

Potential Differences and Methods of Determining Gypsy Moth Female Flight Capabilities: Implications for the Establishment and Spread in Novel Habitats

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Abstract: The introduction of the Asian gypsy moth into novel environments continues with frequent interceptions in North America. There is a concern that these subspecies will pose a greater threat to the forests and urban environments of North America than the established gypsy moths (*Lymantria dispar dispar* L.), due to their greater capacity for female flight. Asian gypsy moth populations vary in many key traits, including female flight capabilities. The potential impacts of female flight, in combination with the other key traits, on the ecology and spread of this insect are first discussed in this communication. This also provides the first review of most of the current literature on the variations in flight capability and flight distance of gypsy moth populations, as well as variation in other traits of concern and the potential methods of identification, with special attention paid to the Asian subspecies *Lymantria dispar japonica* Motschulsky and *Lymantria dispar asiatica* Vinkovskij. There are currently good tools for identifying the general origin of introduced gypsy moth populations, but these do not provide enough information to effectively manage introductions. Gypsy moth key traits differ among populations, even within each subspecies of the gypsy moth, so introduction of gypsy moths from other world areas into locations where the gypsy moth is already present could result in unwanted changes in gypsy moth biology. It also appears that the introduction of flight-capable females could enhance a population's dispersal capability and require modifications to management protocols used for flightless females. Therefore, rapid tools to assess key traits in introduced populations are needed to adequately plan for, or deal with, new introductions into novel habitats.

Keywords: Asian gypsy moth; *Lymantria dispar*; invasive species; forest pests



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1. Introduction

The gypsy moth, *Lymantria dispar* (Lepidoptera: Lymantriidae), is a forest insect capable of causing severe harm to ecosystems and individual trees through repeated defoliation [1,2] and is responsible for substantial economic losses caused by subsequent damage [2]. Liebhold et al. [3] estimated that caterpillars of gypsy moth populations currently present in North America can feed on over 300 (~100 preferred species) shrubs and tree species. Furthermore, Keena and Richards [4] found that gypsy moths of European and Asian origin were able to establish on North American tree types even without prior exposure to the host species, thus making them a threat to temperate forests around the globe.

Three subspecies of gypsy moth have been described: European gypsy moth (*Lymantria dispar dispar*), Asian gypsy moth (*Lymantria dispar asiatica*), and Japanese gypsy

moth (*Lymantria dispar japonica*) [5,6]. The European gypsy moth (EGM) is native to Eurasia, where it is found from Portugal, in the west, to the Ural Mountains, in the east [7]. This subspecies was introduced into North America in 1869 and is now established in many forests of the Eastern United States and Canada [8,9]. The native range of the Asian gypsy moth (AGM) extends east from the Ural Mountains through Russia, China, Korea, and into portions of Japan, while the Japanese gypsy moth (JGM) is limited to the main islands of Japan and the Kuril Islands of Russia [5].

The European gypsy moth (EGM) was first introduced into the northeastern state of Massachusetts, in the United States, in 1869, by a French mathematician, in order to conduct breeding experiments with a native silkworm. During this time, gypsy moth larvae escaped and became established in the surrounding area. Twenty years after this initial establishment, a large outbreak occurred and attracted public attention. Over the next 100 years, the gypsy moth continued to expand into the forests of the Eastern United States and Canada, despite numerous eradication efforts led by local and federal agencies [10]. Within the United States, the rate of spread of EGM averaged 9.45 km/year between 1900 and 1915, 2.82 km/year between 1916 and 1965, and 20.78 km/year between 1966 and 1990 [11]. McManus and Csoka [10] believe that the increased rate of spread that occurred in the second half of the 19th century could be due to the following: (1) a decrease in the use of DDT and other chemical pesticides; (2) the fact that newly infested areas contained contiguous forests dominated by oak species (*Quercus* spp.), the EGM's preferred host; and (3) severe outbreaks that occurred in the 1970s and early 1980s that increased the spread rates of the insect. After finding that stratified dispersal was important in the spread of EGM [11], a barrier-zone management program called "Slow the Spread" was instituted that has reduced the annual rate of spread to <4 km per year [12]. Moreover, outbreaks in established areas are now better regulated in a density-dependent form by two host-specific pathogens, (1) the fungus *Entomophaga maimaiga* Humber, Shimazu, and Soper (Zygomycetes: Entomophthorales) and (2) a nucleopolyhedrosis virus, *LdNPV* [13,14]. In addition, aerial application of *Bacillus thuringiensis* Berliner serovar *kurstaki* (Btk) is currently the most effective control method to suppress gypsy moth outbreaks in Europe [15]. In areas with established gypsy moth populations, outbreaks generally occur every five to ten years [16] and last between one and three years [17,18]. However, the longest outbreak periods were recorded to be 12.5 and 13.0 years in Poland and the Czech Republic, respectively [19]. The outbreak period decreases from the northwest to the southeast regions of Europe. For example, in Central Europe, the period is approximately 12 years, while in Mediterranean and Balkan regions, it is seven to eight years [20]. A study by Hlasny et al. [21] found outbreak periods to be strongly related to geographical gradients and revealed substantial spatial and temporal variability in the outbreak patterns in the regions surrounding the Carpathian Mountains.

