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RESEARCH ARTICLE

Mechanisms and consequences of flight polyphenisms in an outbreaking bark beetle species

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ABSTRACT

Flight polyphenisms naturally occur as discrete or continuous traits in insects. Discrete flight polyphenisms include winged and wingless morphs, whereas continuous flight polyphenisms can take the form of short- or long-distance fliers. The mountain pine beetle (*Dendroctonus ponderosae*) exhibits polyphenic variation in flight distance but the consequences of this flight variation on life history strategies of beetles is unknown. This study assessed the effect of flight on two particular aspects of beetle biology: (1) an energetic trade-off between flight distance and host colonisation capacity; and (2) the relationship between flight distance and pheromone production. A 23 h flight treatment was applied to a subset of beetles using computer-linked flight mills. After flight treatment, both flown and unflown (control) beetles were given the opportunity to colonise bolts of host trees, and beetles that entered hosts were aerated to collect pheromone. A trade-off occurred between initiation of host colonisation and percentage body mass lost during flight, which indicates energy use during flight affects host acceptance in female mountain pine beetles. Furthermore, production of the aggregation pheromone *trans*-verbenol by female beetles was influenced by both percentage body mass lost during flight and flight distance. Male production of *exo*-brevicomin was affected by beetle condition following flight but not by the energy used during flight. These novel results give new insight into the polyphenic flight behaviour of mountain pine beetles. Flight variation is adaptive by acting to maintain population levels through safe and risky host colonisation strategies. These findings suggest mechanisms that facilitate the extremities of the continuous flight polyphenism spectrum. These opposing mechanisms appear to maintain the high variation in flight exhibited by this species.

KEY WORDS: Mountain pine beetle, Dispersal, Pheromone, Host colonisation, Polyphenism, Scolytinae

INTRODUCTION

Polyphenisms are traits that exhibit two or more distinct phenotypes from a single genotype in response to environmental conditions. The link between phenotype and environmental factors promotes individual success under changing environmental conditions (Simpson et al., 2011). Although these distinct phenotypes may be advantageous for certain functions under different conditions, they may develop at a cost to other life history traits (Kopp and Tollrain, 2003; Karlsson et al., 2008).

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Flight is costly, and trade-offs between resource allocation to flight and other life history traits (Karlsson and Johansson, 2008), such as host colonisation (Latty and Reid, 2009, 2010) and reproduction (Roff and Fairbairn, 1991), are common. The most notable flight polyphenism in insects is the occurrence of winged and flightless morphs within the same species. Although many polyphenisms are discrete, continuous flight polyphenisms can also exist as short- versus long-distance fliers (Karlsson and Johansson, 2008; Simpson et al., 2011). Most studies focus on understanding the effects of discrete flight polyphenisms on subsequent life history strategies of adult insects (Cisper et al., 2000); the effects of continuous flight polyphenisms remain less studied.

Continuous flight polyphenisms occur in aggressive tree-killing bark beetle species in the genera *Dendroctonus* and *Ips* (Coleoptera: Curculionidae, Scolytinae) (Jones et al., 2019), which influences obligatory dispersal for host colonisation and reproduction (Raffa et al., 2005). Successful attack of a host tree requires the production of aggregation pheromones to attract conspecifics for mass attack (Safranyik et al., 2010). The pioneering beetle (females in *Dendroctonus* and males in *Ips*) releases aggregation pheromone that triggers the mass attack by both sexes (Raffa et al., 2015). Beetles of the same sex as the pioneer initiate new attacks along the tree bole, while beetles of the opposite sex enter existing galleries to mate (Gitau et al., 2013). Bark beetles synthesise pheromone components *de novo* or through the activity of microbial symbionts (Cale et al., 2019), but also require monoterpene precursors from the host tree for pheromone synthesis (Blomquist et al., 2010).

Differences in pheromone production by beetles, however, have some fitness consequences (Raffa, 2001). If production is low, beetle aggregation on the host tree will fail; this will result in adult mortality as a result of exposure to toxic host secondary compounds (Raffa and Berryman, 1982).

The host colonisation process is costly and depends on the physiological condition of the adult bark beetles arriving at the host after dispersal (Reid et al., 2017). Several hypotheses have been put forward to explain the relationships between dispersal behaviour, host choice and host colonisation in bark beetles (Latty and Reid, 2010). The ‘desperation’ hypothesis states that beetles with low energy reserves enter a tree independent of host quality decisions because low energy reserves prohibit further flight (Byers, 1999). The ‘safe site’ hypothesis posits that beetles enter high-quality hosts to promote mate attraction and successful attack (Latty and Reid, 2010). The ‘condition matching’ hypothesis suggests that host colonisation by the beetle should interact with the quality of the host tree; as a result, beetles in good energetic condition can enter well-defended trees (Chubaty et al., 2014).

The mountain pine beetle, *Dendroctonus ponderosae* (Hopkins 1902), is native to western North America, and has expanded its range eastward and northward (Cullingham et al., 2011) following the most recent population outbreak that started in the early 2000s, and killed millions of pine trees (Safranyik et al., 2010). Dispersal

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by flight dictates the spread of this species and it is arguably the least understood aspect of mountain pine beetle ecology (Chen and Walton, 2011).

