Energy use by the mountain pine beetle (Coleoptera: Curculionidae: Scolytinae) for dispersal by flight

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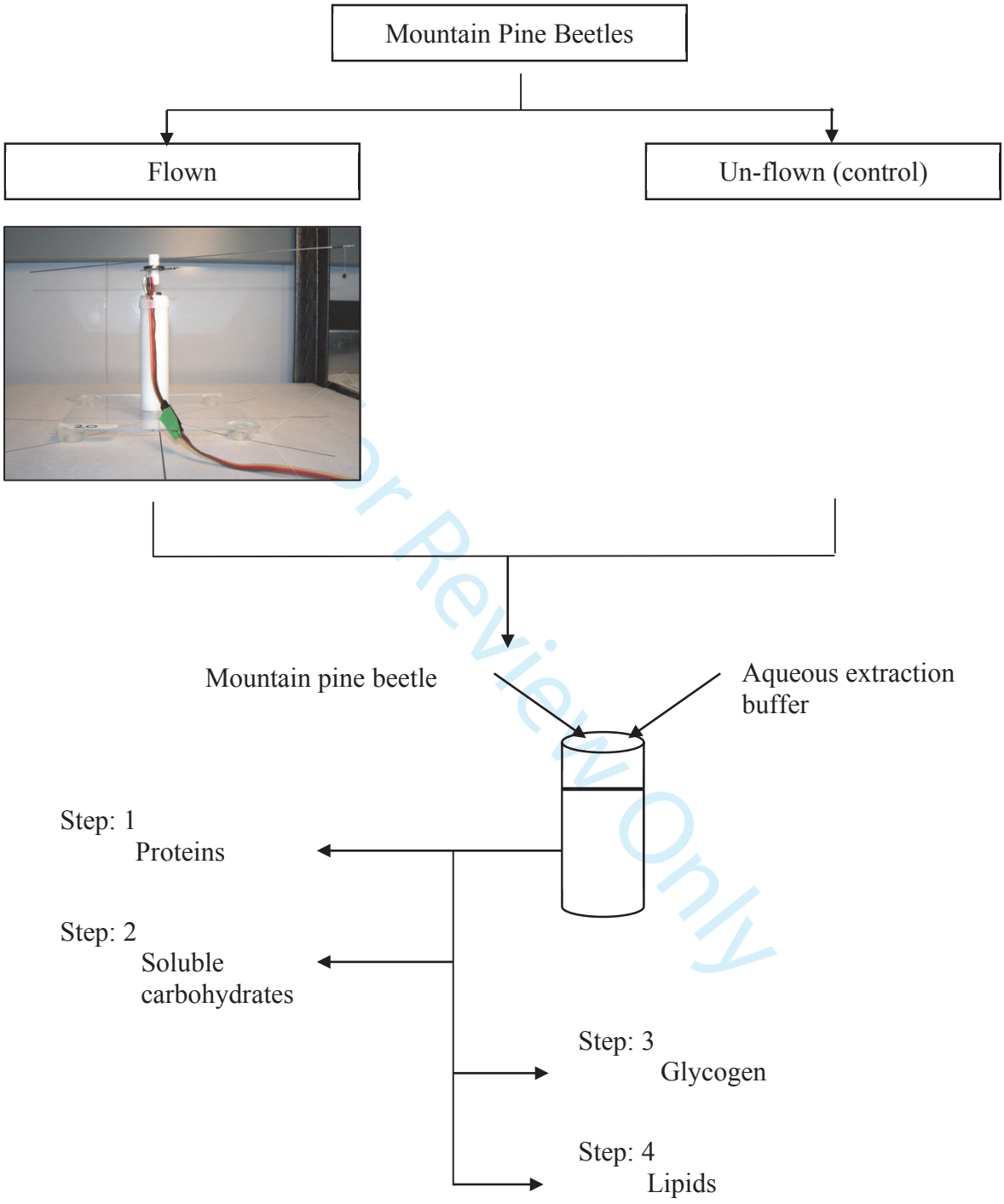

Research Highlights

- We compare the level of energy substrates, proteins, carbohydrates, and lipids of mountain pine beetle flown on flight mills with un-flown control beetles using a colorimetric method.
- Flight-ready mountain pine beetles contain more protein and lipid than carbohydrate. Both female and male mountain pine beetles use lipids and carbohydrates as energy sources during flight.
- There is variation in the energy substrates used by male and female mountain pine beetles during flight.

