

## ORIGINAL ARTICLE

## Size-dependent flight capacity and propensity in a range-expanding invasive insect

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**Abstract** For capital-breeding insects, all resources available for adult metabolic needs are accumulated during larval feeding. Therefore, body size at adult eclosion represents the total energetic capacity of the individual. For female capital breeders, body size is strongly correlated with lifetime fecundity, while in males, body size, which correlates with fitness, is less understood. In capital-breeding species with wingless, flightless, or dispersal-limited females, flight potential for male Lepidoptera has important implications for mate-finding and may be correlated with body size. At low population densities, failure to mate has been identified as an important Allee effect and can drive the success or failure of invasive species at range edges and in species of conservation concern. The capital-breeding European subspecies of *Lymantria dispar* (L.), was introduced to North America in 1869 and now ranges across much of eastern North America. In *L. dispar*, females are flightless and mate-finding is entirely performed by males. We quantified male *L. dispar* flight capacity and propensity relative to morphological and physiological characteristics using fixed-arm flight mills. A range of male body sizes was produced by varying the protein content of standard artificial diets while holding other dietary components constant. Wing length, a proxy for body size, relative thorax mass, and forewing aspect were all important predictors of total flight distance and maximum speed. These results have important implications for mate-finding and invasion dynamics in *L. dispar* and may apply broadly to other capital-breeding insects.

**Key words** flight mill; *Lymantria dispar*; life-history; invasion dynamics; selective pressure

## Introduction

Variation in resources and their allocation affect the quantity and fitness of offspring that individuals produce in a given environment (Boggs & Freeman, 2005; Boggs, 2009). For holometabolous insects, including Lepidoptera, energy and nutrients acquired in the larval stage are later redistributed to fuel the formation of

adult body structures during the pupal stage and meet adult metabolic and reproductive needs (Boggs, 2009). While income breeding species feed as adults and use accumulated resources to supplement reproduction, capital breeders depend entirely on resources accrued as larvae for all adult functions (Tammaru & Haukioja, 1996; Davis *et al.*, 2016). Thus, in capital breeders, body size is indicative of the total energy and material reserve available for adult dispersal, mate-finding, and reproduction.

Estimating fitness for female capital-breeding insects is done relatively easily by counting the number of eggs a female produces (Hough & Pimentel, 1978; Tammaru *et al.*, 1996a; Tisdale & Sappington, 2001; Parry *et al.*,

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2001). On the other hand, determining correlates of male fitness by quantifying the number of eggs fertilized following a controlled mating event or by measuring male flight capacity is more difficult and is done less frequently (e.g., Boggs & Gilbert, 1979; Svård & Wiklund, 1989; Tammaru *et al.*, 1996b; Tobin *et al.*, 2014). Male flight may be particularly important for capital-breeding species where males do most or all of the mate-finding (Berec *et al.*, 2018; Javoiš *et al.*, 2019). Mate location is critical for overcoming mate-finding Allee effects in small isolated populations or those at low density near range edges (Contarini *et al.*, 2009; Yamanaka & Liebhold, 2009; Rhainds, 2010). Therefore, factors that influence male flight capacity and mate-finding ability in capital-breeding species (e.g., resource acquisition and body size) may impact persistence of small populations and establishment in range-expanding populations.

The European strain of *Lymantria dispar* was introduced to North America in 1869 and now ranges from northern Minnesota to the Outer Banks of North Carolina (Grayson & Johnson, 2018). Adults are sexually dimorphic and, in North America, have flight-capable males and winged but flightless females (Doane & McManus, 1981). Thus, energy expended by adult females is allocated primarily to the production and emission of pheromone and the manufacturing and provisioning of eggs, whereas males allocate their resources to flight. While the strong linear relationship between female pupal mass and fecundity is well established (e.g., Hough & Pimentel, 1978; Faske *et al.*, 2019), no studies to our knowledge have assessed the relationship between body size and flight capacity for male *L. dispar*. Given that insect body size is often correlated with metrics of flight capacity (e.g., Davis *et al.*, 2012; Evenden *et al.*, 2014; Jones *et al.*, 2016), and flight-related morphology such as wing load and forewing aspect ratio affect flight capability in other Lepidoptera (Le Roy *et al.*, 2019), male *L. dispar* flight capacity may also be affected by body size and flight-related morphology.