There are ecological costs to the EGM invasion. In general, the EGM displays a preference for certain tree species during outbreaks [10], and these tree species are defoliated at higher rates than species that are consumed by only some larval stages or species rarely, if ever, consumed by any larval stage [22]. Once defoliated, the weakened trees are far more susceptible to other agents, such as boring insects and parasitic fungi, which cause eventual tree mortality [10]. Consequently, EGM has caused a decrease in the dominance of their preferred host species, specifically oaks, in Eastern North American forests and could result in a long-term shift in species composition [23].

Substantial economic costs are also associated with the EGM invasion of North America. These costs generally come from the following sources: (1) timber value loss due to tree mortality or a reduction in growth; (2) residential-property value loss caused from dead or dying trees; (3) household expenditures for tree care; (4) local government expenditures for tree removal and replacement, or treatment; and (5) federal government expenditures for research, regulation, management, and outreach. Aukema et al. [24] estimated that the EGM was responsible for \$254 million in damages on an annual basis, with a considerable proportion of this (\$120 million) coming from residential property value loss.

The AGM was not introduced into North America until much later. The first recorded introduction occurred in 1991, near the port of Vancouver, in British Columbia, Canada [25]. Shortly after this, individual AGMs were discovered in the United States, in Washington and Oregon, in addition to other parts of British Columbia. It is thought that ships from Eastern Russia transported AGM larvae to ports along the Pacific Northwest Coast. These infestations were eradicated quickly through treatment and trapping. However, since this initial introduction, AGM has been detected and eradicated on at least 20 separate occasions throughout the United States [25]. In 2020, an eradication program occurred in Western Washington State for recently discovered introductions of EGM with AGM genetic traits, as well as the Hokkaido gypsy moth (*Lymantria umbrosa* Butler) [26].

If AGMs were to establish in North America, there is a high probability that the ensuing economic and ecological damage would exceed that of the EGM for several reasons (Figure 1). Although males of both AGM and EGM can fly, AGM females are capable of flight, while EGM females are not [7]. Studies have also found that Asian and Japanese female gypsy moths display an attraction to light sources, which has commonly led to egg deposits on ships and vehicles [27,28]. In addition, AGMs have a wide geographic range that encompasses various forest ecosystems. If introductions were to occur with AGMs from multiple origins, they might produce offspring with a wider host range than the original populations [4]. Gypsy moth populations also vary in both the larval and egg responses to temperature. The amount of chill an egg mass requires before hatching varies between populations across all subspecies. Hence, if genotypes that require less chill were introduced into already infested areas, variation in egg hatch within the population could increase, and a non-diapausing strain could develop in uninfested areas with mild climates [29]. Some AGM larvae take a longer time to develop and grow to larger sizes, and this could cause greater defoliation at lower numbers than the EGM larvae [30]. However, with longer time to grow and develop, there is increased opportunity for natural enemies and other factors to influence population dynamics.

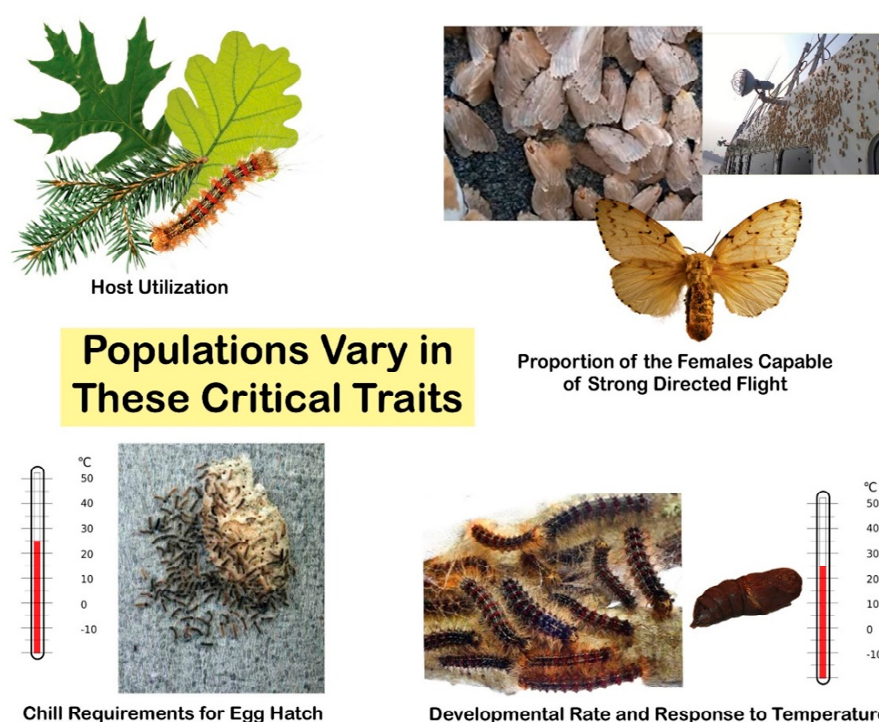


Figure 1. Gypsy moth populations vary in several key traits that can alter the dispersal capabilities or their potential to cause damage in novel habitats.