Energy use by the mountain pine beetle (Coleoptera: Curculionidae: Scolytinae) for dispersal by flight

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Abstract

The mountain pine beetle, *Dendroctonus ponderosae* Hopkins is a major native pest of pine (*Pinus* Linnaeus (Pinaceae)) in western North America. Host colonization by mountain pine beetle is associated with an obligatory dispersal phase, during which beetles fly in search of a suitable host. Mountain pine beetles use stored energy from feeding in the natal habitat to power flight before host colonization and brood production. Lipids fuel mountain pine beetle flight, however, it is not known if other energy sources are also used during flight. Here, we compare the level of energy substrates, proteins, carbohydrates, and lipids of individual mountain pine beetles flown on flight mills with un-flown control beetles. We use a colorimetric method to measure the entire metabolite content of each individual beetle. This study reveals that mountain pine beetles are composed of more protein and lipid than carbohydrate. Both female and male mountain pine beetles use lipids and carbohydrates as energy sources during flight. There is variation between sexes, however, in the energy substrates used for flight. Male mountain pine beetles use protein, in addition to lipids and carbohydrates, to fuel flight, while protein content is not different between flown and control females.

Key words

Dendroctonus ponderosae, metabolites, lipids, carbohydrates, proteins, trehalose, glucose, glycogen, insect dispersal, insect flight

Introduction

Insect dispersal is linked to physiological traits of individuals. Flight-capable insects have functional wings and flight muscles, and a large amount of fuel to power flight (Clobert, 2012). Insect flight activity demands a lot of energy, during which the metabolic rate increases 50- to 100-fold (Beenakkers *et al*., 1984), over non-flight metabolism. Movement to and from foraging sites and to new habitats can cause energy allocation trade-offs between dispersal and reproductive output (Zhao and Zera, 2002). Insect flight muscles contain small amounts of energy reserves and the energy required to fuel extended flight is provided by energy substrates circulating in the hemolymph, which are constantly replenished by the fat body (Beenakkers *et al*., 1984). Insects store energy reserves in the form of glycogen and triglycerides in adipocytes in the fat body. Energy substrates (i.e. lipids, carbohydrates, proteins) that fuel insect flight exhibit high interspecific variation. Some insects use only one energy substrate to fuel flight while others exploit more than one source of energy during different stages of flight (Arrese & Soulages, 2010; Beenakkers *et al*., 1984).

Carbohydrates are the major substrate for flight in most species of Diptera and Hymenoptera while many species of Lepidoptera and Orthoptera use both carbohydrates and lipids (Beenakkers *et al*., 1984). Lipids and carbohydrates are gained through larval (Arrese & Soulages, 2010; Coll & Yuval, 2004) and/or adult feeding (Arrese & Soulages, 2010; Hanski *et al*., 2006). The carbohydrate trehalose, the main blood sugar in insects, is a general substrate for insect flight. Short-distance flyers, such as cockroaches, use trehalose as the major fuel for flight (Elliott *et al*., 1984), and long distance flyers (e.g. locusts, mosquitoes) use trehalose to initiate flight, but switch to lipid reserves for longer flights (Kaufmann & Briegel, 2004; Van der Horst *et al*., 1980). Long distance flyers (e.g. Lepidoptera and Orthoptera) mostly use lipids as the main energy source to fuel flight (Beenakkers *et al*., 1985; Canavoso *et al*., 2003; Elliott & Evenden, 2009; Gade & Auerswald, 2003; Kaufmann

& Briegel, 2004; Ziegler & Schulz, 1986). More than 90% of the lipids stored in the fat body are neutral lipids in the form of triglycerides (Bailey, 1975; Canavoso *et al*., 2001). The amino acid proline is synthesized from fatty acids in the fat body of insects, and is a source of energy in the flight muscles (Arrese & Soulages, 2010), especially in beetles (Goldsworthy & Joyce, 2001).