Fixed-arm flight mills provide a valuable avenue for investigating effects of physiological, morphological, and environmental variation on flight capacity and dispersal under controlled conditions (Minter *et al.*, 2018; Naranjo, 2019). Although tethered flight mills have been used to determine the effect of morphological and physiological characteristics on females of the Asian subspecies of *L. dispar*, a strain with strong female flight ability (Shi *et al.*, 2015; Yang *et al.*, 2017), to our knowledge, none have done so for any strain of male *L. dispar*. Our study assessed the relationship between resource acquisition and allocation and male flight capacity for the European *L. dispar*, an economically and ecologically im-

portant invasive capital breeder. Specifically, we used flight mills to assess the direction and influence of several morphological and physiological characteristics on male *L. dispar* flight performance. We predicted that: (i) due to greater stored energetic resource availability with increasing male body size, increasing body size would correspond to greater flight distance, speed, and flight propensity; and (ii) variation in wing and thoracic morphology would affect male flight distance and speed.

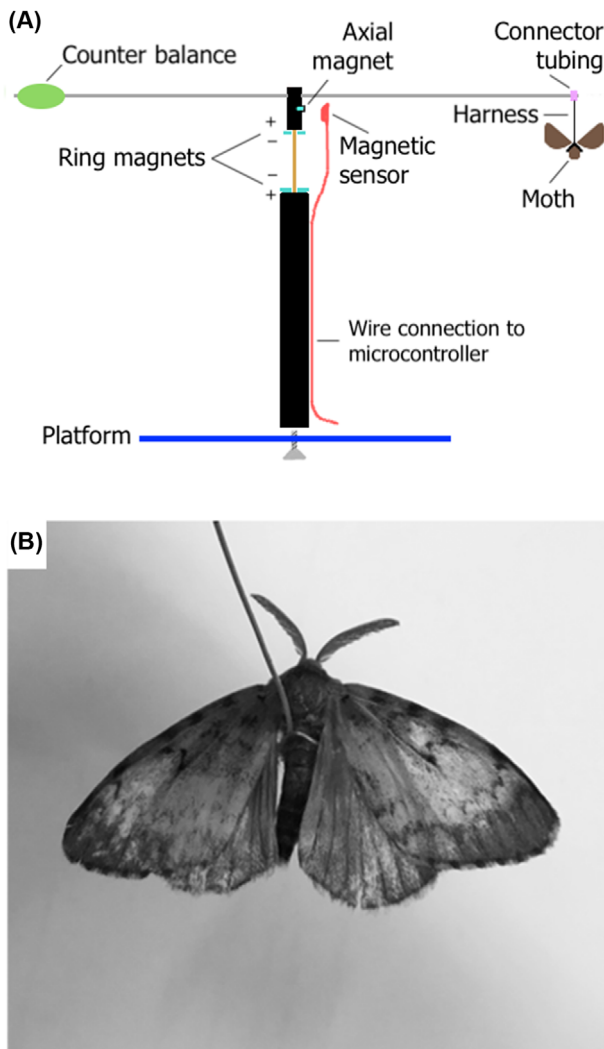
## Materials and methods

### *Insect rearing*

In natural environments, the mass of male *L. dispar* pupae and adults varies greatly as a function of diet quality, population density, and temperature experienced as a larva (Carter *et al.*, 1991; Lindroth *et al.*, 1997; Lazarevic *et al.*, 2004; Jahant-Miller *et al.*, 2020). To investigate the effects of male size on flight capacity required methodology to produce a range of sizes while minimizing the effects of other confounding factors on flight metrics. To produce a range of male sizes for experimentation, we used an agar-based artificial *L. dispar* diet (Leonard & Doane, 1966) and manipulated protein content while holding all other nutrients constant. Males were reared on four diet formulations (high protein, medium-high protein, medium-low protein, low protein; Table S1). To compensate for reduced protein content, the equivalent amount by weight of food grade cellulose powder was added to keep the weight ratio of other ingredients equal across all diet mixes. Fresh diet was poured in 0.47 L plastic-lined paper soup containers and provided to larvae *ad libitum*. Larvae were reared in a growth chamber at 25°C and a photoperiod of 15 : 9 (L : D). Upon pupation, individuals were weighed to the nearest 0.0001 g and returned to the growth chamber in individual 59.15 mL plastic containers until adult emergence.

