Morphological variation associated with dispersal capacity in a tree-killing bark beetle *Dendroctonus ponderosae* Hopkins

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ABSTRACT

1. Intrinsic factors influencing dispersal of insect pests during outbreaks are poorly understood, yet these factors need to be quantified to parameterize dispersal in models that predict population spread. Our study related wing and body morphology of female mountain pine beetles (Dendroctonus ponderosae) to flight distance, as measured by flight mill bioassays.

2. Beetles that flew long distances (> 11 km) had greater body weight and larger wings than beetles that flew short distances (< 1 km). These heavier female beetles should also be more capable pioneers, as other studies have shown that body weight is positively correlated with lipid content.

3. Wing and body morphology of females are significant predictors of flight distance; heavy beetles with large wings generally flew further than smaller beetles, although this relationship is heteroscedastic. Dispersion of flight distance values increases with wing loading (weight/wing area) due to a cohort of 'lazy' individuals that fly short distances (< 1 km) regardless of flight phenotype.

4. Observed morphology explained less than 20% of the variation in flight capacity, indicating substantial contribution from other intrinsic factors that remain to be investigated. This study may have implications for dispersal modeling, providing estimates of flight capacity using morphological measurements.

Keywords: bark beetle, *Dendroctonus ponderosae*, morphological variation, dispersal kernel, flight, wing morphology, dispersal modeling, body weight, heteroscedasticity, pest

INTRODUCTION

Dispersal leading to range expansion can exert selective pressures on the physical traits associated with dispersal phenotypes (Olivieri and Gouyon, 1997; Hassall 2015). In many insects, traits like wing morphology, flight muscle and body condition can influence flight capacity and dispersal potential (Azevedo *et al.*, 1998; Hassall, 2015). Measurements of such flight-related traits can be used to infer an individual's ability to disperse, and may also be correlated with successful establishment after dispersal (Manning and Reid, 2013; Evenden *et al.*, 2014). Models that predict dispersal capacity by pest species have become important tools for environmental conservation and resource management through the refinement of damage prevention, especially in cases of range expansion and invasion of novel habitats (Safranyik and Carroll, 2006).

The mountain pine beetle (MPB) *Dendroctonus ponderosae* Hopkins (Coleoptera: Curculionidae) is a tree-killing bark beetle that attacks and kills pine trees across western North America. MPB has recently expanded its range eastward (Safranyik *et al.*, 2010). This spread is threatening to continue; historically, MPB distribution has been limited by climate rather than host availability (Safranyik, 1978), but climate change has made previously unsuitable regions available to this species (Carroll *et al*., 2004). Milder climate and protection of large overmature trees from natural fires have created conditions in which the beetles can thrive and reproduce to reach epidemic population levels (Taylor and Carroll, 2004; Safranyik and Carroll, 2006). This has led to the destruction of over 723 million m^3 of merchantable pine in British Columbia and Alberta during the last outbreak (NRCan, 2017), with subsequent ecological and economic impacts (Kurz *et al.,* 2008; Corbett *et al.,* 2015). These losses can be reduced through effective allocation of management resources based on early risk detection (Safranyik and Carroll, 2006). Resource allocation is increasingly important in epidemic scenarios in which total control of MPB is not realistic. In such scenarios, equipment and man-power must be allocated in accessible areas that will maximize the impact on MPB spread while minimizing cost. Predictive modelling of MPB spread is one means of risk detection, and such models need to be parameterized with variables such as regional climate and dispersal capacity (Atkins, 1961; Robertson *et al.*, 2007; Aukema *et al.*, 2008; Goodsman *et al.,* 2016). Climatic data are often available, but the ability to identify MPB dispersal phenotypes is lacking.

The variable dispersal behaviour of MPB poses a challenge for predicting its range expansion. Beetles can utilize updrafts to disperse above the forest canopy (Jackson *et al., 2008;* de la Giroday *et al*., 2012), but, research has shown that a majority of dispersal can take place below the canopy (Safranyik *et al.*, 1992). In below-canopy dispersal, many beetles ignore susceptible host trees immediately upon emergence in order to colonize new locations (Safranyik *et al.,* 1989; Robertson *et al.*, 2007). Below-canopy dispersal is self-propelled and can be parameterized in vitro with computer-linked flight mill bioassays. Many models predict MPB dispersal at both landscape and stand levels based on spatio-temporal patterns (Robertson *et al.,* 2007; Aukema *et al.*, 2008; Chen and Walton, 2011), climate (Aukema *et al.*, 2008), host vigor (Lewis *et al.*, 2009) and other environmental conditions (Safranyik *et al.*, 1989; Robertson *et al.,* 2007; Ainslie and Jackson, 2011). Inclusion of flight mill data can improve model accuracy by providing detailed data on flight behavior and patterns such as stopping frequency and length of uninterrupted flight times (Goodsman *et al.*, 2016). Finding the relationships of MPB wing and body morphology to measured flight capacity should improve our ability to predict bark beetle dispersal in nature.

In insects, wing morphology (*e.g*, shape and size) and flight muscle mass are often correlated with dispersal capability. Factors such as variance in aspect ratio (ratio of wing length to width) have been linked to greater dispersal in populations at higher latitudes (Azevedo *et al.*, 1998; Hassall, 2015). For example, small wing aspect ratio and large relative wing size can increase the flight capacity of *Drosophila* in cool climates (Azevedo *et al.*, 1998; Frazier *et al.*, 2008). In several insect species, such as milkweed bugs, neotropical butterflies and sand crickets, there are positive relationships between wing span and flight frequency, duration and speed (Palmer and Dingle, 1989; Dudley 1990; Fairbairn and Roff, 1990). Along with wing size, thoracic muscle mass can influence flight capability in butterflies (Dempster *et al.*, 1976; Chai and Srygley, 1990). In bark beetles, body mass correlates with flight distance and duration (Williams and Robertson, 2008; Evenden *et al.*, 2014). In general, body mass has been a primary factor in body condition quantification (Elkin and Reid 2004; Elkin and Reid, 2005). Body condition is considered to represent the intrinsic resources available for growth, survival and reproduction, and is consequently important for successful colonization (Boggs, 1992).