After emerging from the natal host, mountain pine beetles exhibit two patterns of dispersal within the stand: spot growth and spot proliferation (Robertson et al., 2007). Spot growth involves short distance movements from the natal host to a reproductive host located only a few metres away. Spot proliferation results from beetle flight past suitable hosts followed by host selection much further away from the natal host. Understanding the mechanism underlying these flight polyphenisms in the mountain pine beetle and the cascading effects of flight polyphenisms on subsequent host selection and colonisation is essential for understanding population dynamics of the beetle (Robertson et al., 2007). Although some variation in flight distance is explained by lipid content (Evenden et al., 2014), energy reserves alone do not account for the large degree of flight variation exhibited by the mountain pine beetle (Shegelski et al., 2019). One explanation of the varied flight behaviour in mountain pine beetle populations is that beetles may require a flight period before becoming responsive to semiochemicals (Gray et al., 1972), similar to other bark beetle species (Thompson and Bennett, 1971). Beetles with high lipid levels need to expend energy before settling on a host, which could explain flight variation over geographic and temporal scales (Robertson et al., 2007).

While beetle body condition (high lipid to body volume ratio) affects host colonisation behaviour in mountain pine beetle (Elkin and Reid, 2005), it is unknown whether the same lipid resources consumed during flight (Evenden et al., 2014) are also allocated to host colonisation. Although metabolic costs associated with pheromone production may be insignificant (Pureswaran et al., 2006), mountain pine beetle aggregation pheromones are produced and/or stored in the fat body (Song et al., 2014; Chiu et al., 2018). It is unknown whether lipid use during flight influences the production of the male-produced aggregation pheromone *exo*-brevicomin, or the storage and use of *exo*-brevicomin and the female-produced aggregation pheromone *trans*-verbenol. Mountain pine beetle reproduction is also linked to body condition; beetles in poor condition produce smaller eggs (Elkin and Reid, 2005), and there is a trade-off between energy use during flight and offspring production (Wijerathna et al., 2019).

In this study, we tested the influence of flight polyphenisms on (1) female beetle host acceptance and (2) male and female production of aggregation pheromones. The aim was to reveal the relationship between energy use during the obligatory dispersal phase of mountain pine beetle and the subsequent host colonisation process.

MATERIALS AND METHODS

Collection of beetles

Beetle-infested lodgepole pine, *Pinus contorta* var. *latifolia* Douglas, was collected as 50 cm long cylindrical cross-sections of a tree bole, hereafter referred to as ‘bolts’. Bolts were collected from three trees at each of three sites in Hinton, AB, Canada (53°20.530 117°35.208, 53°22.825 117°32.561 and 53°16.527 117°39.916) in June 2018, and from two trees at each of two sites in Slave Lake, AB, Canada (54°51.751 115°09.751 and 54°53.842 115°08.708) in November 2017. The localities were chosen to ensure that beetles collected were in the epidemic population range of Alberta. Only mass-attacked trees (>40 attacks m^{-2}) that were larger than 27 cm diameter at breast height were felled. Two, 50 cm bolts from each tree, removed from 1–2 m above the ground were transported to the University of Alberta. Cut ends of the bolts were sealed with paraffin wax (parowax®) to minimise desiccation, and bolts were stored at 5°C until July 2018 when bioassays were conducted.

When beetles were needed for bioassays, bolts were removed from cold storage and placed in 121 l emergence bins fitted with a glass jar. Mountain pine beetles are positively phototactic and when they emerge from bolts they follow the light towards the glass jar where they are collected. Bins were housed at 21°C under a 16 h:8 h light:dark cycle. Emerging beetles caught in the glass jars were collected daily, separated by sex, labelled and placed in 1.5 ml microcentrifuge tubes with a small strip of paper to hold on to (Evenden et al., 2014). Beetles were stored at 4°C before use in the bioassay at 3–5 days post-emergence from the bolt.

Flight mills

Flight on flight mills was used as an experimental treatment to assess the impact of flight on subsequent host colonisation and pheromone production (Fig. 1). Beetles (3–5 days post-emergence) were weighed to the nearest 0.01 mg (Mettler Toledo XPE205 Microbalance, Columbus, OH, USA) and assigned randomly to one of two treatments: 23 h flight period (flown) or 23 h without the opportunity to fly (control). Beetles in the flown treatment were tethered using a 2 cm long, 30 gauge aluminium wire (0.02 mm diameter) with a small loop at the end. The loop was attached to the pronotum of each beetle using Press-Tite Contact Cement (LePage, Mississauga, ON, Canada) so that elytra movement was not restricted. Twenty-two tethered beetles were positioned on flight mills on each of 13 days, and given the opportunity to fly during the 23 h treatment period. Control beetles were housed with a piece of paper in perforated 1.5 ml microcentrifuge tubes in the flight mill room during the treatment period. The flight mill room was kept at 23°C with a 16 h:8 h light:dark cycle. The distal end of each tether was attached to the flight mill arm at a ∼100 deg angle using a small piece of wire insulation. Light (550 lx) was provided by high flicker frequency fluorescent bulbs (Evenden et al., 2014).