### *Flight mill design*

Flight mills were assembled using the design described by Jones *et al.* (2010; Appendix 1). During each assay, a moth was attached distally to a rotating arm (18.34 cm diameter) and a magnetic sensor detected a small magnet below the flight mill arm at each full rotation as the moth powered the mill (Fig. 1A). The magnetic sensor was connected to a microcontroller (Fig. S1) that read up to eight sensors and converted rotation and time information to a USB signal for logging by a terminal program (TeraTerm) on a personal computer. Opposite-facing



**Fig. 1** (A) Schematic of fixed-arm flight mill with tethered moth. Rotating arm is held above mill using opposite-facing magnets to reduce rotational friction. A magnetic sensor detected magnetic impulse at each full rotation and a computer program linked to flight mill recorded each rotation with a timestamp. (B) Male *L. dispar* with inverted y shaped harness glued to descaled first abdominal segment. Image was taken following 24 h flight assay.

magnets reduced rotational friction by levitating the flight mill arm above the axle.

#### Flight assay

Pupae were checked at least twice daily for adult emergence. Moths that emerged and were not flown right away were held individually in small plastic containers at 4 °C

until ready to be flown. All flight assays were initiated within 24 hours of emergence. Prior to harness placement, moths were cold anesthetized at approximately –18 °C for up to 2 min, then weighed to the nearest 0.000 1 g. Inverted y-shaped wire harnesses (Fig. 1B) were glued dorsally to the descaled first abdominal segment using cyanoacrylate gel super glue. Moths were reweighed with the harness and attached to the distal end of the flight mill arm using thin plastic tubing. A piece of soft polymer clay equal to the weight of the moth and harness was attached to the opposite end as a counterbalance.

Flight assays began at the start of the photophase (light period) and ran for 24 h with photoperiod of 15 : 9 (L : D). Photophase and scotophase temperatures were 25 °C and 15 °C, respectively, which are similar to ambient temperatures *L. dispar* encounter in their invasive range. During flight assays, total distance and duration of each individual flight was recorded, along with morphological (pupal mass, moth mass, wing length, width, and area, thorax width, height and shape, relative thorax mass, relative abdominal mass, and forewing aspect) and physiological (mass lost during flight, relative mass lost during flight) moth characteristics. Moths that had stopped flying were encouraged to fly every 15 min using gentle tactile stimulation (i.e., paintbrush touches to the legs, posterior edge of the wings, or antennae) for the first 8 h of each flight assay. Moths that became detached from the flight mill during the flight assay or that did not fly at all due to injury during preflight procedures were excluded from analyses. Following the 24-h flight assay, moths were removed from flight mills, frozen, and reweighed to quantify mass lost during flight. The right forewing of each male was removed and scanned, and the digital image of each wing was analyzed using ImageJ software to determine wing length, wing width, and wing area. Thorax mass and abdominal mass were measured by carefully separating each tagma using a scalpel and weighing them individually on a digital balance to the nearest 0.000 1 g. Wing load was calculated as initial moth mass divided by two times the wing area ( $\text{g}/\text{cm}^2$ ). Forewing aspect ratio was calculated as four times the squared length of the forewing divided by forewing area ( $\text{mm}/\text{cm}^2$ ). Relative thorax mass was calculated as the mass of the thorax divided by the preflight moth mass, and relative abdominal mass was calculated as the mass of the abdomen divided by the preflight moth mass. Thorax width and height were measured to the nearest 0.01 mm using a digital caliper. Thorax shape was calculated as the width of the thorax divided by the height. Mass lost during flight was measured as the initial mass of the moth minus the mass of the moth measured immediately after the flight assay, and ratio of mass lost was