Dispersal is necessary in MPB as adults must find a new host tree in order to reproduce (Safranyik and Carroll, 2006), and females are pioneers in host colonization. Females initiate the attack once they arrive on a host and release aggregation pheromones to attract conspecifics (Blomquist *et al.*, 2010), and bark beetle body condition determines the quality and quantity of their offspring (Kautz *et al*., 2016). Correlations among lipid content, attack success and reproduction in bark beetles have been well documented (Elkin and Reid, 2005; Reid and Purcell, 2011; Manning and Reid, 2013; Kautz *et al.,* 2016); larger, heavier beetles have greater lipid content (Evenden *et al.*, 2014), resulting in increased fecundity (Elkin and Reid, 2005), and greater ability to resist host defences (Reid and Purcell, 2011). Larger females therefore are potentially better pioneers through their capacity to facilitate range expansion.

As the condition of females is influential in colonizing novel regions, this study aims to identify and quantify the morphological factors associated with female MPB dispersal capacity. These morphological features of females could provide a way to characterize the flight potential of individuals and dispersal capacity of populations (Hill *et al.*, 1999b; Hassall *et al.*, 2009). Given that flight performance is often associated with phenotypic traits such as wing size and body condition, larger individuals with larger wings should have greater dispersal capacity.

METHODS

Beetle data collection

All MPB used for this study were collected near Grande Prairie, Alberta, Canada (55.17 N, 118.80 W). The sample group was reared from bolts collected in October 2015. Four lodgepole pine trees infested by MPB were selected (3 from site 1 at 54.57 N, 119.42 W; and 1 from site 2 at 54.19 N, 118.68 W), and two 50-cm bolts were cut from each tree at 1m above ground level. All bolts were sealed with paraffin wax on the cut ends upon felling. These bolts, containing larval MPB, were stored at 4°C until use.

Bolts were removed from 4°C and placed in separate 136 L opaque plastic emergence chambers at 24±1°C in April 2016. Emerged beetles were collected daily. A total of 173 females were collected and flown; however, 49 beetles that flew less than 10 m, 44 of which did not fly at all, were removed from this study in order to exclude individuals that had irregular flight behaviours that were unlikely to be related to observed morphological features. Overall, the experimental group consisted of 124 female beetles, including 53 collected from site 1 bolts and 71 from site 2 bolts. Individual beetles were separated by sex based on the incidence of beetle stridulation, which is considered to be ~98% accurate for identifying males (Rosenberger *et al.*, 2016). This was performed at 24±1°C. The beetles were then placed in 2 mL centrifuge tubes with a small piece of paper, and stored at 4°C to reduce use of metabolic resources until the flight assay (Evenden *et al.*, 2014), which was performed 3 to 5 days post emergence.

Flight distance, duration and frequency data was collected for female beetles using the methods for computer-linked flight mill bioassays of Evenden *et al*. (2014). Flight distance was calculated as the total distance flown over a flight period, flight duration was calculated as total time spent in-flight, and flight frequency was calculated as the number of times flight was initiated after a minimum 5 second period of non-flight. Females were weighed prior to flight (Mettler Toledo, XS105 accurate to 0.01mg), and body dimensions (total length and pronotum width) were measured using digital calipers (Pro.Point 1-150mm, accurate to 0.01mm; remeasuring of 10 randomly selected specimens verified 99.01% precision). Beetles were then attached to a 2 cm long tether of 0.32 mm-diameter aluminum wire using LePage® Heavy Duty Contact Cement. Tethers were carefully attached to the pronotum to ensure no interference of elytra and wing movement. Length of the flight bioassay was 22 hours. This duration was chosen to allow for processing of samples and switch over of specimens between flight cohorts. During the flight assay the flight mill chamber had a photoperiod of 16:8 (L:D) h and a temperature of 22.5 °C. Flight assays began 2 hours after initiation of the light phase of the photoperiod, which allowed time to process the previous flight group, and to prepare the next group of beetles for the 22-hour flight assay. Female beetles were flown in groups ranging from 8 to 20.

After flights were complete, beetles were weighed again and stored in 85% ethanol at -20°C. For morphological data collection, wings were removed and suspended in a 50% ethanol solution. Wings were unfolded using paintbrushes and positioned, then removed from the solution by sliding a small strip of card stock below the wing and gently lifting it, maintaining the unfolded wing shape. The wings were fixed to the card stock strip by applying a thin layer of diluted glue with a fine paintbrush. Dried wings were scanned and measured using ImageJ version 1.51j8 (Schneider *et al.*, 2012).

Sixty-two MPB individuals were available for muscle dissection. The metathoracis medianus, lateralis posterior and lateralis medius flight muscles were identified for dissection based on Reid (1958) and Chapman (1998). After removal of the wings and elytra, the metathorax was opened by removing the dorsal tergites. Once the flight muscles were exposed, fibre length and lateral width of the metathoracis medianus were measured using an ocular micrometer in a dissection microscope under 50x magnification. The anterior edge of a medial segment of the metathoracis medianis was then detached from the metathoracic prephragma and its transverse thickness was measured. Subsequently, the transverse thickness and lateral width of the lateralis medius and the lateralis posterior were measured.

The lateralis medius was then carefully separated from the metacoxa, extracted from the metathorax, placed flat and its length measured. Measurements of the volume of individual muscles were then summed into 2 groups: dorsal longitudinal flight muscle volume, which included the metathoracis medianus muscle group, and dorsal ventral flight muscle volume, which included measurements of the lateralis medius and the lateralis posterior. Individual muscle measurements were each included in preliminary models, but were not found to provide additional explanatory power individually. To simplify variables and interactions in the presented models, these measurements were consolidated by calculating the volume of an elliptical cylinder, representing the flight muscle volume, and this value was used for further analyses.