One of the most important variables used in estimating the spread of gypsy moths is flight ability and flight distance of female moths. While current spread and distribution

modelling approaches have used general flight distance estimates [31], more specific information would help to improve projections of future potential distribution and spread. However, there does not appear to be a distinct line separating the flight capacity of each gypsy moth subspecies. While dividing gypsy moths into three subspecies can be useful for descriptive purposes, traits can be passed between subspecies through hybridization [32]. Because of this, it is especially important to examine flight capability on a distinct population basis instead of limiting the inquiry to a subspecies level. The overall objective of this communication is to document what is currently known regarding the flight capability, and potential flight distances, of females from different gypsy moth populations. We also place this information on flight capability within the context of other factors important to a program targeted at preventing the establishment and spread of flight-capable females in novel habitats. This includes assessing the current ability to distinguish populations with flight-capable females and evaluating the effects of female flight on the population dynamics (establishment and spread) in novel habitats.

2. Gypsy Moth Population Dynamics (Establishment and Spread) and Potential Impacts of Female Flight

Gypsy moths represent one of the most studied and best-documented biological invasion systems in North America [33]. Novel introductions and establishments are regularly monitored through extensive surveys, and this information is used to understand population dynamics and other factors affecting establishment, such as dispersal ability, environmental tolerance, and host preferences [9]. Gypsy moth population dynamics are complex due to the fact that there are multiple natural enemies that regulate population density or reduce outbreak populations [34]. Gypsy moth populations immediately after outbreaks are mostly regulated by two host-specific entomopathogens, *Entomophaga maimaiga* and the nucleopolyhedrosis virus *LdMNPV* [35]. Outbreaks have been recorded in areas that have at least 20% of the moths' preferred host species [36]. However, there are multiple other key factors involved in influencing the population dynamics of the species. One of these factors is land use. A study by Lentini et al. [37] found that outbreaks are generally more frequent in overexploited forests and woodlands (<25% canopy cover rate) than natural ones. Moreover, gypsy moth population dynamics may be further subjected to changes depending on control measures [15]. A study by Mannu et al. [15] found a forward shift in abundance peak with early *Btk* applications during the population growth, leading to postponed outbreak peak by two to three years, whereas *Btk* applications during the culmination phases were found to be more effective in suppressing gypsy moth populations.

A recent finding showed that gypsy moth establishment and spread dynamics are very different along the species' leading edge [33,38]. Gray et al. [34] studied the relationship among gypsy moths, forest composition, stand architecture, and natural enemies. They found that habitat structure did not influence natural enemy populations and that the pre-eruptive phase was different from the pre-establishment phase due to high mating success. Moreover, they found high mating success and adult female survival in the pre-eruptive phase across all of the sites and habitat structures. This hints at the inability to find mates at very low densities (pre-establishment), resulting in the Allee effect, a positive relationship between individual fitness and population density [38] that may be a limiting factor in the establishment of newly founded gypsy moth populations [39]. In this scenario, an individual of a species under the influence of an Allee effect will experience a decrease in its fitness level when the population density is low. In the case of invasive species, low density at initial introduction provides an opportunity for Allee effects to modify the invasion dynamics and could cause longer lag times, slower spread, and reduced establishment probability [40].

Mate-finding failures, as a source of Allee effect, are also an important factor determining the spread and establishment of gypsy moths in flightless female populations of North America [41,42]. It has been found that both Allee effects and stratified diffusion into new areas play roles in gypsy moth dispersal, so tactics based on these findings have been used

along the leading edge to slow the spread. Isolated colonies of gypsy moths with flightless females are prone to Allee effects and extinction given their low abundance. In this case, mating success seems to be a prominent density-dependent factor affecting sexually active sporadic populations [43]. Sharov et al. [44] found a significantly higher likelihood of mating in higher-density populations. Gypsy moth fecundity decreases rapidly in adults greater than two days old, which means there is limited time for mating before death [45]. Consequently, female movement may or may not be helpful in low-density populations.