**Table 1** Summary statistics for dependent (total distance, maximum speed attained) variables and wing length.

Flight capacity metric	Mean $\pm$ SD	Median	Minimum	Maximum
Total distance (km)	19.96 $\pm$ 16.74	15.98	1.24	68.22
Maximum speed (m/s)	1.21 $\pm$ 0.37	1.21	0.32	1.93
Wing length (mm)	2.06 $\pm$ 0.21	2.03	1.64	2.53

calculated as mass lost during flight divided by initial moth mass.

Flight propensity was measured as the number of flights consisting of more than 100 full rotations initiated upon tactile stimulation divided by the total number of touches for each individual moth. One hundred rotations was equal to approximately 58 m of linear flight.

#### Variable selection

Total distance flown and maximum speed were used as dependent variables. Based on biological significance, wing length, which correlates strongly with pupal mass (Tobin *et al.*, 2016; Jahant-Miller *et al.*, 2020), was chosen to represent body size, and proportionate mass lost during flight (RatioMD), forewing aspect ratio (FWa), and relative thorax mass (RTM) were also included in the model and each represented a different physiological or morphological component. Variables selected for the full models were not strongly correlated ( $P < 0.4$ ). Flight mill identity was included in both models as a random effect to capture any variation in measured flight capacity metrics caused by any physical differences in flight mill design. Since insects do not need to produce lift during tethered flight, wing load was not included in flight capacity analyses.

#### Statistical analysis

All data analyses were performed in R (version 3.6.1, R Core Development Team, 2019). Significance level for all analyses was  $\alpha = 0.05$ . Effects of morphological and physiological characteristics on metrics of male flight capacity were analyzed using mixed effects linear regression analysis. Fixed effects and pairwise interaction effects were tested using stepwise model selection (package *lmerTest*; Kuznetsova *et al.*, 2017). For each dependent variable, full and reduced models were compared using Akaike information criterion and analysis of variance (ANOVA) hypothesis testing. Model normality and homoscedasticity were checked using residual plots. Total distance flown was square root transformed

to improve normality. Effect of wing length on flight propensity was compared using one-way ANOVA.

## Results

A total of 96 male *L. dispar* were flown. Mean pupal mass of flown moths was  $0.41 \pm 0.013$  g (mean  $\pm$  SE) and ranged 0.19–0.73 g, values that encompass much of the variation seen in males from natural populations (Jahant-Miller *et al.*, 2020). Mean wing length was  $2.06 \pm 0.021$  cm and ranged 1.64–2.53 cm (Table 1). High protein diet produced the greatest mean pupal mass ( $0.4362 \pm 0.014$  g), followed by medium-high ( $0.4002 \pm 0.048$  g), medium-low ( $0.3409 \pm 0.027$  g), and low ( $0.1823 \pm 0.014$  g). High, medium-high, and medium-low protein diets supported relatively high survival rates, while low protein diet did not. As a result, no moths reared on low protein diet were used in flight assays. In total, high protein, medium-high, and medium-low diets produced 70, 11, and 15 moths flown in flight assays, respectively.