A small magnetic transmitter positioned on the flight mill arm detected the arm rotation propelled by beetle flight. The transmitter directed the signal to the attached computer. LabView software (National Instruments Corporation, Austin, TX, USA) measured each revolution of the flight mill arm (94.4 cm in circumference). Output included the duration and number of revolutions for each flight burst initiated by the beetle. Total flight distance and duration, as well as flight velocity and number of flight bursts were calculated from this output.

After the 23 h treatment period, the tether was removed from each flown beetle, and both flown and control beetles were weighed to the nearest 0.01 mg. Beetles that died or became detached from tethers during flight treatment were not included in the subsequent bioassays or statistical analyses.

Inoculation material

In July 2018, three uninfested lodgepole pine trees were felled at each of three sites (53°20.530 117°35.208, 53°22.825 117°32.561 and 53°16.527 117°39.916) in Hinton, AB, Canada. Trees were chosen based on size and overall appearance; only those that were healthy looking (i.e. green needles and no large wounds) and larger than 27 cm diameter at breast height were felled. From each tree, three 50 cm bolts were harvested between 1 and 2.5 m above the ground. Bolts were transported to the University of Alberta, where the cut ends of each bolt were sealed with paraffin wax and stored at 5°C until Aug 2018 when needed for bioassays.

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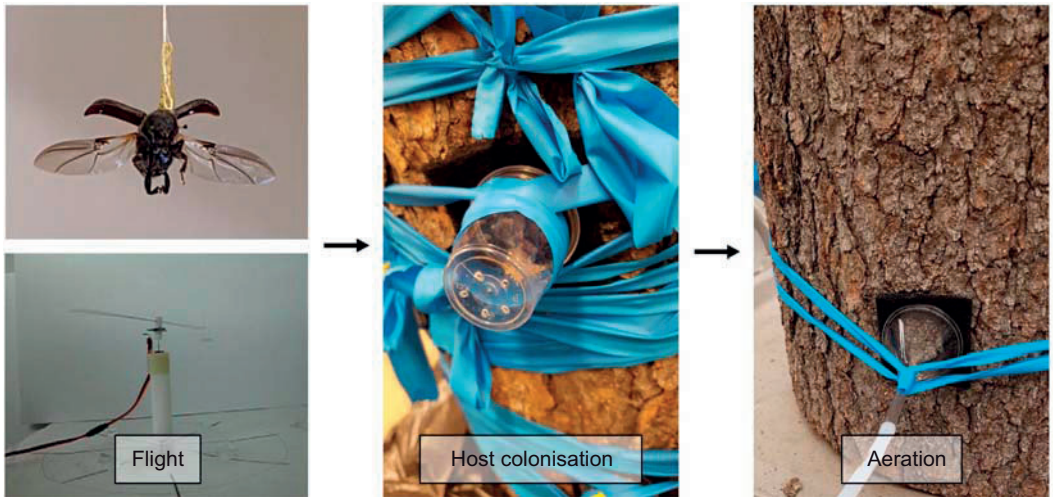

Fig. 1. Experimental set-up for host colonisation and aeration experiments. For host colonisation, mountain pine beetles (*Dendroctonus ponderosae*) flown on flight mills for 23 h were subsequently placed into small cups tied to the side of a healthy bolt. Females that entered within the first 24 h were paired with a flown male and subjected to the collection of pheromones in the aeration part of the bioassay.

Host colonisation experiment

The first experiment tested the hypothesis that flight treatment influences subsequent host colonisation behaviour by female mountain pine beetles (Fig. 1). Host colonisation was measured as the capacity to enter lodgepole pine bolts and the time taken for successful host entry. Uninfested bolts were removed from cold storage 24 h prior to beetle inoculation. Ten clear plastic cups (30 ml) were positioned 10 cm from the bottom of the bolt and secured with flagging tape. A charcoal filter (Paasche Charcoal Filter, Kenosha, WI, USA) skirt was placed between the bolt and the cup to fill any gaps.

Immediately following flight treatment and measurement of post-treatment mass, each female beetle was introduced into one of 10 individual cups positioned on a lodgepole pine bolt. Flown and control beetles were placed in alternating order on each bolt. Beetle activity was monitored for 72 h following the initial placement in the cup or until host entry or death. Boring dust within the cup indicated host entry. Data for beetles that escaped from the cups (34 flown beetles and 33 control beetles escaped) were removed.

Pheromone production experiment

A second experiment tested the hypothesis that flight treatment affects pheromone production by mountain pine beetles following successful host entry (Fig. 1). A subset of female beetles, from both treatment groups (flown n=12, and control n=9), that entered host material within 24 h of inoculation were used in aeration bioassays to measure semiochemicals released by the beetles.