Statistical Analysis

All statistical analyses were conducted in R version 3.3.2 (R Core Team 2017). Model R^2 values were obtained using the R package piecewiseSEM (Lefcheck, 2015) which finds R^2 values for non-fixed effect linear models, based on Nakagawa and Schielzeth (2013) and Johnson (2014). Flight models were created and analysed using R packages nlme (Pinheiro *et al.*, 2017) and lme4 (Bates *et al.*, 2015).

ANOVA hypothesis testing was used to determine whether differences exist between the strongest and weakest fliers observed in this study. Samples were separated by quartiles based on flight distance in order to apply the ANOVA tests. The upper quartile beetles, with flight distances > 11.13 km (n=31), represented strong fliers. They were compared to the lower quartile beetles that represented weak fliers, with flight distances < 1.05 km, (n=31; this number excludes any beetles that flew less than 10 m). Muscle volume was also compared using ANOVA hypothesis tests on the top and bottom quartiles, but with smaller sample sizes since fewer beetles with dissected flight muscle were available. The top quartile group contained 16 beetles, with flight distances > 8.0 km, and the bottom quartile group contained 16 beetles, with flight distances < 3.5 km. Morphological features tested included body weight, body size (calculated as ellipsoid volume[$\frac{4}{3} * \pi * body\ length * pronotum\ width^2$]), preflight weight, postflight weight, density ($\frac{preflight\ weight}{body\ size}$), absolute weight lost, proportional weight lost, wing size (measured as two-dimensional surface area), and wing shape (calculated as aspect ratio: $\frac{wing\ length}{wing\ width}$).

A linear mixed effects model was produced using distance flown as the dependent variable. Each included emergence bolt and flight mill as random variables. Each full model included the independent variables: pre-flight weight, body size, wing size, age (days since emergence) and wing shape, as well as all two-way interactions. Step-wise model simplification was achieved by removal of the least significant variables (highest p-values) until only significant ($p<0.05$) variables remained, and reduced models were compared to previous models and the full model using AIC and ANOVA hypothesis testing until the most parsimonious model was found. Following model refinement methods by Crawley (2013), interactions between variables were preferred for removal over basic variables in instances where both variables and interactions had a p-value greater than 0.05. All models were tested for significance using F-tests, and the included independent variables were tested with ANOVA. The residuals of each regression model were observed for random dispersal, and a variance inflation factor test was performed on each model before and after refinement using the R package *fmsb* (Nakazawa, 2017)

We tested the significant regression variables of distance flown for heteroscedasticity using Breusch-Pagan tests. When heteroscedasticity was present, quantile regressions were used to regress the data at separate quantiles of the independent variable, using the R package quantreg (Koenker, 2016). Because variables can only be tested independently in quantile regression, a third variable, wing loading ($\frac{preflight\ weight}{wing\ area}$), was introduced to combine the significant independent variables into a single term. Tests were conducted at the quantiles of 0.12 to 0.96 in increments of 0.12 in order to maximize resolution while retaining an adequate number of samples per quantile. When heteroscedasticity was observed, ANOVA hypothesis testing was used to compare slopes to an average quantile slope.

RESULTS

Morphology Associations

Variability in the following data is reported as standard error. Female MPB flew an average distance of 7.4±0.58 km over the 22-hour flight period, with a maximum flight distance of 28.81 km. On average, beetles flew for 3.70±0.29 hours and initiated flight 176±27.52 times; 81.8% of the total flight distance occurred during the light stage of the photoperiod. Strong fliers flew longer, and more frequently, than weak fliers ($F_{(1,60)}$=402.230, P<0.0001; and $F_{(1,60)}$=7.433, P=0.0084, respectively). Strong fliers flew an average of 7.60±0.36 hours with an average frequency of 144.8±29.65 flights, while weak fliers flew an average of 0.170±0.03 hours with 55.5±12.67 flights. Forty-four beetles did not fly at all, while 5 flew less than 10 m. Dispersal distance data was not normally distributed (Figure 1).

Insert Figure 1

Long distance and short distance fliers were morphologically different (Figure 2). Beetles that flew longer distances had greater body weight, both preflight ($F_{(1,60)}$=9.021, P=0.0039) and postflight ($F_{(1,60)}$=6.637, P=0.0125), as well as greater body density ($F_{(1,60)}$=15.741, P=0.0002). Long distance fliers also lost more absolute weight ($F_{(1,60)}$=10.990, P=0.0016) and proportional weight ($F_{(1,60)}$=5.467, P=0.0227) during the flight bioassay. Long distance fliers tended to have larger wings, but this only bordered significance ($F_{(1,60)}$=3.740, P=0.0578; Figure 2). There was no significant difference between long and short distance fliers in wing shape ($F_{(1,60)}$=1.453, P=0.2328), body size ($F_{(1,60)}$=1.332, P=0.2530), or flight muscle volume ($F_{(1,30)}$=0.1008, P=0.7530). Plots containing all 124 samples showing the relationship between total distance flown and preflight weight, postflight weight, wing area, body density, proportional weight lost and absolute weight lost can be seen in Supplementary Figure S1. The age of beetles in this study (3-5 days) had no relationship with flight distance ($F_{(1,60)}$=0.440, P=0.5095).

Insert Figure 2

Regression Models

After model refinement, flight distance of MPB was significantly related to both wing size ($F_{(1,121)}$=8.512, P=0.0042) and preflight weight ($F_{(1,121)}$=12.466, P=0.0006). Heavier beetles with larger wings flew farther than lighter beetles with smaller wings. Wing data was essential in predicting flight performance; exclusion of wing size data resulted in a significant loss of explanatory power in the flight distance model ($F_{(121,122)}$=8.512, P=0.0042), as well as an increase in AIC (AIC 2516.0 to 2522.4). Ultimately, these linear mixed effects models explained only a limited amount of the flight performance in female MPB. The models predicted 19% of variation in flight distance (R^2=0.1910).

A variance inflation factor (VIF) of 10 or more indicates multicollinearity; VIF tests showed that there was no multicollinearity before or after model refinement (VIF=1.32 and VIF=1.17, respectively).