#### Flight capacity

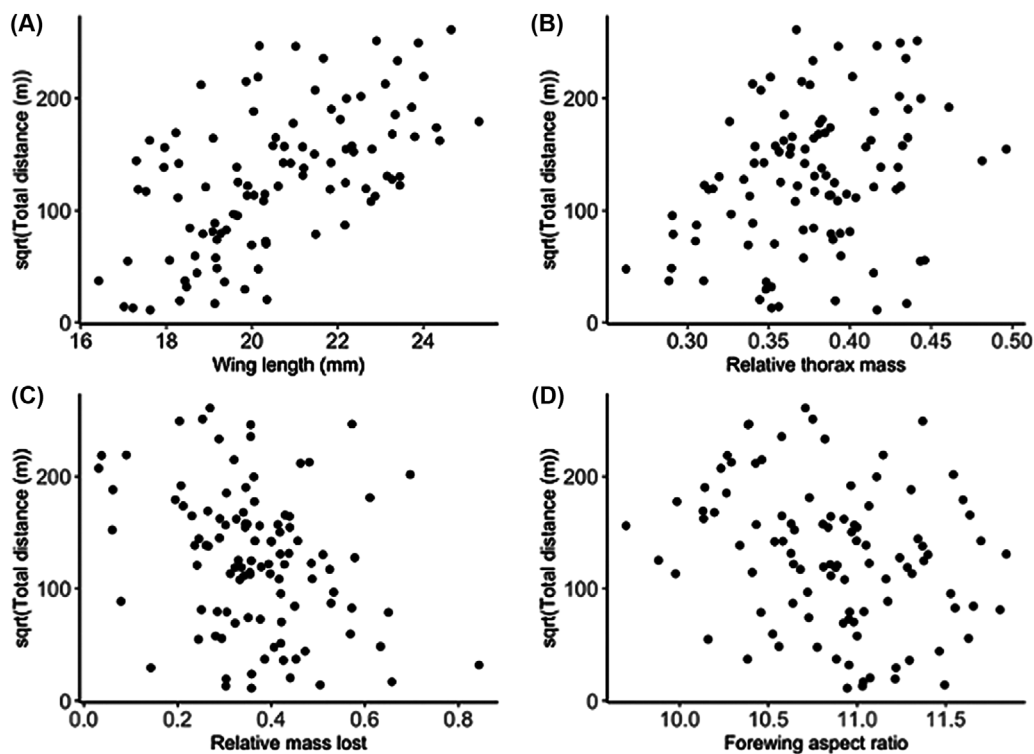
Wing length was the strongest predictor for both flight capacity metrics (total distance and maximum speed) in the mixed regression models (Tables 2, 3). Total distance flown ( $F_{1,99} = 98.47$ ,  $P < 0.0001$ ; Fig. 2A) and maximum speed ( $F_{1,93} = 80.17$ ,  $P < 0.0001$ ; Fig. 3A) were significantly impacted by wing length. Large moths in the upper quartile for wing length ( $n = 27$ ,  $X_{WL} = 2.33$  cm [2.22–2.53 cm]) flew on average 31.6 km (11.6–68.2 km), or 282% more than small moths in the lower quartile ( $n = 24$ ,  $X_{WL} = 1.80$  cm [1.64–1.89 cm]) which flew on average 11.2 km (0.12–44.9 km). Forewing aspect ratio had a significant negative effect on total distance ( $F_{1,91} = 30.32$ ,  $P < 0.0001$ ; Fig. 2D) and maximum speed ( $F_{1,93} = 17.70$ ,  $P < 0.0001$ ; Fig. 3B). Reduced forewing aspect was associated with greater flight speed and greater total distance flown. Relative thorax mass had a significant positive effect on total flight distance ( $F_{1,91} = 28.17$ ,  $P < 0.001$ ; Fig. 2B).

**Table 2** Intercept and slope coefficients for total distance (m) mixed linear regression. Total distance was square root transformed to improve residual normality.

Explanatory variable	Estimate	SE	Lower CI (5%)	Upper CI (95%)	<i>t</i> (df = 91)	<i>P</i>	Est. model <i>R</i> <sup>2</sup>
Intercept	75.77	110.41	-137.95	46.41	0.69	0.49	0.59
Wing length	204.78	20.63	161.58	239.59	9.92	<0.001	
Relative mass lost	-14.16	24.58	-120.40	-6.67	-0.58	0.57	
Forewing aspect ratio	-50.94	9.25	-67.40	-32.53	-5.51	<0.001	
Relative thorax mass	507.00	95.52	289.33	651.88	5.31	<0.001	