The role of the Allee effect may be diminished when flight-capable females are present. Flight-capable females fly to lights (the moon or manmade light sources) or light-colored surfaces to lay their eggs. Virgin females fly less frequently than mated females, as they call for a mate first [46] (Figure 2). However, virgins that are not mated quickly will eventually fly to another location, to call for a mate. Since both sexes are attracted to lights, virgin females may be increasing their chances of finding a mate by flying to nearby light sources, where males will also congregate [27]. Once mated, females fly and congregate near lights, to lay their eggs, effectively creating local concentrations of flight-capable individuals. These concentrations could be within an already infested area with mixed-flight individuals, or out ahead of the generally infested area, if moving into new habitats. These local concentrations could then coalesce and increase the rate of expansion just as the small populations ahead of the leading edge in flightless female populations increase the speed of invasion into new areas. With flighted females, however, the number of these small populations outside the generally infested area could be higher, and they could be further from the leading edge of the infestation. There has been evidence of this already occurring when it was observed that female flight capability was maintained at higher levels (even up to 70%) than random mating within European populations where flighted types and non-flighted types were sympatric [7]. When individuals from populations with flight-capable females are mated with individuals from populations where females were flightless, the first-generation females are only capable of a descending glide, and only about 15% of the female progeny of matings between the hybrids are capable of strong directed flight [32].

In addition to the Allee effect, climate suitability is also an important factor in determining the establishment probability of gypsy moths when suitable hosts are present [31]. In the past, various authors have used phenology (Gypsy Moth Life Stage model) and climate matching models (MaxEnt) to assess the establishment probability of gypsy moths [31,47]. These studies show that the potential southern margin of gypsy moth spread in the USA will be limited by insufficient chilling that terminates diapause, while exposure to extreme cold temperatures will result in egg mortality in the northern regions of North America [48]. Moreover, cold winter temperatures were found to be associated with reduced summer trap captures of EGMs [49]. Interestingly, May et al. [50] found no evidence of metabolic adaptation to colder environments based on their comparisons between northern and southern populations of North America gypsy moths. Moreover, Tobin et al. [51] found that the average development rates of North American gypsy moths were faster in areas experiencing higher temperatures despite larvae being exposed to repeated supraoptimal temperatures. Here, Tobin et al. [51] provide evidence of ongoing gypsy moth southern range margin retraction and suggest higher optimal temperatures during larval and pupal development period to be associated with gypsy moth range margin retraction. The authors further suggest that higher temperatures could exceed the positive effects on gypsy moth population growth. Moreover, recent works by Faske et al. [52] and Banahene et al. [53] provide evidence of local physiological adaptation under warmer climates and occurrence of strong temperature-dependent selective pressures in the southern populations of gypsy moth, respectively.

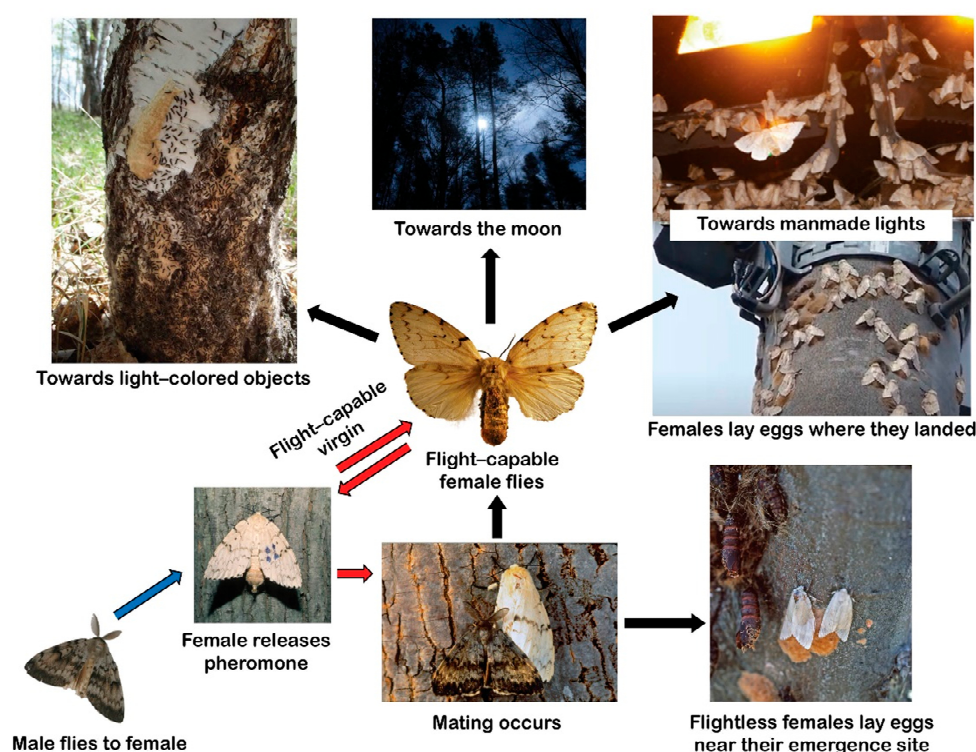


Figure 2. Schematic showing the sequence of mate finding, mating, female flight, and oviposition for populations with flight-capable or flightless females. Note that flight-capable virgin females will fly after calling for a few hours, when no mating occurs, but they will resume calling in the new location. Strong directed female flight to lights or light-colored objects occurs after mating, and females will congregate, in order to oviposit.