Bark beetles (Curculionidae: Scolytinae) use both carbohydrates and lipids to fuel flight (Byers & Liifqvist, 1989; Chen *et al*., 2010; Evenden *et al*., 2014; Kinn *et al*., 1994; Thompson & Bennett, 1971), but lipids are the major source and most of these are in the form of triglycerides (Kinn *et al*., 1994). Flight capacity of *Dendroctonus* beetles is related to body lipid content, in which individuals with higher lipid content fly faurther (Chen *et al*., 2011; Evenden *et al*., 2014; Kinn *et al*., 1994; Williams & Robertson, 2008) and longer (Chen *et al*., 2011; Hodges & Barras, 1974; Kinn *et al*., 1994; Williams & Robertson, 2008) than beetles with low body lipid levels. Carbohydrates are used by some *Dendroctonus* (Coleoptera: Curculionidae) species during flight initiation (Chen *et al*., 2010; Thompson & Bennett, 1971).

Mountain pine beetle, *Dendroctonus ponderosae* Hopkins is a major native pest of pine (*Pinus* Linnaeus (Pinaceae)) in western North America. Trees over an area of 18 million hectares have been killed by mountain pine beetle during the most recent outbreak which started in the late 1990s in western North America (Cullingham *et al*., 2011, Aukema *et al*. 2006www.nrcan.gc.ca). Host colonization by mountain pine beetle is associated with an obligatory dispersal phase (de la Giroday *et al*., 2012, Gray *et al*., 1972), during which beetles fly in search of a suitable host. Females are the pioneers in host location and colonization. Upon reaching a suitable host and initiating feeding, females release the aggregation pheromone, *trans*-verbenol, which attracts both sexes of beetle to initiate the mass attack of the tree (Pitman, 1968). Arriving males produce another aggregation

pheromone, *exo*-brevicomin, which attracts mostly females. As tree colonization progresses, both sexes produce the anti-aggregation pheromone verbenone and males produce frontalin to discourage further host colonization (Pureswaran *et al*., 2000). Mountain pine beetles use stored energy from feeding in the natal habitat during this obligatory flight period before brood production (Bentz, 2006). Lipids fuel mountain pine beetle flight (Evenden *et al*., 2014), however, it is not known if they use additional energy sources during flight. In mountain pine beetle, lipid content is lower in beetles that have flown on flight mills than in un-flown control beetles, and flight distance is negatively correlated with beetle lipid content remaining after flight (Evenden *et al*., 2014). Low levels of energy resources after the obligatory dispersal phase may affect beetle host colonization behaviour and subsequent reproduction (Wijerathna *et al*., 2019, Chiu *et al*., 2017, Reid *et al*., 2017, Chubaty *et al*., 20141409, ,-200914; Elkin & Reid, 2005, Kautz *et al*., 2014, Latty & Reid, 2010, Reid *et al*., 2017,Elkin & Reid, 2005 Wijerathna et al. 2018).

Here, we compare the energy budget of individual beetles flown in a flight mill bioassay to that of beetles not given the opportunity to fly. We used a colorimetric method to measure the energy budget of each individual beetle. Protein, carbohydrate and lipid content of individual mountain pine beetles were compared between the two treatment groups.

Materials and Methods

Beetles

Mountain pine beetle-infested lodgepole pine bolts were obtained from five different sites (3 trees/site) near Grande Prairie, AB (55.1699°N, 118.7986°W) in October 2013. One, 50 cm bolt from 1 m above the soil surface was cut from each tree. Bolts were transported to the laboratory at the University of Alberta where the ends were sealed with paraffin wax before storage at 5°C so that beetles could experience winter conditions (Lusebrink *et al*. 2013).