**Table 3** Intercept and slope coefficients for maximum speed (m/s) mixed linear regression.

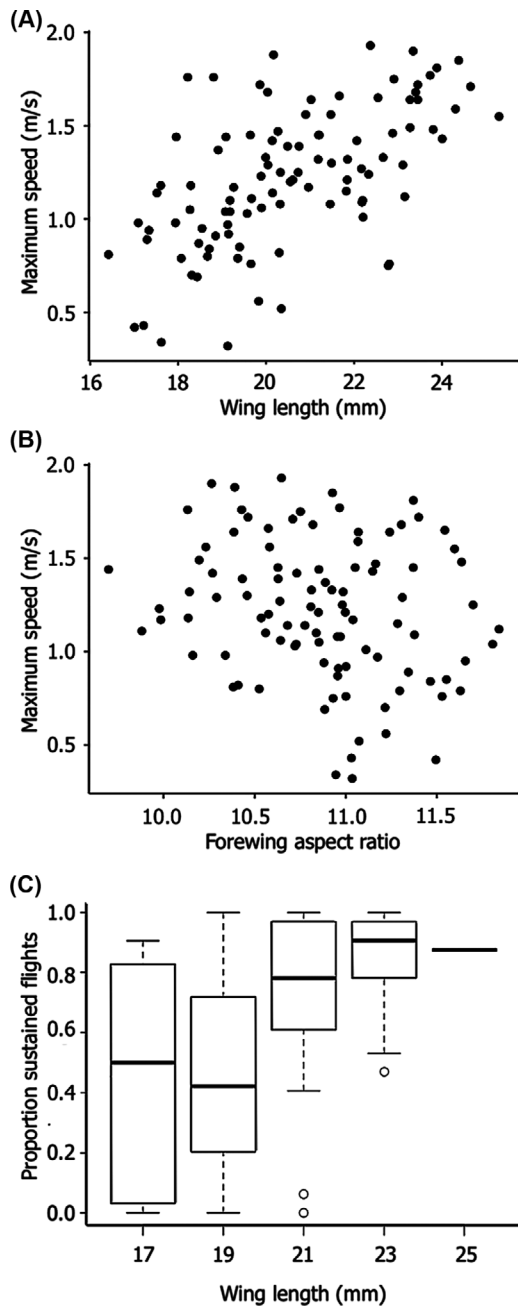
Explanatory variable	Estimate	SE	Lower CI (95%)	Upper CI (95%)	<i>t</i> (df = 91)	<i>P</i>	Est. model <i>R</i> <sup>2</sup>
Intercept	1.53	0.68	0.20	2.86	2.25	0.03	0.48
Wing length	1.21	0.13	0.94	1.47	8.95	< 0.001	
Forewing aspect ratio	-0.26	0.06	-0.37	-0.14	-4.21	< 0.001	

**Fig. 2** Scatter plots for the effect of wing length, forewing aspect ratio, relative thorax mass, and relative mass lost during flight on distance flown.

### Flight propensity

All but five moths recorded at least one response flight consisting of more than 100 full rotations; wing lengths

of those that did not were 17.0, 17.22, 17.62, 19.13, and 20.34 mm. Wing length had a significant effect on proportion of sustained flights initiated ( $F_{4,91} = 9.43$ ,  $P < 0.001$ ; Fig. 3C); males with greater wing length were



**Fig. 3** Scatter plots for significant factors impacting maximum speed attained by male *L. dispar*, including (A) wing length and (B) forewing aspect ratio. Raw data are plotted to visualize effects of wing length and forewing aspect ratio on maximum speed. (C) Boxplot depicting relationship between mean proportion of sustained flights initiated and wing length.

more likely to initiate sustained flight than males with smaller wings.

## Discussion

Insect body size is often correlated with greater flight distance and speed (e.g., Dingle *et al.*, 1980; Shirai, 1991; Bruzzone *et al.*, 2009; Evenden *et al.*, 2014; Fahrner *et al.*, 2014; Jones *et al.*, 2016). This study is the first to assess the impact of body size and flight morphometrics on measures of flight capacity for male *L. dispar*. Wing length, a proxy for body size, was the most important predictor for both metrics of flight capacity; large moths were more likely than small moths to initiate sustained flight, and once initiated, larger moths tended toward longer, faster sustained flights than smaller moths. The greatest distance flown in the present study was more than 68 km and was accomplished by one of the largest moths (24.6 mm wing length). However, many midsize moths (19–21 mm wing length) in our study also flew total distances greater than 30 km. Moths with wing lengths between 19 mm and 21 mm are common in both endemic and high-density populations (Tobin *et al.*, 2016; Jahant-Miller *et al.*, 2020). Due to altered physical properties of tethered flight relative to free flight, such as a forced flight path, lack of natural flight stimuli, and the fact that tethered insects do not need to produce lift, extrapolation of tethered flight distances to flight potential in natural settings should be done with caution (Minter *et al.*, 2018).