Heteroscedasticity and Quantile Regression

Breusch-Pagan tests showed potential heteroscedasticity in the relationship of distance flown to preflight weight ($BP_{(1)}$=10.447, P=0.0012) and wing area ($BP_{(1)}$=4.028, P=0.0447), which were more prevalent when both variables were tested simultaneously as wing loading ($BP_{(1)}$=16.811, P<0.0001).

Insert Figure 3

Preflight weight and wing area were first tested with quantile regression separately (Figure 3). Distance flown had a significantly different response to preflight weight at the 96% quantile ($F_{(1,247)}$=5.399, P=0.0210), but showed no significant difference at the 12% quantile ($F_{(1,247)}$=3.369, P=0.0676). Preflight weight had a significantly stronger effect on flight distance when it was greater, with an increase of 1.63 km in flight distance per 1 mg increase in beetle weight, while it otherwise had an average increase of 0.67 km per 1 mg increase in preflight weight.

When both variables were combined into wing loading, the tests for heteroscedasticity revealed a general trend in which the dispersion of distance flown values increased with wing loading.

Insert Figure 4

Distance flown had a significantly different response from the average at both the 12% quantile ($F_{(1,247)}$=12.132, P=0.0006; Figure 4 and the 96% quantile ($F_{(1,247)}$=4.495, P=0.0350; Figure 4). At the 96% quantile, the predicted distance flown increases by 38.5 km per 1 mg/mm^2 increase wing loading, while at the 12% quantile this relationship was reduced to an increase of 1.6 km per 1 mg/mm^2. Otherwise, the average increase was 19.3 km per 1 mg/mm^2 wing loading.

DISCUSSION

Morphological Associations with Dispersal Capacity

We found that heavier beetles with larger wings flew farther than lighter beetles with smaller wings. After simplification, linear mixed effects models showed that the primary variables explaining distance flown were wing size and preflight body weight. Increased dispersal capacity by heavier individuals has also been found in other studies (Hill *et al.*, 1999a; Williams and Robertson, 2008; Evenden *et al.*, 2014). Although body weight was a significant predictor of dispersal in this study, body size was not related to flight distance. Reid and Purcell (2011) found no relationship between body size and fat content, but demonstrated that relative body weight is correlated with fat content. Other research has also shown the relationship between weight and lipid content (Williams and Robertson, 2008; Evenden *et al.*, 2014), implying that denser beetles have a greater store of flight fuel per unit of body size that allows them to fly further. This is also supported by our findings of increased density in long distance fliers.

An increase in stored lipids can also influence dispersal behaviour in bark beetles through behavioural responses to olfactory stimuli. Some newly emerged beetles will ignore both visual and chemical cues from susceptible hosts (Shepherd, 1966; Safranyik *et al.,* 1989; Eidson *et al*., 2017). In the Douglas-fir beetle, *Dendroctonus pseudotsugae*, beetles with higher fat content are less responsive to aggregation pheromones and spend more time selecting a host to colonize (Atkins, 1966; Bennett and Borden, 1971), thereby increasing dispersal; depletion of fat reserves through flight initiates a behavioural response in which the beetles are more responsive to olfactory cues. Fat content likely has a similar effect on dispersal in MPB, as well as impacting colonization success (Seybold *et al.*, 2006; Reid and Purcell, 2011) and reproduction (Manning and Reid, 2013) after dispersal.

We have shown that long range dispersing beetles not only have higher preflight weight, but postflight weight as well, which can influence the success of pioneers. Successful colonization requires large amounts of stored fat to detoxify monoterpenes produced by the host tree (Reid and Purcell, 2011), and to produce offspring (Elkin and Reid 2005; Manning and Reid, 2013). Greater postflight weight provides a substantial advantage to MPB, since the heaviest individuals are most likely to disperse long distances and also have the greatest chance of succeeding in host colonization.

While weight is important in dispersal capacity of MPB (Evenden *et al.*, 2014), the inclusion of wing morphology significantly increased the predictive power of the regression models tested in this study. Wing size is often important for flight performance in insects (Betts and Wootton, 1988; Taylor and Merriam, 1995; Hill, 1999a), and larger wings increase lift and carrying capacity (Wootton, 1992), allowing longer distance travel with a greater load of resources for dispersal and colonization, such as lipids. Large wings are also important for flight capacity in cold climates; cold temperatures can reduce flight muscle function and wing beat frequency, but larger wings allow for increased lift generation with lower input power (Azevedo *et al.*, 1998; Frazier *et al.*, 2008). Individuals with such an advantage could have contributed to the recent range expansion of MPB north into the Northwest Territories (NRCan, 2017), and eastward across the Rocky Mountains, although this hypothesis remains to be tested.

The ability to predict range expansion can be challenged by difficulties in acquiring data for predicting dispersal phenotype; however, morphological data related to dispersal capacity can be obtained from specimens that are already available from population monitoring. Currently, 4-inch diameter disks are collected from infested trees in many mass attack regions, and larvae are observed under the bark to determine the reproductive success of a population (Alberta Agriculture and Forestry, 2016). Quantification of the relationship between larval instars and adult size is needed, but, with some modification of sampling procedure, collected data, such as larval weight, could be incorporated into this data collection and subsequently used in dispersal models that distinguish between regional populations.

Although not investigated in this study, wing size may also affect above-canopy passive dispersal. Range expansion in MPB can also occur through long distance dispersal events in which beetles are caught in updrafts and carried up to 300 km (Cerezke, 1989; Jackson *et al*., 2008). Certain behaviours exhibited by MPB hint at adaptations to passive dispersal: 44 of the beetles that were tethered to flight mills did not actually fly, but many of these displayed a “drifting” behaviour in which they would open the elytra and spread their wings fully, without flapping. Similar behaviors have been documented by Atkins (1959) when testing flight preparation and response in the Douglas-fir beetle, *D. pseudotsugae.* They found that some beetles fully extended their wings, but did not vibrate them, when tossed in the air. We suggest that such behaviour, when caught in an updraft, could maximize the exposed wing area while minimizing energy consumption. Associations between stationary wing size and passive dispersal distance occur in winged seeds of plants, (Augspurger, 1986) and this relationship could be similar in insects dispersed by the wind.