Beetle Flight Treatment

After removal from cold storage 4-6 months later, the infested bolts were placed at room temperature in separate 121 L bins made of opaque plastic and fitted with emergence jars. The emergent adult beetles were separated by sex (Lyon, 1958). Before flight treatment, beetles were stored at 4°C in microcentrifuge tubes (2.0 ml) with a piece of paper to provide a surface to which beetles could cling (Evenden *et al*., 2014). Beetles 3-5 days post emergence were weighed to the nearest 0.01 mg (Mettler Toledo, XS105, Columbus, OH) and prepared for flight by attaching a tether of 0.2-mm-diameter aluminum wire with a 0.4-mm-diameter loop to the beetle pronotum with Press-Tite Contact Cement (LePage, Mississauga, ON, Canada). Flight experiments were conducted in a controlled ~~environmental chamber~~flight mill room maintained at 24°C and 16L: 8D photoregime (621 lux from high flicker frequency fluorescent lights during the photophase). Tethered beetles were attached to the distal end of each flight mill arm by inserting the straight portion (2 cm) portion of the aluminum tether at an approximately. The beetles were flown for 23 h. The flight treatment was initiated 4 h after the beginning of the photophase. Males and females were flown on alternate days (n=3-15 per day) to avoid the influence of sensory cues from the opposite sex that might influence flight. As beetles propelled the mill arms, a magnetic sensor on each flight mill indicated the arm rotation of each mill to the computer. One revolution of the mill arm was 94.2 cm. The software (LabView, National Instruments Corporation, Austin, TX) output included number of revolutions, longest single flight and flight duration. The flight distance was calculated by multiplying the number of revolutions by 94.2 cm.

A random sample of beetles was selected to serve as control beetles. Control beetles were tethered in the same manner, but the tether was then removed from the beetle and beetles were kept individually in perforated microcentrifuge tubes (2.0 ml) during the flight

period in the same environmental chamber that housed the flight mills. Beetles were weighed and stored in 2-ml Eppendorf vials at -20°C immediately after flight treatment.

Biochemical Analysis

The colorimetric method was used to estimate the total energy budget of each individual mountain pine beetle in both treatments (flown and control) (Foray *et al*., 2012). Carbohydrates (glucose, glycogen and trehalose), lipids and proteins were measured for each individual beetle. The Bradford assay was used to determine the protein content (Fig. 1). Glucose and trehalose content were then determined using a hot anthrone reaction (van Handel, 1965; van Handel, 1985a). In the next step, glycogen was measured using another hot anthrone reaction. The lipid in each insect was assessed with the vanillin assay procedure (van Handel, 1985b). The vanillin assay was used instead of petroleum ether extraction (Evenden *et al*., 2014) because petroleum ether method only allows the extraction of lipids. The purpose of this study was to extract total energy budget from the same individual in a step vise procedure.

Biochemical experiments were carried out on 61 flown (n_{female}= 32, n_{male}= 29) and 43 un-flown (control) (n_{female}= 23, n_{male}= 20) beetles. To correct for a possible effect of body mass on energetic condition, each flown and control beetle was weighed to the nearest 0.01mg before the biochemical analysis. The metabolite content (µg) per insect body weight (mg) was calculated and the mean comparisons were conducted between flown and control beetles. The standard curves were set up to check for linearity (Foray et al., 2012) in each type of assay (ranges of R^2: protein curves= 0.96, carbohydrates curves= 0.92-0.95, lipid curves=0.94-0.95). Beetle weight before analyses ranged from 4 - 15.5 mg, which permitted the use of similar volumes of solutions as in Foray et al. (2012). Each insect was placed individually in a 2-ml Eppendorf tube containing a stainless steel bead and 180 µl of aqueous buffer solution (100 mM KH_2PO_4, 1mM dithiothreitol (DTT) and 1mM

ethylenediaminetetraacetic acid (EDTA)). Each individual beetle was crushed by shaking the tube for 30 s at 25 Hz (BIO 101, SAVANT).

Bradford assay for protein content

Samples were centrifuged separately (180*g* at 4°C) (Eppendorf 5415) and 2.5 µl of each supernatant was transferred into a 96-well microplate without removing the lipid layer from the surface of the supernatant. Two hundred and fifty µl of Bradford micro-assay reagent (B6916: Sigma) was added to each well and incubated at room temperature for 15-20 min. Protein concentration was determined spectrophotometrically at 595 nm (Molecular Devices, ThermoMax, California) using a dilution series of bovine serum albumin dissolved into the same buffer as the standard.