Other significant predictors for metrics of flight capacity included forewing aspect ratio for speed and distance, and relative thorax mass for distance, and relative mass lost during flight for distance. Aspect ratio describes the shape of a wing where higher values indicate a longer, narrower wing and lower values indicate a shorter, broader wing. For aircraft and some flying animals including insects, aspect ratio is negatively correlated with flight speed and positively correlated with flight efficiency during gliding flight because longer, narrower wings produce less drag (Saville, 1957; Dudley & Srygley, 1994; Davis *et al.*, 2012). Energy storage for insect flight and flight musculature are thoracic components and flight metrics such as distance and speed are often correlated with increased relative thorax mass (Dudley & Srygley, 1994; Marden, 2000; David *et al.*, 2015). Relative mass lost during flight had a negative relationship with total distance flown, indicating that larger moths may use a smaller proportion of their stored resources than smaller moths and may have the capacity to continue flying longer than our flight assay allowed.

Most female *L. dispar* mate only once and appear non-selective, thus, the first male to reach the female has a reproductive advantage (Doane & McManus, 1981). This suggests that being the fastest flier with the greatest endurance would be ideal; however, large body size can generally only be attained at the cost of longer larval development. All else being equal (i.e., foliar quality, temperature, etc.), genetically larger individuals take more time to develop than smaller conspecifics (Hough & Pimentel, 1978; Teder *et al.*, 2014, but see Thompson *et al.*, 2020 for latitudinal differences among populations). Adaptation towards greater flight capacity may ultimately put larger males at a mating disadvantage unless environmental conditions require mate-finding flights that cover greater distances, such as in low-density populations. Conversely, as a protandrous species, larger male *L. dispar* could gain a mating advantage over smaller males if delaying emergence improves synchrony with female emergence (Robinet *et al.*, 2007), especially in low-density populations. While body size is the greatest factor impacting flight capacity, changes to wing or thorax morphology can also alter *L. dispar* flight ability and could do so without affecting development time.

Density-dependent shifts in mating or mate-finding morphology occur in other arthropods. In the amphipod *Gammarus roeselii*, individuals exhibit differential allocation to mating morphology in response to population density (Lipkowski *et al.*, 2019). In high-density *G. roeselii* populations where mating competition is relatively intense, individuals express larger antennae to increase mate selectivity and larger grasping legs to reduce the likelihood of having a mate stolen. In a wing-dimorphic planthopper *Prokelisia dolus* Wilson, flight-capable males in low-density populations with fully developed wings had greater mate-finding ability compared to wingless morphs, but at the cost of reduced fecundity (Langellotto & Denno, 2001).

Morphological and physiological adaptation that facilitates dispersal has been documented for several plant and animal phyla at invasion fronts, as well as areas in which species frequently undergo extinction and colonization events (Phillips *et al.*, 2006; Darling *et al.*, 2008; Forsman *et al.*, 2010; Schäfer *et al.*, 2018). In cane toads, *Rhinella marina* (Linnaeus), for example, dispersal ability increases with leg length and more recently established populations (e.g., those at the invasion front) in Australia have longer legs than populations behind the invasion front (Phillips *et al.*, 2006). Similar intraspecific variation in dispersal-related morphology and physiology has been reported for the butterfly *Melitaea cinxia* (Linnaeus) and for in-

vasive starlings, *Sturnus vulgaris* Linnaeus, in Australia (Haag *et al.*, 2005).