Heteroscedasticity in Flight Distance and Future Directions

Many factors can affect flight performance, and although the morphological variation observed in our study follows a normal distribution, the distribution of flight capacities follow a well-known non-normal pattern of dispersal, the dispersal kernel (Figure 1; Bateman, 1950; Chapman *et al.,* 2006). We found that many individuals fly only short distances on the flight mill while very few fly long distances. There are population-wide advantages to this distribution of dispersal behaviours, as it gives the greatest survivorship in the largest portion of the population while still allowing some risk for colonization.

Beetles that fly short distances use less lipid (Williams and Robertson, 2008; Evenden *et al.*, 2014) and tend to be more successful in colonizing host trees than similarly sized beetles that burn more of their lipid reserves to disperse (Reid and Purcell, 2011; Manning and Reid, 2013; Kautz *et al.,* 2016). In our study, we found that body weight and wing size were associated with flight distance and, consequently, a population of larger individuals with larger wings is likely to have a greater proportion of long distance dispersers; however, the strong positive skew in the dispersal kernel of MPB causes inconsistencies with predictions based on linear relationships with morphology.

Even with both wing and body morphology included, the power of these models to predict flight distance remains relatively low. Part of the weakness in the relationship of morphology with flight distance is due to heteroscedasticity in the coefficients of the variables. Quantile regression shows that preflight weight and wing area, when considered separately, do not show a clear relationship with flight distance (Figure 3); however, when combined as wing loading, the heteroscedastic nature of the relationship becomes evident (Figure 4). When wing loading is low, morphology has little power to predict dispersal, but the relationship at high wing loading values has a significantly higher slope, indicating that small increases can have a significant positive impact on dispersal capacity (Figure 4). This inconsistent relationship is caused by an increase in the dispersion of flight distance at different quantiles of wing loading, and is likely due to the large cohort of ‘lazy’ individuals that fly only short distances regardless of high wing loading (Figure 4). These ‘lazy’ beetles increase the disparity between individuals with low and high wing loading within each quantile, which subsequently increases the quantile slope coefficient. This creates difficulties in predicting the relationship between morphology and flight capacity, and indicates that there is more than morphology associated with dispersal capacity. The natural tendency to disperse is an important contributor to flight distance in other insects (Steyn *et al.*, 2016), and further research should focus on identifying factors that contribute to variation in dispersal behaviour, such as the ‘lazy’ dispersal phenotype seen in our study.

Other factors involved in dispersal capacity in the natural environment that could not be tested in this lab study include abiotic conditions such as temperature (Atkins, 1959; Aukema *et al.*, 2008), wind (Safranyik *et al.*, 1989; Ainslie and Jackson, 2011), and humidity (Safranyik and Carroll, 2006). Biotic factors that can influence dispersal include host presence, density and distance from the beetle (Atkins, 1966; Robertson *et al.*, 2007), wing flexibility (Mountcastle and Combes, 2013), joint resilin content (Haas *et al.*, 2000), as well as parasitic infections (Everleigh *et al*., 2007). Some of the variation in the relationship between beetle morphology and flight performance may be mediated by behaviour. Atkins (1966) has shown that different female dispersal behavioural types exist within Douglas-fir beetle populations, but the behaviours exhibited by these types did not appear to have a strong correlation with body weight. Our results similarly reveal that some 'lazy' individuals do not fly to their capacity as indicated by wing size and body weight. Although morphological characteristics demonstrably have an impact on flight capacity in MPB, other factors that govern dispersal-related behaviour, such as gene expression, should also be investigated.

Conclusions

Body weight and wing size contribute to dispersal capacity in mountain pine beetle, and such data can potentially be useful in improving dispersal model quality (Goodsman *et al.*, 2016). Although many other factors must also be considered in the parameterization of dispersal models, we have shown that large female beetles with large wings generally have increased dispersal capacity, flying farther than smaller beetles with smaller wings; autonomous populations in dispersal models can be assigned flight capacities based on morphology observed *in situ* through use of samples already being collected for monitoring purposes.

As large females are better colonizers (Seybold *et al.*, 2006; Reid and Purcell, 2011; Manning and Reid, 2013), our study expands understanding of the mechanisms by which MPB establish new populations and increase their range; however, due to the heteroscedasticity of the data, morphological variation alone provides limited power to predict flight capacity; many individuals fly short distances despite having the morphology of long distance dispersers. This suggests that, even in a controlled laboratory setting, other intrinsic factors also influence flight and dispersal behaviour. Further research should target these intrinsic influences so that we can better understand the elements governing dispersal-related behaviours of MPB and other economically significant insect species.

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Figure Captions

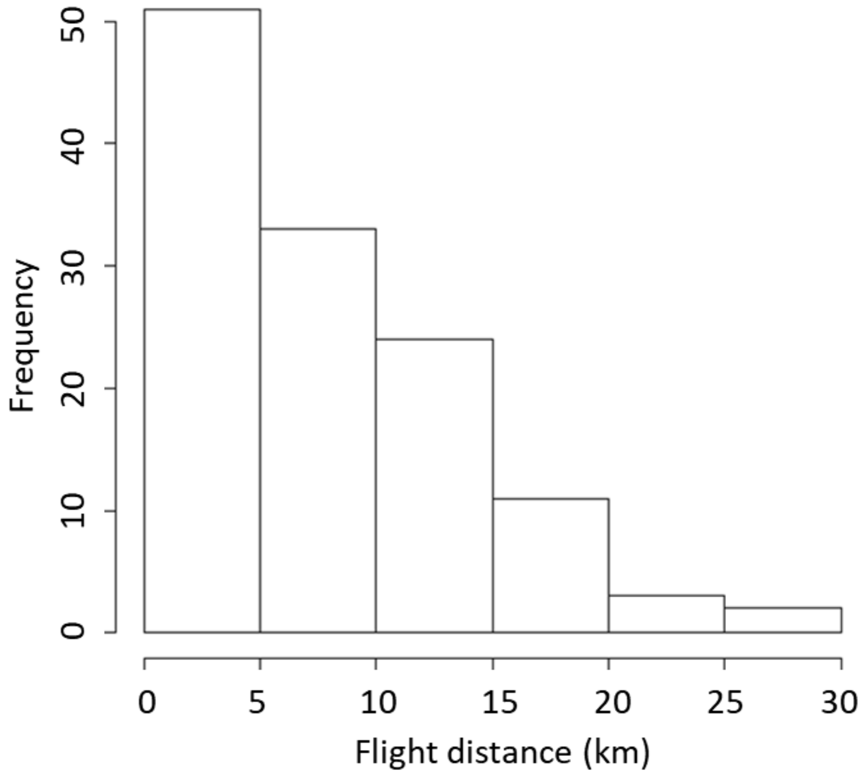
Figure 1. The positive skew in the distribution of flight distances for 124 female mountain pine beetles. Beetles that flew less than 10 m were excluded from this study; the minimum flight distance is 0.01 km.