Just like their EGM counterparts, the larvae and pupae of gypsy moth from Asian origins with flight-capable females may struggle to survive and develop in regions that experience extended periods of temperatures $\geq 30^{\circ}\text{C}$ [30]. The developmental rates and responses to temperature of many AGM populations are very similar to those of EGM populations, with the exception of the most slowly developing ones from the *L. dispar japonica* subspecies [30]. In addition, the variation between AGM populations in both host utilization and egg chill requirements is already present between EGM populations [4,29], so establishment potential will depend in part on the traits of the source AGM population and the climate and hosts present in the area where it is introduced. AGM populations should be able to establish in most cases where EGM can establish, based on modeling that has been done [54].

Environmental cues may also impact flight behavior in gypsy moths. Temperature and light intensity may play important roles in determining flight propensity [55]. Iwaizumi and Arakawa [56] collected egg masses from four different localities in Japan: Chiba, Kobe, Hachinohe, and Tomakomai. From the reared larvae, female flight duration under yellow and black light sources was examined. The authors observed yellow fluorescent light that cut out ultraviolet radiation had a suppressive effect on female flight. The relatively high light intensity of 10 lux most likely suppressed the nocturnal flight behavior of the tested females. Iwaizumi and Arakawa [56] found that females from three of the populations flew an average of 200 m, while females from the Tomakomai population had a mean flight distance of roughly 600 m.

Chen et al. [57] measured the flight propensity of female AGM from seven different populations under three combinations of light intensities. Three of the populations originated in China, two from Russia, and one each from Japan and South Korea. Seventy-two percent of all females tested had the ability of directed flight. The authors also found that the range of light intensities used within the study (0.05, 0.10, and 0.40 lux) did not have a

significant effect on flight propensity. This agrees with research by Charlton et al. [55] that found female gypsy moth flight to begin when light intensity falls below 2 lux.

3. Variation in Female Flight Capability and Flight Distances

3.1. Female Flight Capability

Keena et al. [46] investigated the propensity and capability of flight in female gypsy moths from populations established in Russia and North America, as well as the flight capability of hybrids bred from these two populations. While the majority of female moths from the Russian populations were able to fly, females gathered from the population established in the United States were not flight capable. To further analyze how hybridization could affect flight capability, the authors bred four new populations and assessed flight abilities. Parents used during breeding, as well as the percentage of offspring capable of sustained flight (FL) and those capable of gliding flight (GF), are shown below in Table 1.

Table 1. Origin and flight-capacity percentages of North American, Russian, and hybrid gypsy moths [18].

Origin		Sustained Flight	Gliding Flight
Female	Male		
Russia	Russia	88%	11%
Russia	North America	0%	51%
North America	Russia	2%	65%
North America	North America	0%	0%

Gypsy moths with both parents from North America were not capable of sustained or gliding flight. However, over 50% of the crosses of North American and Russian populations were able to glide. This suggests that if individuals from flight-capable populations become established in North America and mate with the currently established gypsy moth populations, it is likely that the first-generation offspring would have substantially improved gliding abilities. The possibility of sustained flight amongst hybrids is less established. While only 2% of the Russian–North American crosses were able to fly within this study, a separate investigation found that 8% of captive hybrid females were able to attain sustained flight if they were fed foliage, while no hybrids were flight-capable when reared on an artificial diet [58]. This suggests that female flight capacity of gypsy moths of all strains may be underestimated in studies if an artificial diet is used.

Female gypsy moth flight capacity has been further explored in a study that cross-bred gypsy moths that have established in North Carolina, USA, that are incapable of flight with individuals from Mineralni, Russia, that have a high capacity for ascending flight [32]. The authors compared the parents, reciprocal F1 hybrids, double reciprocal F2 hybrids, and all potential backcrosses. Heritability of female flight was found to be at least 0.60, while relative wing size had a heritability of 0.70. However, overall, flight capacity is determined by a combination of behavior, flight musculature, and wing size, which is controlled by multiple genes. The authors go on to suggest the following:

*“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”.*

Conversely, if a population of gypsy moths with flight-capable females were to become established in an area of North America where gypsy moth populations were not present, flight-capable females could remain in the population for many generations [32]. Introduction of AGM strains into North America in locations with established EGMs would most likely create hybrid populations. The probability of hybridization will be highest when both

introduced and native populations have similar developmental responses to temperature and numbers of instars, since these traits can have an impact on the seasonal dynamics of the gypsy moth. These hybrid populations would have reduced flight capability, as compared to their Asian parentals [46], and may also incorporate other undesirable traits from the invading population, depending on the genetics of the trait. For example, the phenotype that requires less chill before eggs are able to hatch is a dominant trait that, once introduced, will increase in the population [29]. Subsequent introductions of other AGM strains could further increase the EGM population's flight capacities and introduce other traits that could alter the population's biology and behavior further.