In low-density populations, emigration can exacerbate inverse density-dependent Allee effects such as mate-finding success and cooperative feeding behaviors, and ultimately lead to range retraction (Taylor & Hastings, 2005; Tobin *et al.*, 2009). However, in *L. dispar*, male dispersal from established populations in the range interior to the range margin could improve mating success by alleviating Allee effects caused by mate-finding failure typical of very low-density populations (Sharov *et al.*, 1995; Liebhold & Bascompte, 2003; Contarini *et al.*, 2009; Tobin *et al.*, 2009). Increased dispersal ability of male *L. dispar* from just behind the invasion front to nascent satellite populations ahead of it has the potential to increase establishment success and the rate of range expansion.

Size-dependent effects of flight ability on mate-finding may be relevant to other capital-breeding Lepidoptera, especially populations experiencing Allee effects and those in which females are non-dispersing prior to mating. A body size effect on mate-finding success has been shown in some other capital-breeding Lepidoptera. For example, male autumnal moth *Epirrita autumnata* Borkhausen mating in low-density field populations were larger than mean population size, indicating that there may be a size-dependent advantage, although it is unclear whether it is from mate-finding or mating competition (Tammaru *et al.*, 1996b). Evenden *et al.* (2015a) identified a mate-finding Allee effect in a capital-breeding spring defoliator, *Malacosoma disstria* Hübner; caged females were mated more frequently in high-density populations than low. Although large males originating from endemic populations were more likely to initiate mate-searching flight behavior than small males from outbreak populations, there was no apparent mate-finding advantage for larger body size (Evenden *et al.*, 2015a, Evenden *et al.*, 2015b). Mated males of the saturniid *Rothschildia lebeau* Guerin-Meneville tended to be larger on average than non-mated males in a field study that used caged females to attract endemic males (Agosta, 2010).

Adaptive shifts in wing morphology facilitating dispersal ability at the invasion front are unknown in *L. dispar*. Counterselection favoring small male body size may be driven by short growing seasons and cool climates encountered by range-expanding populations in northern regions. In addition, a significant component of *L. dispar* range expansion is driven by human-vectored movement of life stages (Sharov & Liebhold, 1998; Bigsby *et al.*, 2011; Tobin & Blackburn, 2014). Movement of propagules from established areas to range front populations may swamp any adaptive change. Despite known barriers

to adaptation, recent studies indicate that rapid adaptation is occurring in *L. dispar* populations in response to environmental conditions at range margins (e.g., Thompson et al., 2017, 2020; Faske et al., 2019). Future studies should take advantage of common garden studies, which disentangle genetic and environmental effects on body size, to detect potential shifts in *L. dispar* body size or flight morphology in this and other range-expanding invasive insects.

## Acknowledgments

Many thanks to Melody Keena (USFS), Ann Hajek and Tonya Bittner (Cornell University) for their advice on improving larval rearing procedures. We thank Toomas Tammaru (University of Tartu) for graciously reviewing and providing substantive comments on an earlier version of the manuscript. Kristine Grayson (Richmond University), David Althoff (Syracuse University) and Melissa Fierke and Colin Beier (SUNY-ESF) provided helpful comments on earlier versions of the manuscript. Thanks to Kiyoto Tanemura (Michigan State University) for help with writing R code and to Dr. Lianjun Zhang (SUNY-ESF) for input on statistical analyses. Our lab technician Marina Wittman provided valuable assistance weighing and mixing diet ingredients. Funding was provided by Macrosystems Biology (Grant 1702701 to Kristine Grayson, Sal Agosta, and Dylan Parry) and Gypsy Moth Slow-the-Spread Foundation, Inc (Grant 19-01-13 to DP).

## Author contributions

CJM and DP conceived and designed the experiments. RM designed the flight mill apparatus and provided technical assistance. CJM performed the experiments and analyzed the data. CJM and DP wrote the manuscript.

## Disclosure

The authors declare no competing or financial interests.

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Manuscript received April 8, 2021

Final version received June 28, 2021

Accepted July 13, 2021

## Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Appendix 1** Detailed flight mill design.

**Fig. S1** Microcontroller schematic.

**Table S1** Formulations for gypsy moth lab diet with varying quantities of protein.