Figure 2. Morphological differences between 31 longest distance (> 11.13 km) and 31 shortest distance (< 1.05 km) dispersing female mountain pine beetles. Long distance dispersers have greater preflight and post flight weight, wing size, proportional and absolute weight loss. Points represent group averages and bars show standard error. Comparisons were done using ANOVA. All graphs depict significant results.

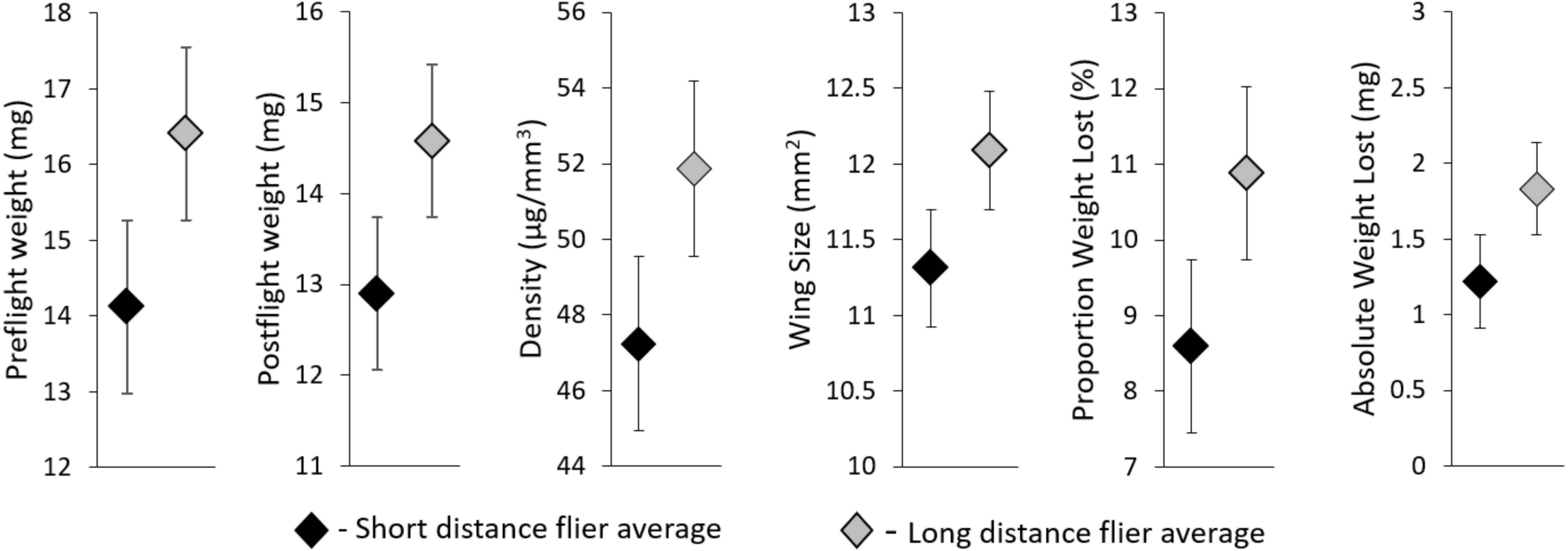
Figure 3. Quantile regressions showing the relationships of (A) preflight weight and (B) wing size with flight distance at 12% quantiles of 124 female mountain pine beetles. The solid horizontal line is the average relationship between flight distance and wing loading (dashed line is standard error). Grey shaded area represents the 90 percent confidence band for the quantile estimates. Preflight weight shows a significant deviation from the average relationship at the 96% quantile, showing that higher preflight weight has an increasing effect on flight distance. Although other potential sources of heteroscedasticity may exist, no other significant differences in slope coefficients were found. The asterisk in (A) indicates a significantly different quantile from the average.