Hot anthrone reaction for carbohydrate content

After the completion of the Bradford assay, 20 µl of 20% sodium sulphate solution (S421-500: Fisher) was added to the homogenate, to dissolve all the carbohydrates. Then 2.5 µl of the extraction buffer solution was added to reach a final solution of 0.2 ml of 2% Na_2SO_4 which was mixed with 1500 µl of a chloroform-methanol solution (1:2 v/v) to solubilize the lipids and water soluble carbohydrates. Samples were vortexed and then centrifuged for 15 min at 180*g* and 4°C to separate glycogen from the supernatant. The supernatant was transferred into a new tube for subsequent analysis and the pellet was kept for determination of glycogen content. To determine glucose content, 150 µl of the supernatant was transferred into a different Eppendorf tube that was left to evaporate for approximately 50 min at room temperature until a volume of ~10 µl was reached. Two hundred and forty µl of 1.42 gl^{-} anthrone (AAA1911814: Fisher) reagent (Foray *et al*., 2012) was added to the tube and tubes were incubated for 15 min at room temperature. Tubes were further incubated at 90°C in a water bath for another 15 min. Absorbance of the solution was read at 625 nm by transferring 200 µl to a borosilicate microplate using D- glucose (D-16500:

Fisher) as the standard. Similarly, 150 µl of supernatant was used to determine the trehalose content. Seventy-five µl of 1N HCL was added to the tube and incubated at 90°C for 7 min (van Handel, 1985a). After this, 75 µl of 1N NaOH was added and incubated for another 7 min at 90°C in a water bath. Anthrone (500 µl) was added to the tubes and heated at 90°C in a water bath for another 17 min. Two hundred µl of the sample was used for the absorbance reading using D- trehalose (BP2687-10: Fisher) as the standard. Glycogen content of each insect was assayed by twice washing the pellets using 400 µl X 2 of 80% methanol. The washing steps included vortexing followed by centrifuging 5 min at 16000*g* and the supernatant was removed. Then 1 ml of anthrone was added to the pellet, followed by 15 min of incubation at 90°C. Samples were cooled on ice to stop the reaction and 200 µl was transferred to borosilicate microplate. Absorbance was read at 625 nm with glucose as the standard.

Vanillin assay for lipid content

First, 100 µl of the remaining supernatant was transferred to an Eppendorf tube and heated at 90°C until complete solvent evaporation. Ten µl of 98% sulphuric acid (SA818-1: Fisher) was added to the tube and was incubated at 90°C for 10 min in a water bath. After cooling, 190 µl of vanillin (1.2 g/l) (AC14082-1000) was added to the tube and incubated for 15 min at room temperature. Two hundred µl of the solution was then transferred to a borosilicate microplate and absorbance of lipids was measured spectrophotometrically at 525 nm using triolein (44895-U: Sigma) as the standard.

Data Analysis

Data were analyzed using R v. 3.1.1 2014.07.10 (R Core Development Team 2014). Initial models contained all explanatory variables and interactions. In all analyses, model simplification was achieved based on ANOVA hypothesis testing ($p < 0.05$) for full and

reduced models, until the most parsimonious model remained using backward model selection.

A generalized mixed effect~~linear~~ model analyzed the total energy budget of mountain pine beetle in the biochemical assay. The R package nlme was used to analyze the generalized mixed effect model (Pinheiro *et al*., 2018). The amount of the metabolites as a proportion of body weight was the dependent variable. The metabolite type (protein, lipid, carbohydrates; glucose, glycogen and trehalose), flight treatment and beetle sex were treated as fixed effects and tree bolt from which beetles emerged and individual beetle nested within each tree bolt were~~as~~ treated as ~~a~~ random factors. We report the results of the minimal model.

The energetic condition of experimental beetles was analyzed using separate generalized mixed effects models for each energy substrate (Pinheiro *et al*., 2018). The amount of lipid, proteins, and carbohydrates (glycogen, glucose, trehalose) was compared between flown and control beetles using separate models for each substrate (Table 1). The models specified each metabolite as a proportion of body weight as the dependent variable. The fixed independent factors ~~was~~ere flight treatment (flown, control) ~~and beetle sex~~ with tree bolt from which beetles emerged specified as a random factor. Beetle sex was occasionally used as a fixed factor of some models (Table 1). Response variables were not normally distributed. For this reason, gamma error distributions were used in each model (Table 1).