3.2. Female Flight Distance

The nocturnal flight activities of female gypsy moths from Yokohama City and Chiba City, Japan, were assessed by Iwaizumi et al. [56]. Female moth behavior was monitored in a cage under different photoperiodic conditions. Mean flight distances of 226 and 269 m were observed for virgin females and mated females, respectively. However, the maximum recorded value of 746 m for virgin females is closer in agreement with Liebhold et al. [59], who estimated that female gypsy moths in Kanazawa, Japan, had a maximum flight distance per night of around 1 km.

Yang et al. [60] evaluated the effect of mating status and age on the flight activity of female gypsy moths from seven populations within China. Using a computer-linked flight mill, the authors found flight activity peaked when females were one day old and decreased thereafter. However, no observable difference was seen between mated and unmated females. Total flight distance varied among the populations. One-day-old females from Guizhou, China, had a mean flight distance of 7.5 km, with one individual even reaching 10.67 km, while one-day-old females from Inner Mongolia flew an average of 3.95 km. However, the authors caution that these distances may not represent entirely realistic conditions, as the tethering used within the flight mill experiment most likely increases flight distances. It is also important to consider the life cycle of gypsy moths in estimating their spread. The time females spend in the moth stage is, in part, dependent on how soon they mate, as individuals die after depositing their eggs. Gypsy moth adults live no more than three days, and generally only one day [61].

To accurately predict the likely rate of spread of an AGM outbreak in North America, information on potential flight distances is needed. Table 2 summarizes all the literature on flight distances for studies conducted with gypsy moth populations from China, Japan, and Eastern Russia. By using data collected at ports in the Republic of Korea, Japan, and Russia [62,63], minimal potential flight distances can be estimated. Average distances between general egg collection areas and forest edge were 1.7 km in the Republic of Korea, 3.1 km at Japanese ports, and 0.5 km in Russia. Flight distances for all ports are shown in Table 3. These numbers could provide a rough estimate for flight distances for these populations, but because the actual distances between the forests and the ports differ between countries, care should be taken in using this data.

Table 2. Flight distances of gypsy moth populations in China, Japan, and Eastern Russia.

Authors	Year	Population	Distance	Notes
Rozkhov and Vasilyeva	1982	Asia (unspecified)	100 km	
Baranchikov	1986	Russia Far East	3–5 km	
Savotikov et al.	1995	Asia (unspecified)	20–40 km	
Liebhold et al.	2008	Japan (Kanazawa)	≤1 km	max distance, one night
Iwaizumi et al.	2010	Japan (Yokohama City, Chiba City)	746 m	max distance, one night, virgin female
			511 m	max distance, one night, mated female
			226 m	mean distance, one night, virgin female
			269 m	mean distance, one night, mated female

Table 2. Cont.

Authors	Year	Population	Distance	Notes
Iwaizumi and Arakawa	2010	Japan (Tomakomai)	659 ± 335 m	mean distance, one night
		Japan (Hachinohe)	188 ± 202 m	mean distance, one night
		Japan (Kobe)	356 ± 351 m	mean distance, one night
		Japan (Chiba)	255 ± 116 m	mean distance, one night
Yang et al.	2017	China (Jining, Inner Mongolia)	3.95 ± 0.29 km	mean distance, one night; one-day-old females; measured with flight mill
		China (Sandeli, Liaoning)	6.63 ± 1.40 km	mean distance, one night; one-day-old females; measured with flight mill
		China (Yanzikou, Beijing)	5.56 ± 1.16 km	mean distance, one night; one-day-old females; measured with flight mill
		China (Longhua, Hebei)	4.03 ± 0.99 km	mean distance, one night; one-day-old females; measured with flight mill
		China (Lianyungang, Jiangsu)	5.79 ± 1.44 km	mean distance, one night; one-day-old females; measured with flight mill
		China (Liuan, Anhui)	6.54 ± 1.12 km	mean distance, one night; one-day-old females; measured with flight mill
		China (Xifeng, Guizhou)	7.50 ± 2.28 km	mean distance, one night; one-day-old females; measured with flight mill

Table 3. Potential flight distance of gypsy moth populations from the Republic of Korea, Japan, and Russia.