A single flown (n=11) and control (n=7) male was introduced into galleries of individual females 24 h after females were introduced into cups. Males were flown the day after females and introduced into the bolts in a different manner. The bark was peeled back slightly around the female entrance hole and boring dust was blown away to reveal the exact point of entrance. Males were gently pushed into the female's entrance hole. Once the male was firmly positioned within the entrance hole, the set-up described below was assembled for aeration.

Aerations were conducted using the methods described in Erbilgin et al. (2014). Once female beetles entered the bolt, the clear plastic cup was removed, and replaced with a glass funnel (DWK Life Sciences Kimble K2895045, 45 mm diameter, 50 mm stem). The glass funnel was positioned over a charcoal filter skirt pressed tightly against the bolt and secured with flagging tape. The stem of the glass funnel was connected to a small, 10 cm portion of PTFE tubing (Cole-Parmer, 3/16 in×1/4 in, RK-06605-32). A second piece of PTFE tubing was attached to PVC tubing (Fisherbrand, 3/16 in inner diameter, 1/16 in wall) that was subsequently connected to a laboratory bench vacuum. To collect the semiochemicals released by the beetles, Porapak Q tubes (6×110-mm, 2 sections: 75/150 mg sorbent, 20/40 mesh) were inserted between the two portions of PTFE tubing. Over a 4 h duration, the vacuum pulled air (100 ml min^{-1}) over the site of beetle entry to trap semiochemicals produced by the beetle pair into the attached Porapak Q tube. After the 4 h aeration, Porapak Q tubes were removed from the PTFE tubing and were capped, wrapped in tinfoil and stored at −80°C until extraction. Repeated aerations measured pheromone production at 12, 24, 36, 48, 72, 96 and 120 h after introduction of females into cups. Males were introduced 24 h after females, so the 12 h time point contained emissions from females only; the subsequent collections were conducted on beetle pairs.

Chemical extraction and analyses

Each Porapak Q tube from each aeration sample was scored with a glass cutter to remove the adsorbent beads from the tube into a 2 ml Axygen microtube that was placed onto dry ice. The stock solution of the extraction solvent contained 500 ml dichloromethane (DCM, HPLC Grade, Fisher Scientific) with 5 µl of heptyl acetate (purity >98%, Sigma-Aldrich) to act as an internal standard. A 1 ml sample of the stock solution was dispensed (0.5–5 ml dispenser, Dispensette Organic, Eppendorf) into each 2 ml microtube containing adsorbent material from each sample. Microtubes containing adsorbent material and stock solution were vortexed for 30 s at maximum speed (3000 rpm; VWR Pulsing Vortex Mixer) and were then placed into a sonicator (Symphony) for 10 min. Microtubes were centrifuged for 15 min at 0°C at 16,100 rcf (Eppendorf AG 2231) to create two phases. Dichloromethane with the extract was collected from the lower phase.

To filter the extract, the solvent solution was pipetted into a modified pipette (Fisher Scientific, borosilicate glass, 13-67-20A) containing a small amount of glass wool to act as a filter. Filtered

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extract was collected in 2 ml Autosampler vials (Fisher Scientific, 9 mm/Amber-ID, 03-391-9) that were capped (Autosampler caps, 9 mm screw thread/PTFE/Silicone, 03-391-14) and stored at −40°C until chemical analyses.

Chemical analyses were performed using a Gas Chromatograph/Mass Spectrometer (GC/MS, 7890A/5975C, Agilent Technologies, Santa Clara, CA, USA) with a HP-CHIRAL-20β column (i.d. 0.25 mm, length 30 m, Agilent Technologies). Helium was the carrier gas with a flow rate of 1 ml min^{-1}; 2 µl samples from each extract were injected in a pulsed splitless mode. The oven temperature started at 45°C for 2 min, increased to 70°C by 20°C min^{-1}, increased to 90°C by 10°C min^{-1}, increased to 120°C by 2°C min^{-1}, increased to 150°C by 3°C min^{-1}, and then increased up to 230°C by 30°C min^{-1} and held for 1 min. The data were acquired using both SIM and SCAN modes. SCAN mode was conducted to identify the compounds of interest, whereas SIM mode was used to quantify the collected data. The quantified compounds included (1) *trans*-verbenol and (2) *exo*-brevicomin. Compounds were quantified by comparison with commercially available standards with a chemical purity >99% (Contech Enterprises Inc., Victoria, BC, Canada).

Statistical analyses

All data analyses were performed in R version 3.4.1 (http://www.R-project.org/). The explanatory variable, percentage mass lost during the flight treatment, was calculated by dividing the difference between pre- and post-treatment mass by pre-treatment mass, and multiplying this value by 100. Data were tested for normality and heteroscedasticity using visual techniques and Shapiro–Wilks test. Because of the confounding nature of the variables (percentage mass lost, pre-treatment mass and distance flown), the effects of these independent factors were analysed in separate models to avoid spurious associations.