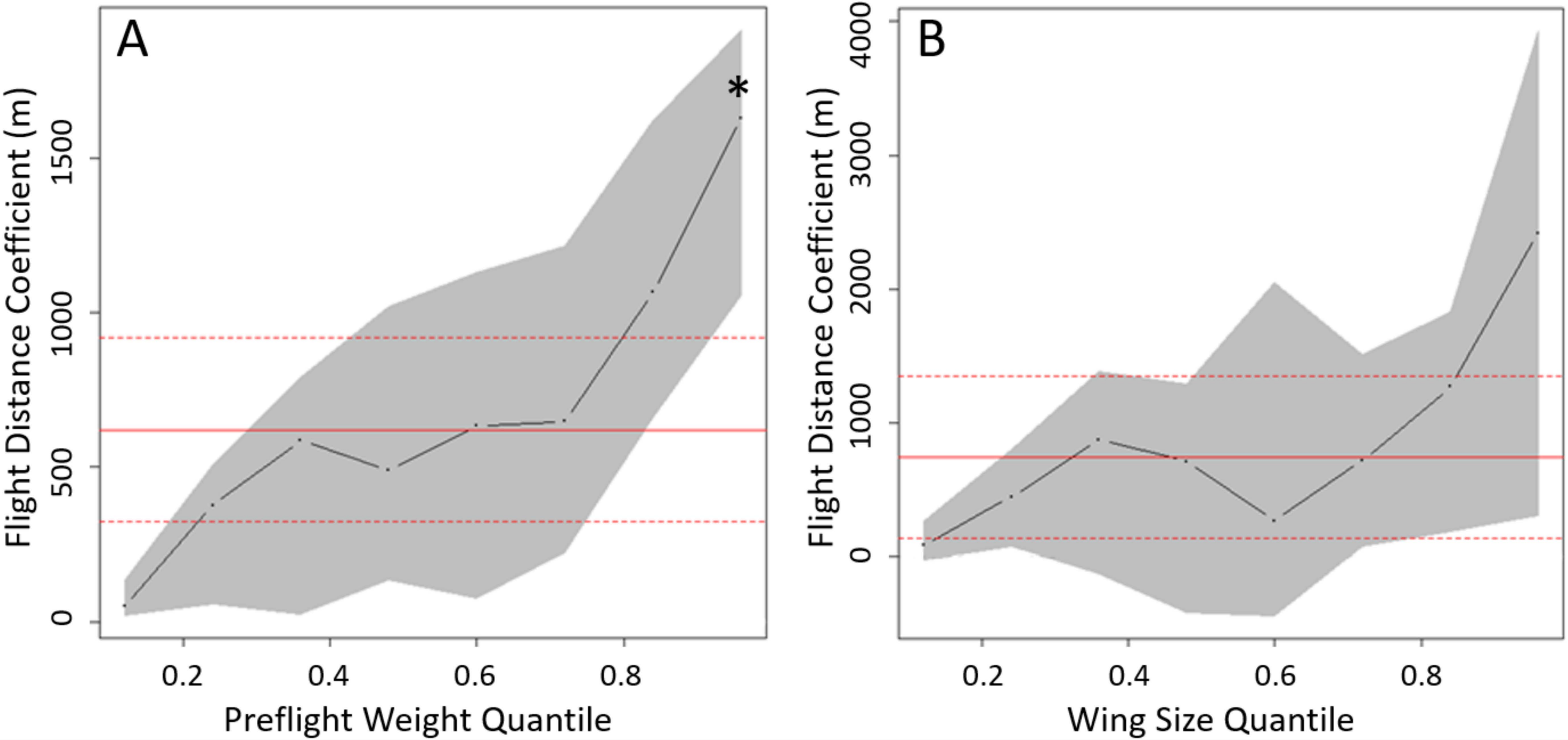
Figure 4. (A) Wing loading vs flight distance of 124 mountain pine beetles, with regression lines of the lowest 12% of dispersers (i) shown as a dotted trendline, and the highest 12% of dispersers (ii) shown as a dashed trendline. (B) Regression at each 12% quantile shows a significant change in the relationship,

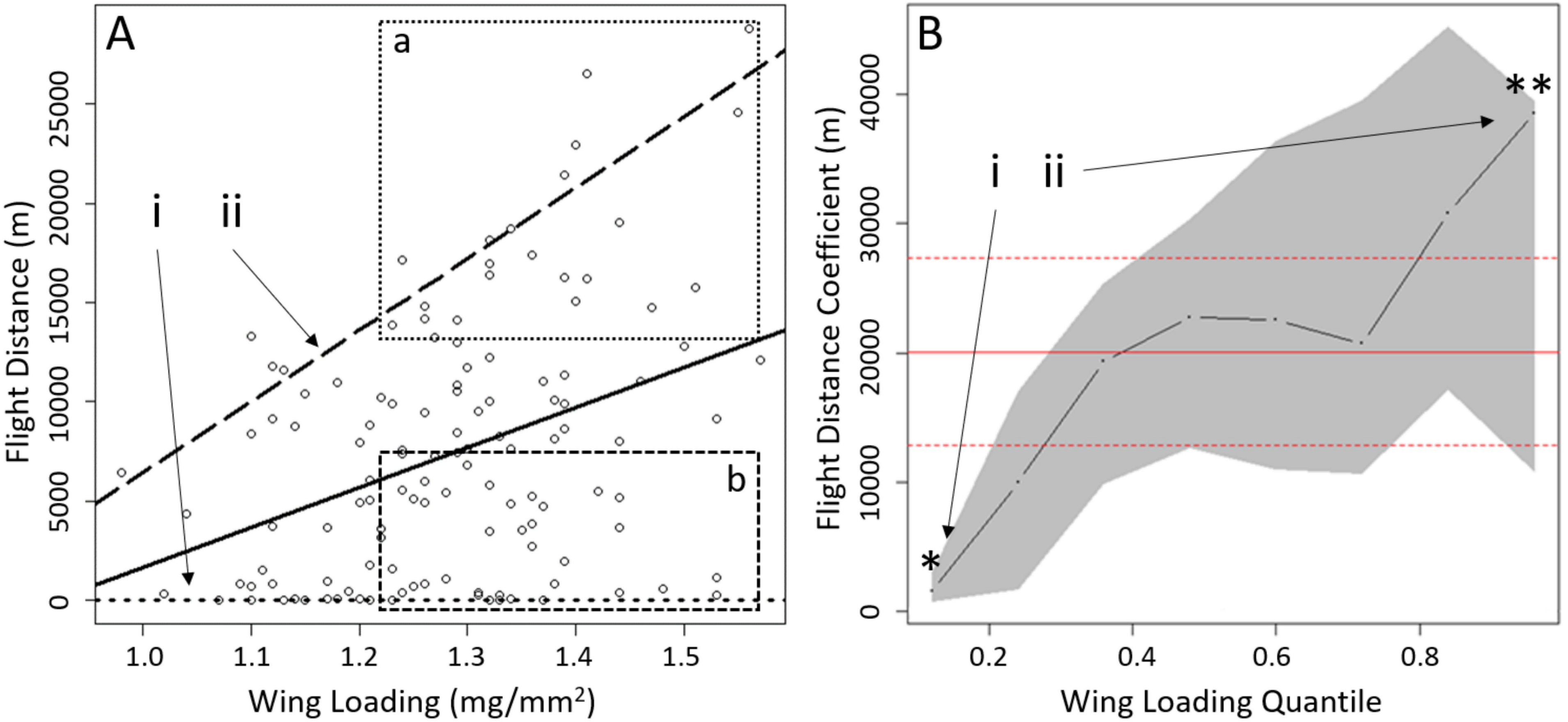
resulting in heteroscedasticity. The solid horizontal line is the average relationship between flight distance and wing loading (dashed line is standard error). Grey shaded area represents the 90 percent confidence band for the quantile estimates. At lower quantiles of wing loading (i) the relationship approaches 0; at higher quantiles (ii) the relationship becomes more pronounced. All strong fliers show high wing loading ((A), box ‘a’), but many “lazy” individuals have high wing loading and do not fly long distances ((A), box ‘b’), causing heteroscedasticity in the relationship. Asterisks in (B) indicate quantiles with significantly different coefficients from the average.