Country	City Port	Distance from Port to Forest Edge (km)	Notes
Republic of Korea	Donghae	2	
Republic of Korea	Okgye	1	
Republic of Korea	Incheon	2	
Republic of Korea	Pyongtaek	2–5	Depends on size of forest needed
Republic of Korea	Busan	2	
Republic of Korea	Pohang	2	
Republic of Korea	Ulsan	3	
Republic of Korea	Gunsan	1–3	Depends on size of forest needed
Republic of Korea	Mokpo	1–2.5	Depends on size of forest needed
Republic of Korea	Gwangyang	1.5	
Republic of Korea	Yeongilman	1.5	
Republic of Korea	Onsan	2–4.5	Depends on size of forest needed
Republic of Korea	Daesan	1	
Japan	Kokura	3–5	Depends on size of forest needed
Japan	Ube	4	
Japan	Oita	3–8	Depends on size of forest needed
Japan	Hiroshima	3–4	Depends on size of forest needed
Japan	Matsunaga	2	
Japan	Tsuruga	1.5	
Japan	Kanazawa	1–7	Depends on size of forest needed
Japan	Chiba	5–8	Depends on size of forest needed
Japan	Fushiki	7	
Japan	Toyama-shinko	6–14	Depends on size of forest needed
Japan	Sakata	0	Port directly next to forest
Japan	Hachinohe	5	
Japan	Nagahama	4.5	
Japan	Aomori	4	
Japan	Hakodate	0.5–4	Depends on port location used
Japan	Tomakomai	3	
Japan	Otaru	1	

Table 3. Cont.

Country	City Port	Distance from Port to Forest Edge (km)	Notes
Russia	Vladivostok	3.5–9	9 k to closest trap; 3.5 k to closest forest edge
Russia	Nakhodka	0–3	3 k to closest trap; 0 k to closest forest edge
Russia	Vostochny	0.5	
Russia	Olga	0	Port directly next to forest
Russia	Slavyanka	1	
Russia	Zarubino	0	Port directly next to forest
Russia	Posyet	0	Port directly next to forest
Russia	Plastun	0	Port directly next to forest
Russia	Vanino	0.2	
Russia	Kozmino	0	Port directly next to forest
Russia	Korsakov	0.5	

All of these factors combined suggest that it may be appropriate to use a range of techniques when estimating flight capacity and flight distances of AGMs. Further research is required to classify all populations, using genetic markers and flight distance to improve predicted rate of spread accuracy associated with introduced gypsy moths in novel environments.

4. Identification of Gypsy Moth Subspecies and Specific Traits within Populations

4.1. Genetic and Genomic Analyses of Subspecies and Populations

Understanding the underlying mechanisms of flight capacity is an important goal. Various approaches have been used, from physiological inquiries to genomics. The ability to identify subspecies is of vital importance in stopping the spread of flight-capable AGMs. However, easily observed morphological differences between subspecies are minimal [64]. Genetic methods of identification have been developed which help to differentiate between subspecies and populations [65–67] and therefore aid in determining flight capability and potential flight distance.

Gypsy moth genetic variation and distribution of female flight has been described by Keena et al. [7]. Gypsy moths were obtained from 46 locations around the world, representing the current established range of the species. Female flight capability, pre-flight behaviors, female muscle strength, and female wing size were evaluated for all 46 strains. For 31 of these strains, the authors determined the mitochondrial DNA (mtDNA) haplotype, the nuclear DNA genotype at one locus, and the microsatellite genotype at four loci. Female gypsy moths that were capable of directed flight came from Northeastern Europe, Siberia, and Asia. Wing musculature and size were found to be useful in predicting female flight capacity within populations. An analysis of all DNA haplotypes and genotypes was able to place 94% of individuals into their general geographic origin: Asian, Siberian, European, or North American. However, a clear genetic distinction was not found between European strains that differed in flight-capable females.

By analyzing microsatellite loci and mitochondrial DNA sequences, Wu et al. [68] detected four genetic clusters of *Lymantria dispar*, which correspond to the general place of origin for the named gypsy moth subspecies—Western Europe, the Asian continent, and the islands of Japan. The fourth cluster was found for gypsy moth populations established in North America and was most likely caused by a population genetic bottleneck after initial establishment on the continent. Wu et al. [68] also found evidence of continuing hybridization between subspecies, with high levels of genetic variation in East Asian gypsy moth populations. Analysis of genome-wide single nucleotide polymorphisms (SNPs) is a powerful approach that also enables clear distinction of subspecies and geographic variants, while revealing introgression near the geographic boundaries between subspecies [69]. These polymorphisms could be translated into genomic bio-surveillance tools [70].

Receptors in the olfactory systems of insects have been shown to be important in flight orientation [71]. Thus, it could be possible to differentiate between populations

containing flight-capable and flightless gypsy moth females by examining olfactory genes. McCormick et al. [72] studied the differences between olfaction-related genes in gypsy moth females from one flightless and two flight-capable populations. The flightless population, considered to comprise EGMs, came from New Jersey, USA, while the flight-capable populations had origins in Northern Japan and Far Eastern Russia and were considered to comprise JGMs and AGMs, respectively. By using next-generation sequencing, they identified 115 chemosensory genes and conducted a principal component analysis to determine gene-expression patterns. The olfactory-related genes of JGMs appeared more divergent, whereas the AGM and EGM populations shared many commonly expressed genes. This suggests that these differences are not related to flight capability [72].