The effect of flight treatment on female beetle host acceptance was analysed using a contingency table. Dichotomous entry data in the host colonisation experiment was analysed using a binomial distribution in a generalised linear mixed effects model with natal bolt and reproductive bolt defined as random factors in each model. The response variable, host entry, was assessed in three separate models: (1) host entry explained by percentage mass lost by both flown and control female beetles, during the flight period; (2) host entry explained by distance flown by female beetles during the flight period; and (3) host entry explained by pre-treatment mass of both flown and control female beetles. For model 1, percentage mass lost was square-root transformed to meet the assumption of normality; for model 2, distance flown was transformed to the fourth root to meet the assumption of normality. Cox proportional models are regression models used to determine the relationship between survival time and predictor variables. In the case of this study, the 'survival' term was defined by entry success and time until entry. Thus, instead of the 'survival' term representing the length of time until death, it represented the length of time until host entry. Four cox proportional models were used to analyse entry success and time until host entry in relation to: (1) square-root transformed percentage mass lost for all beetles; (2) percentage mass lost for flown beetles; (3) fourth-root transformed distance flown; and (4) pre-treatment mass for all beetles. For the beetles that entered, the relationship between time until entry and percentage mass lost was analysed using a mixed effects linear model separately for flown and control beetles. As multiple models were used to analyse these groups separately, a Bonferroni correction of α=0.025 was applied to determine significance. Both natal and reproductive hosts were included as random factors in both analyses.

Pheromone production data were analysed using linear mixed effects models with natal bolt and reproductive bolt defined as random factors in each model. The response variable, total *trans*-verbenol production across all aeration time points, was assessed in three separate models: (1) *trans*-verbenol production as explained by percentage mass lost during treatment, by both flown and control female beetles; (2) *trans*-verbenol production as explained by distance flown by female beetles during the flight period; and (3) *trans*-verbenol production as explained by pre-treatment mass of both flown and control female beetles. For models 1 and 3, total *trans*-verbenol production was cube-root transformed to meet the assumption of normality. The response variable, total *exo*-brevicomin production across all aeration periods, was assessed in three separate models: (1) *exo*-brevicomin production as explained by percentage mass lost during treatment, by both flown and control male beetles; (2) *exo*-brevicomin production as explained by distance flown by male beetles during the flight period; and (3) *exo*-brevicomin production as explained by pre-treatment mass of both flown and control male beetles.

RESULTS

Host colonisation experiment

Beetles placed on flight mills flew an average of 4.02±0.54 km over the 23 h period (Fig. 2). The minimum flight distance was 0.002 km and the maximum flight distance was 22.26 km. Of the 267 flown and control female beetles used in the host colonisation study, 40% entered the host material within 72 h. Initiation of host colonisation was influenced by flight treatment. Beetles that flew on flight mills were 13% less likely to initiate host colonisation compared with unflown control beetles (χ^2=5.2722, P=0.0216).

Generalised linear models indicated a negative relationship between host entry and the percentage mass lost during the flight treatment (χ^2=31.774, P=1.732×10^{-8}). Female beetles that lost less mass during flight treatment were more likely to enter a host (Fig. 3). No relationships between host entry and distance flown (χ^2=0.0763, P=0.7824) or pre-treatment mass (χ^2=0.5286, P=0.4672) were found.

Cox proportional models showed that percentage mass lost affected host entry and entry time for all beetles (Z=6.264, P=3.74×10^{-10}) and for flown beetles alone (Z=2.184, P=0.029, Fig. 3). There was no relationship, however, between distance flown (χ^2=0.408, P=0.683) or pre-treatment mass (χ^2=0.704, P=0.4820) and host entry. Of the beetles that entered the bolts, the time until entry was negatively influenced by the percentage mass lost during the treatment in flown (χ^2=7.0248, P=0.0080; Fig. 4) but not control (χ^2=0.0093, P=0.923) beetles.

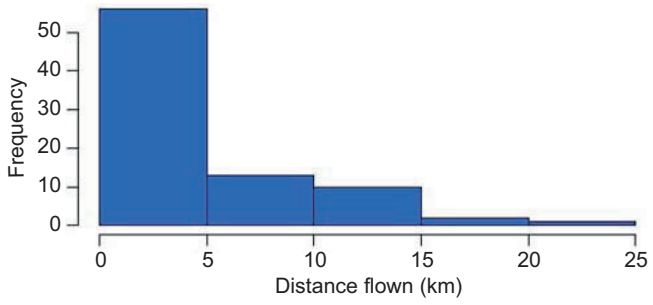

Fig. 2. Histogram of flight distribution exhibited by females flown on flight mills for 23 h. The average flight distance was 4.02±0.54 km, with a minimum flight distance of 0.002 km and a maximum flight distance of 22.26 km (N=83).