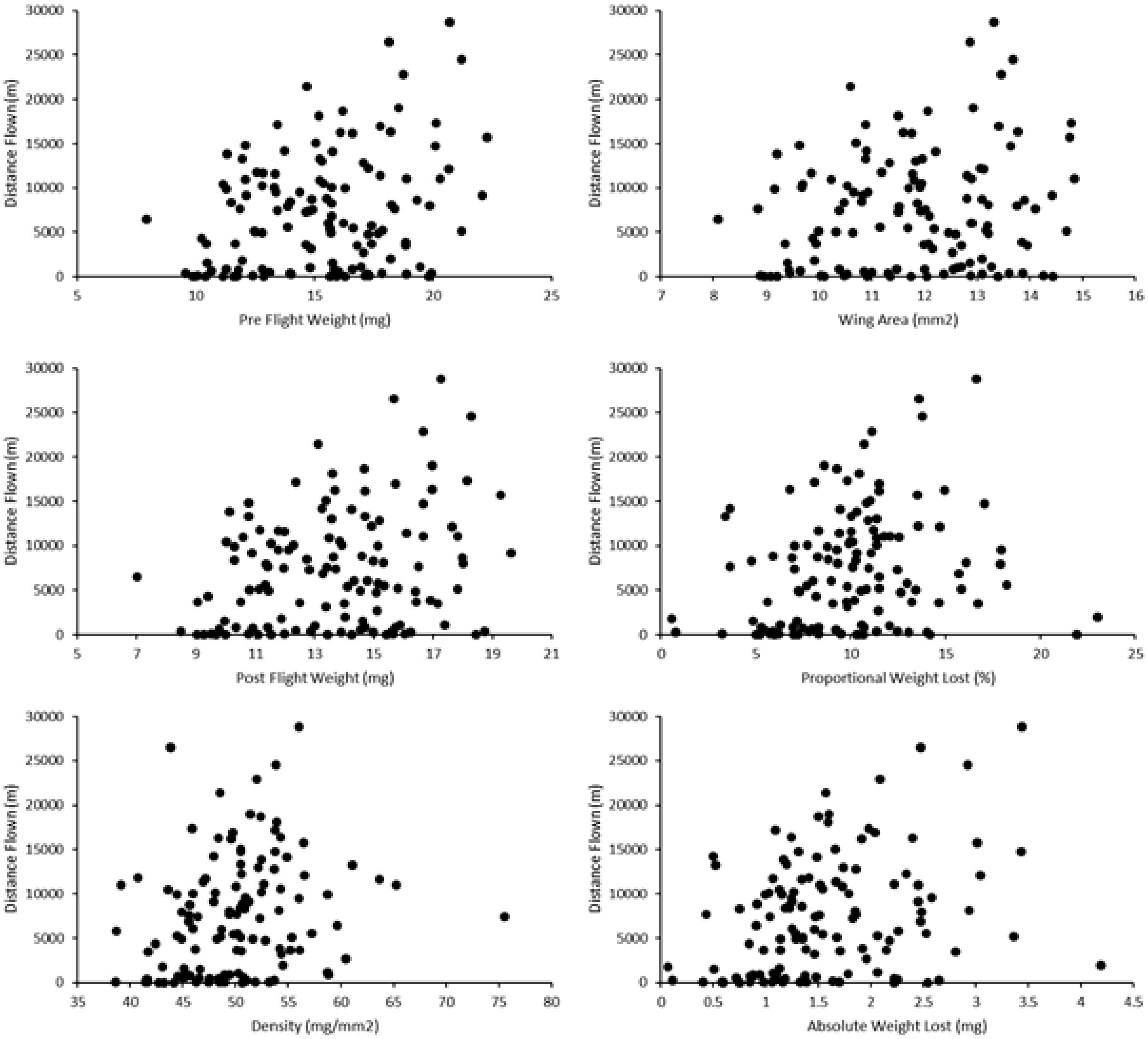


Frequency
0
10
20
30
40
50
0
5
10
15
20
25
30
Flight distance (km)


Preflight weight (mg)
18
17
16
15
14
13
12
Postflight weight (mg)
16
15
14
13
12
11
Density (µg/mm^3)
56
54
52
50
48
46
44
Wing Size (mm^2)
13
12.5
12
11.5
11
10.5
10
Proportion Weight Lost (%)
13
12
11
10
9
8
7
Absolute Weight Lost (mg)
3
2.5
2
1.5
1
0.5
0
◆ - Short distance flier average
◇ - Long distance flier average


A
*
Flight Distance Coefficient (m)
0
500
1000
1500
0.2
0.4
0.6
0.8
Preflight Weight Quantile
B
Flight Distance Coefficient (m)
0
1000
2000
3000
4000
0.2
0.4
0.6
0.8
Wing Size Quantile


A
a
b
i
ii
Flight Distance (m)
0
5000
10000
15000
20000
25000
Wing Loading (mg/mm²)
1.0
1.1
1.2
1.3
1.4
1.5
B
i
ii
*
**
Flight Distance Coefficient (m)
0
10000
20000
30000
40000
Wing Loading Quantile
0.2
0.4
0.6
0.8



Supplementary Figure S1. Plots showing all 124 specimens relating morphological features to the distance flown in meters, as measured on 22-hour computer-linked flight mill bioassays.