Lymantria genomes have been sequenced [73,74], and analyses revealed that the genomes of *Lymantria* are among the largest in the lepidoptera [73]. Genetic comparisons of AGM and EGM genomes found differences in metabolic pathways which suggest strong signatures of energy-related pathways in both subspecies, dominated by metabolic functions related to thermogenesis [73]. Divergent proteins associated with muscle contraction could possibly help to explain differences in wing musculature and, therefore, flight [74]. Further functional studies are needed to confirm these predictions.

4.2. Physical Differences among Subspecies and Populations

Physical characteristics of insects play a large role in determining flight capacity. In order for insects to fly, total flight muscle mass must make up a minimum of 12% to 16% of total body mass [75]. Several studies have sought to examine the physical characteristics of female gypsy moths between flight-capable and flightless populations.

Keena et al. [32] evaluated the muscle strength and wing size of female gypsy moths from North American and Russian populations, as well as various crosses between the two strains. Muscle strength was tested by inverting females onto their backs and evaluating the ease with which they were able to right themselves by beating their wings. Over 90% of the females from the Russian population were able to right themselves within one or two quick wingbeats, while the majority of North American females were unable to do so. More than 50% of the hybrid females were able to right themselves, although many individuals were significantly challenged with the movement. Female wing measurements varied considerably among the populations and crosses. The wings of Russian females were greater in size than those of hybrids, which in turn were greater than those of the individuals from North America.

To further explore the relationship between wing characteristics and female flight capability within gypsy moth populations, Shi et al. [76] conducted a morphometric analysis of 821 gypsy moths from eight different strains. Individuals of both sexes were sourced from one site in the United States, two sites in Europe, two sites in Russia, two sites in China, and one site in Japan. Body mass, length, width of fore- and hindwings, wing area, wing aspects, and wing loads were measured for all individuals. Average wing loading was significantly lower, while average wing area was larger, for females from flight-capable populations. The authors of the study created a model, using female wing load and forewing length, that correctly predicted female flight capability of the populations 97% of the time. This information could be especially useful if used in conjunction with reliable molecular identification methods in determining the flight capability of invasive gypsy moth populations.

4.3. Identification of Flight-Capable Populations

No single tried and true technique exists to identify flight-capable AGM populations and individuals. However, the two methods described above show the most promise. The model developed by Shi et al. [76] could be used to predict flight capability of a population (native or introduced) by measuring female forewing length and wing loading. A disadvantage of using this method is that it is necessary to capture an adult female gypsy moth. While male gypsy moths can be captured with the use of traps baited with

a synthetic sex pheromone called “disparlure” [77], female gypsy moths remain elusive. Black-light traps can capture both male and female AGMs but are generally not considered specific enough for detection purposes [25]. Another method of capturing female gypsy moths involves the collection of egg masses, followed by the rearing of juveniles into adulthood [46]. However, the additional time this would take could hamper detection and eradication efforts.

An alternative method of identifying flight-capable gypsy moth populations was employed by Picq et al. [69]. They used single nucleotide polymorphism (SNP) markers to identify the geographic origins of gypsy moth samples. With this method, genetic material could be taken from both female and male moths, to determine geographic origin. This information could then be compared to known flight-capability information for gypsy moth populations. The disadvantage of this is that it does not directly predict flight capacity. A combination of this approach with genetic markers associated with flight by genome-wide association studies, or by functional analyses, could provide solutions in the future [78].

5. Conclusions: Current Challenges and Future Directions

Gypsy moths from many different populations have been and continue to be introduced into novel environments where they have the potential to establish and spread. There is a suite of traits that are of concern because of how they impact the establishment and dispersal potential of gypsy moth populations, including female flight capability, host utilization, and egg chill requirements. It has become clear that these traits differ among populations, even within each subspecies of gypsy moth, so introduction of gypsy moths from other world areas into locations where the species is already present could result in unwanted changes in gypsy moth biology. It also appears that introduction of flight-capable females, even from Europe, could enhance dispersal capability and require modifications to management protocols. Therefore, information on not only the origin of the population but also its biological traits is needed to determine risks associated with new introductions of gypsy moths into a novel habitat. Having a tool to rapidly identify the female flight capability of newly introduced gypsy moths is a critical component of any system instituted to evaluate the risks associated with the introduction but we currently do not have such a tool. Other rapid tools to assess larval host utilization (particularly conifers) and egg chill requirements would also be useful but are not available. Although much is known about gypsy moths from several world areas, we still do not have enough knowledge to adequately plan for or deal with new introductions into novel habitats.

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