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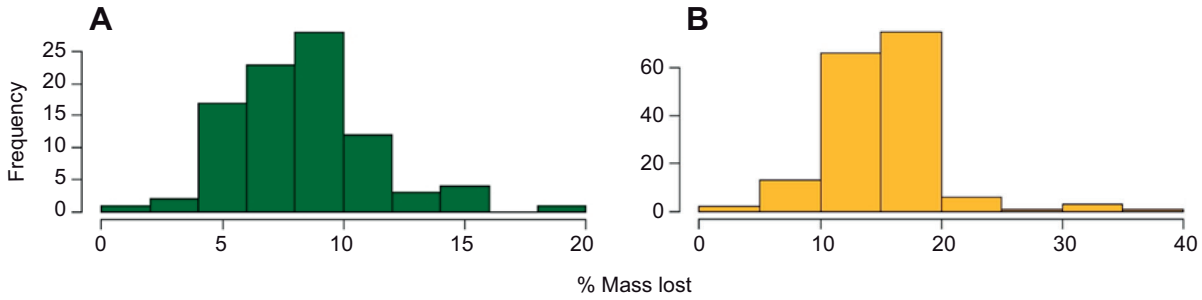

Fig. 3. Histograms of percentage mass lost during flight for female mountain pine beetles that did or did not enter hosts. Female beetles that failed to enter hosts (B) lost more mass on average than those that entered hosts (A) (χ^2=31.774, P=1.732×10^{-8}; N=83).

Pheromone production experiment

The production of *trans*-verbenol by female beetles was influenced by the percentage mass lost during flight treatment (χ^2=3.8706, P=0.04914) and the distance flown (χ^2=5.1584, P=0.0231), but not by pre-flight mass (χ^2=1.1417, P=0.2853). Females that lost more mass and flew further distances produced more *trans*-verbenol (Fig. 5).

The production of *exo*-brevicomin by male beetles was influenced by pre-flight treatment mass (χ^2=5.6937, P=0.0170) and distance flown (χ^2=9.5932, P=0.0020), but not by percentage mass lost during flight (χ^2=0.9912, P=0.3195). Males that weighed more prior to flight treatment produced more *exo*-brevicomin; males that flew further produced less *exo*-brevicomin (Fig. 6).

DISCUSSION

The important life history traits of adult mountain pine beetles include dispersal from the natal host, host colonisation, aggregation triggered by pheromone production, and reproduction after overcoming host defences. The current study uncovered mechanisms by which energy use during flight influences host entry and pheromone production by beetles. The amount of lipids retained by females following flight dictates the outcome of host colonisation success (Chubaty et al., 2014). In the current study, female beetles that lost less than 10% of their body mass during flight were more likely to enter hosts compared with those that lost more than 10%. In mountain pine beetles, mass loss is linked to lipid metabolism during flight (Evenden et al., 2014). Our findings are in agreement with the results of earlier studies on male pine engraver beetles (*Ips pini*) in which beetles that enter host material have 21% more lipid compared with those that do not (Wallin and Raffa, 2000). Certain silvicultural treatments, like stand thinning techniques, can increase flight distance before host colonisation in managed stands. Mountain pine beetles were detected in high numbers in thinned stands (Schmitz et al., 1989) as well as in clear cut stands (Reid, 2008). In these stands, beetles are forced to fly further distances before host colonisation, which could impact the number of successful attacks on trees.

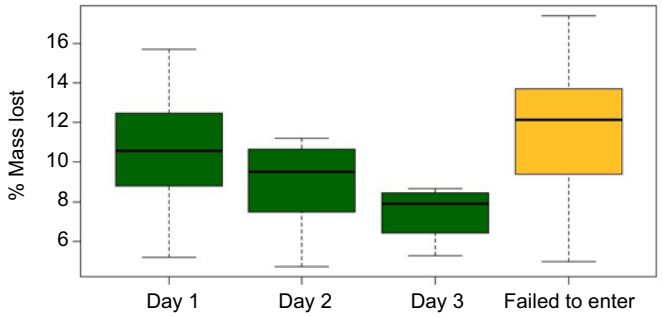

Fig. 4. Box plots of percentage mass lost for flown female mountain pine beetles that entered lodgepole pine hosts at different times post-inoculation. The midline indicates the median and the bottom and top of the box represent the 25th and 75th percentiles, respectively. Whiskers represent the maximum and minimum values. Beetles that entered host material (green bars, n=49) lost less mass during the flight treatment compared with those that subsequently failed to enter hosts (yellow bar, n=34) (Z=2.184, P=0.029). Mass lost after flight influenced the length of time it took beetles to initiate colonisation after flight (χ^2=7.0248, P=0.0080) (green bars).

The speed of host colonisation is also dependent on energy reserves remaining in female beetles after dispersal. We found that the fastest beetles to enter the host had lost the most mass during the flight treatment. This result indicates a trade-off between energy use during flight and host acceptance in female mountain pine beetles, which probably intensifies the flight–reproduction trade-off previously suggested for this species (Wijerathna et al., 2019). These results lend further support to the 'desperation hypothesis' (Latty and Reid, 2010). In contrast to our findings, time to host entry by pine engraver beetles declined with beetle starvation (Wallin and Raffa, 2002), suggesting that energy-use trade-offs may not be consistent across bark beetle species.

