDNA haplotypes broadly separated the *L. dispar* strains into three groups: North American (N−B−), Siberian/European (N+B−), and Asian (N+B+). The JS strain is the only Asian exception, having the Siberian/European haplotype. When additional mtDNA loci were evaluated, *L. dispar* from the JS strain (originating from Hokkaido, Japan) was found to have a unique haplotype not found in other Asian populations and different from the Siberian/European populations (Bogdanowicz et al. 2000). More extensive surveys of Europe have found the North American haplotype in low abundance (<5%) in France, Italy, and Germany (Prasher 1996). Conversely, the Siberian/European haplotype has been found at similar low frequencies in both Canada (Côté 1996) and the United States (Prasher 1996). Rare occurrences of the Asian haplotype have been found in Austria, the Slovak Republic, and near Moscow (Prasher 1996). The B+N− haplotype has been found in Yakovlevka, Russia (Bogdanowicz et al. 1993) and populations from Kyrgyzstan (M.-J.C., unpublished data). The geographic areas where this haplotype is present have not been broadly sampled, so its relative abundance is unknown. Thus, none of these haplotypes is completely fixed across the geographical regions where they predominate.

The A allele at the FS1 nuclear DNA locus increases in frequency from west to east across Eurasia and occurs at low frequencies in some U.S. strains (Prasher and Mastro 1995). Neither allele is completely fixed in a region, but the N allele in North America and the A allele in Siberia and Asia generally occur at frequencies >95% in the populations sampled. The occurrence of the N allele at higher frequencies and the lower female flight seen in the RB population may be evidence for some adults (likely males, because the mtDNA was not the North American haplotype) of U.S. origin escaping from field studies conducted there comparing a U.S. strain with a Siberian strain. The occurrence of all genotypes of the FS1 across Europe supports the idea that these populations may be more variable and could have resulted from some movement and mixing of strains.

The microsatellite data for the four loci evaluated cannot be used to definitively determine the origin of an individual male, but the presence of alleles not found in North American do point to an origin outside of North America. There are three additional alleles, not found in our analyses, that have been found in Eastern Canada at low frequencies: 193 and 197 for the 10F1 locus and allele 149 for the 198 locus (Bogdanowicz et al. 1997; M.-J.C., unpublished data). North American individuals can be fairly accurately distinguished from Asian and Siberian moths but not from European moths using the microsatellite data. To de-

termine the broad geographic cluster to which a newly trapped individual belongs, the individual's data could be run with the strains presented here in a population assignment analysis.

Ninety-four percent of the individuals were accurately placed into their broad geographic group of origin (North American, European, Siberian, and Asian) when all DNA genotypes and haplotypes were used in a population assignment analysis. However, because only three ancestral clusters are predicted, individuals cannot be assigned to a specific origin. Within the European strains, there was no clear distinction between ones with and without flight-capable females. A principle coordinate analysis based on Nei distances was similarly accurate in grouping the strains by geographic regions. If additional markers or substantially more individuals were used, the ability to identify the source population would increase.

The observed pattern of genetic variation and known geographic probability of environment and strain differences can be used as a basis for developing scenarios that would explain the present female flight distribution. Reduced variation in the North American *L. dispar* population compared with the European populations has been documented (Harrison et al. 1983), and this could have been caused by a founder effect and/or subsequent adaptation to a new environment. Additionally, there may have been no flight capable female present in the European strain from which the founders came. Reineke et al. (1999) suggested that population bottlenecks may have occurred in Eurasia when populations retreated to refugia during the glaciation event 10,000 yr ago. It is possible that a predominantly flightless female population existed in a southern European refuge and that it was the primary founder population for much of Europe. Immigration (Spuler 1908) and interbreeding, most likely with a Siberian population based on the shared mtDNA haplotype, may have increased variation in female flight capability in northeastern Europe and resulted in the east to west decline in female flight. This immigration may have been natural, resulting from the extended flights that some females make, or aided by humans. The actual flight capability of females from parts of southeastern Europe and central Asia was not determined because samples were not available, but reports in the literature support this interpretation. Mountain barriers in Europe seem to have limited the spread of flight-capable females into southern Europe. There is only minimal evidence of Asian genes in the European population; a few individuals were assigned to the predominately Asian clusters in our analysis and in earlier reported molecular evidence (Graser 1996, Reineke et al. 1999). Gene flow between Japanese and mainland Far Eastern populations, and between those populations and the Siberian populations, is apparently limited based on DNA genotypes and mtDNA haplotypes. This would be expected because there is a whole series of mountain ranges and deserts that separate the Far East forests from the Siberian forests that would limit gene flow between these geographic areas.

Several factors have been suggested as possible selection factors that maintain female flight in *L. dispar* in these areas: ability to find thermally favorable habitats (Baranchikov 1989) or escape from predators and parasitoids at oviposition sites (Higashiura 1989), the ability to escape deteriorating habitats and colonize new ones (Reineke and Zebitz 1998), and a means of avoiding adult predation (Charlton et al. 1999). The selection for flight capability in females must be strong to maintain high levels in a population because the strategy not to migrate was shown to be evolutionarily stable in the absence of catastrophes (Gyllenberg et al. 2002). An inverse relationship between the dispersal capability of insects and the persistence of habits (Denno et al. 1996) has been documented, and flight is inherited polygenically with gliding as the intermediate trait (Keena et al. 2007). This is further emphasized by the fact that several traits of gypsy moth are associated with the evolution of reduced wings in temperate forest macrolepidoptera: spring feeding larvae, overwintering as eggs, eggs placed in clusters or a single mass, high host breadth, high fecundity, and outbreak population dynamics (Hunter 1995).

The implications for management are clear; no single marker or group of genetic markers currently in use can directly predict female flight capability or the specific population of origin. We can assume female flight is present in the population of origin if an individual is assigned to the Asian or Siberian groups based on genetic markers, but it is not possible to distinguish flight capable individuals from flightless individuals using genetic markers. This is most obvious when European populations with and without flight-capable females are compared. Thus, without a genetic marker directly associated with female flight, or additional genetic markers that can be used to accurately assign individuals to a specific strain of origin, the flight capability of moths classified as European in origin cannot be predicted. Without additional genetic markers, males from traps that have the N+B− mtDNA haplotype, the A FS1 allele, and one or more microsatellite alleles that are rare or non existent in the United States would likely be classified as European (or Siberian) with unknown female flight capability. If these moths are all treated as potentially from populations with flight-capable females, flight will be overestimated and the converse is true if they are treated as coming from populations without female flight. The AFLP primers developed by Reineke et al. (1999) may be useful in determining the specific strain of origin when the genetic marker set currently in use by North American regulatory agencies identifies the individual as being of European origin.

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