Distant dispersal away from the natal tree may increase the need for effective signalling to attract conspecifics to mount a mass attack. We show that female flight distance and energy use are linked to a subsequent increase in *trans*-verbenol production by females following host entry. Release of high concentrations of *trans*-verbenol should increase the success of pioneer beetles that initiate attack on distant hosts to increase the aggregation of conspecifics (Erbilgin et al., 2014). Similarly, attraction of a sister species *Dendroctonus frontalis* increases positively with *trans*-verbenol dose (Shepherd and Sullivan, 2019). Beetles that disperse only a short distance would benefit less from the production of strong pheromone signals.

Female mountain pine beetles release *trans*-verbenol upon initiation of gallery construction and feeding (Pureswaran et al., 2000). *trans*-Verbenol production requires the oxidation of the precursor, α-pinene (Hughes, 1975), obtained from the natal host (Chiu et al., 2018). Additionally, *trans*-verbenol production varies with the concentration of α-pinene present in the reproductive host (Taft et al., 2015), which suggests that the α-pinene necessary for pheromone synthesis could be obtained from both sources. Female mountain pine beetles accumulate α-pinene in the form of monoterpenyl esters, which are fatty acid esters stored in the fat body (Chiu et al., 2018). As we have shown that flight increases *trans*-verbenol production in female mountain pine beetles, the biochemical mechanism dictating this increase may be the result of lipid use during flight through impact on the stored monoterpenyl

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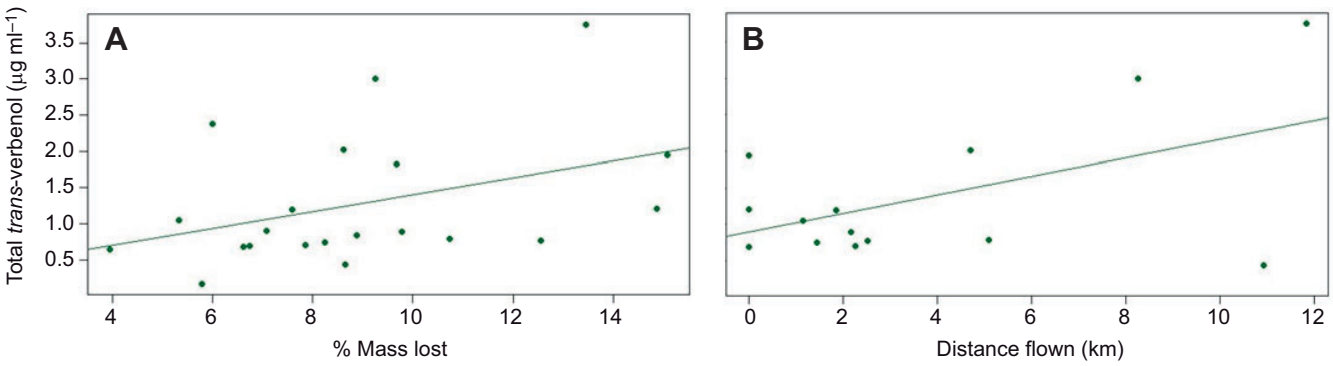

Fig. 5. Percentage mass lost during flight and distance flown versus subsequent *trans*-verbenol production by female mountain pine beetles. Data are shown for both flown (n=12) and control (n=9) beetles in lodgepole pine bolts. (A) Beetles that lost more body mass during the assay produced higher amounts of *trans*-verbenol (χ^2=3.8706, P=0.0491). (B) Flight promoted *trans*-verbenol production in female beetles (χ^2=5.1584, P=0.0231).

esters. High variability in pheromone production, including *trans*-verbenol, occurs in other bark beetles (Pureswaran et al., 2008). Variation in pheromone production can also be linked to body size (Pureswaran and Borden, 2003) and genetics (Domingue and Teale, 2008), but the causes of variation differ with pheromone component identity.

Flight distance negatively affected *exo*-brevicomin production by males. This difference in pheromone production in response to flight between sexes could be due to the timing of pheromone production. Males can begin to produce *exo*-brevicomin immediately upon emergence from the natal host (Song et al., 2014). The complete biosynthetic pathway behind the production of *exo*-brevicomin remains unknown; however, it is synthesized *de novo* from fatty acyl-CoA precursors and stored in the fat body (Song et al., 2014). Energy use during flight could influence *exo*-brevicomin storage in the fat body, with more pheromone released during periods of flight than rest. This may explain why males produce low levels of *exo*-brevicomin when they enter the female nuptial galleries to reproduce (Song et al., 2014). These low levels of *exo*-brevicomin are probably adaptive in mediation of aggregation behaviour as low concentrations of *exo*-brevicomin are more attractive to conspecifics than higher concentrations (Rudinsky et al., 1974). Flight may promote the release of low, attractive quantities of *exo*-brevicomin. Males potentially have a finite amount of pheromone to release based on the condition of the beetle at the time of pupation. Our finding that heavier males produced more *exo*-brevicomin than lighter males supports this idea. The quality of the natal host probably has a large influence on the amount of *exo*-brevicomin males can produce in a lifetime, as good quality hosts produce larger, more robust offspring (Graf et al., 2012). This supports previous findings indicating a marginal link between mountain pine beetle body mass and length with *exo*-brevicomin production (Pureswaran and Borden, 2003). Heavier males fly further than lighter males (Evenden et al., 2014); this difference in flight behaviour could promote optimal levels of *exo*-brevicomin release at the reproductive host.

Interestingly, mass loss during flight influences pheromone production in females but not males. This is potentially due to differential energy use during flight between the sexes. Females rely heavily upon lipids during long-distance flight, while males use both lipids and proteins to power flight (Wijerathna and Evenden, 2019). This is probably driven by variation in the energy needed for host colonisation, as females require proteins for reproduction (Pitt et al., 2014). The reliance on lipids by female beetles for flight probably has a direct impact on mass loss during flight (Evenden et al., 2014), whereas mass loss by male beetles is a combination of the depletion of multiple energy sources (Wijerathna and Evenden, 2019). If lipid use is responsible for differing pheromone titres, the link between mass loss and pheromone production in males would be lost. In the fat body, female beetles store monoterpenyl esters used in the production of *trans*-verbenol (Chiu et al., 2018), while male beetles store *exo*-brevicomin in its final form in the fat body (Song et al., 2014). Lipid use during flight may allow for the release of pheromone from storage in males and reduce the subsequent pheromone titre available to males calling at the new host. In females, as the entire biosynthetic pathway of *trans*-verbenol

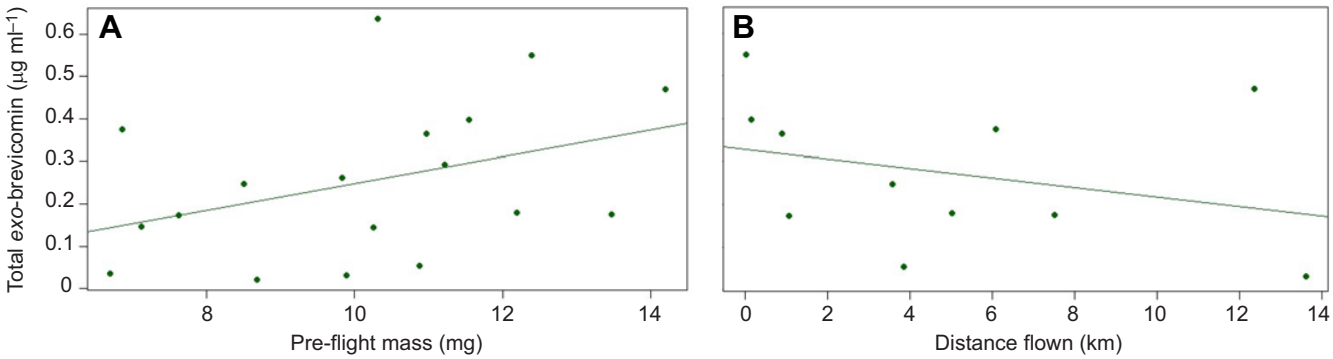

Fig. 6. Pre-bioassay mass and distance flown versus subsequent *exo*-brevicomin production by male mountain pine beetles. Data are shown for flown (n=11) and control (n=7) beetles in lodgepole pine bolts. (A) Heavier beetles produced more *exo*-brevicomin (χ^2=5.6937, P=0.0170). (B) Flight distance was negatively associated with *exo*-brevicomin production in male beetles (χ^2=9.5932, P=0.002).

remains unknown, all that can be concluded is that flight could aid or promote biosynthesis of this compound.

Here, we provide evidence for possible mechanisms that drive flight polyphenisms in bark beetles. The trade-off between energy use during flight and host colonisation could select for short-distance dispersal so that beetles have enough energy to successfully colonise their reproductive host. Alternatively, long-distance dispersal might be adaptive for outbreeding and access to high-quality and abundant hosts (Raffa et al., 1993). Energy use during flight positively impacts subsequent pheromone production in the pioneering female beetles. Increased *trans*-verbenol production will aid beetles in mediating mass attacks at distant hosts; this in combination with other benefits at these distant locations will select for long-distance dispersers. These results provide evidence for mechanisms that promote contrasting selection on flight in bark beetles. Selection at both ends of the polyphenism spectrum maintains high dispersal variability within populations. This intraspecific variation in dispersal strategies promotes an evolutionarily stable strategy for bark beetle populations (Kautz et al., 2016). Understanding variation in spatial movement of bark beetles across landscapes will help to predict future population spread of these aggressive tree pests.

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Competing interests
The authors declare no competing or financial interests.

Author contributions
Conceptualization: K.L.J., N.E., M.L.E.; Methodology: K.L.J., G.I., R.R., N.E., M.L.E.; Formal analysis: K.L.J., R.R.; Investigation: K.L.J., G.I., R.R., N.E., M.L.E.; Resources: G.I., N.E., M.L.E.; Data curation: K.L.J.; Writing - original draft: K.L.J.; Writing - review & editing: K.L.J., G.I., R.R., N.E., M.L.E.; Supervision: N.E., M.L.E.; Project administration: N.E., M.L.E.; Funding acquisition: N.E., M.L.E.

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