Results

The total metabolite content of flight-ready mountain pine beetle is composed of more protein (34.52± 2.53µg) and lipid (17± 1.22µg) than carbohydrate (0.26± 0.2µg) (χ^2~~F~~=3855.55~~231.5~~, df=2, p <0.001) (Fig. 2) (Table 2). An interaction of flight treatment and sex affects beetle protein content (χ^2=7.5003, df=1, p= 0.0061). ~~Flown m~~Males that have flown on the flight mills have lower protein content than control males (χ^2=6.9561, df=1, p=

0.0083), while protein content is not different between flown and control females (χ^2=0.4864, df=1, p= 0.4855) (Fig.3).

Both male and female mountain pine beetles flown on flight mills have less trehalose than control beetles (χ^2=17.8609, df=1, p= 2.38e-05) (Fig. 4), but trehalose content did not differ with beetle sex (χ^2=0.3166, df=1, p= 0.5731). Female mountain pine beetles flown on flight mills have lower glucose content compared to control females (χ^2=34.604, df=1, p= 4.037e-09) (Fig. 4). Glucose content does not differ between the sexes (χ^2=0.3030, df=1, p=0.5820). Glycogen content is marginally higher in flown mountain pine beetles compared to control beetles (χ^2=3.6654, df=1, p= 0.0555) (Fig. 4), but it does not differ between the sexes (χ^2=2.9071, df=1, p= 0.0882). Control beetles had more lipid per unit of body mass than flown beetles (χ^2=9.0554, df=1, p= 0.0026) (Fig. 5). Females had more lipid than males (χ^2=11.3039, df=1, p= 0.0008) in the current study (Fig. 5).

Discussion

This study reveals that the total metabolite content of flight-ready mountain pine beetle is composed of more protein and lipid than carbohydrate (Fig. 2). The high protein content probably reflects the fully developed state of the beetle flight muscles before host colonization (Atkins & Farris 1962). Here we reveal that flight in mountain pine beetle is fueled not only by lipids (Evenden *et al*. 2014), but carbohydrate and protein substrates are also used. Other bark beetles use both carbohydrates and lipids during flight (Byers & Liifqvist, 1989; Chen *et al*., 2011; Kaufmann & Brown, 2008; Thompson & Bennett, 1971; Van der Horst *et al*., 1980). The carbohydrate content of mountain pine beetle is lower compared to proteins and lipids content. Similarly, lower amount of carbohydrates was extracted from *Dendroctonus armandi* and from *Venturia canescens* compared to lipids following the vanillin assay extraction (Foray *et al*., 2012, Chen *et al*., 2011). There is variation between the sexes in the energy substrates used for flight in mountain pine beetle.

Males use protein as a fuel for flight, while protein content is not different between flown and control females. Male and female mountain pine beetles have similar proportions of functional proteins before host colonization, but starvation causes a shift in protein composition that varies by sex (Pitt *et al*., 2014). It remains to be tested if such a shift occurs as a result of flight.

Based on findings of the current study, both female and male mountain pine beetles use carbohydrates during flight. Carbohydrates fuel flight initiation in some bark beetles (Freidman, 1985), whereas lipids are used for sustained flight (Atkins, 1969). *Dendroctonus armandi* use carbohydrates in flight initiation, and flight performance decreases with starvation treatment (Chen *et al*., 2011). Males of *Dendroctonus pseudotsugae* Hopkins use carbohydrates during the initial dispersal process (Thompson & Bennett, 1971). Insects use trehalose at the initiation of flight (Kaufmann & Brown, 2008; Van der Horst *et al*., 1980) and mountain pine beetle might use trehalose to fuel take-off and for initial flight behaviours.

Bark beetles feed on phloem that contains glucose and fructose (Ilse & Hellgren 2007), and the glucose content of individual beetles increases with prolonged feeding (Chen *et al*. 2011). It is likely that female mountain pine beetles use glucose as a source of energy during flight. Similarly, *Dendroctonus armandi* Tsai and Li use carbohydrates to fuel flight, and glucose body content declines with the number of flights (Chen *et al*., 2011). Glucose content does not differ between the sexes in the mountain pine beetle or in *D. armandi* (Chen *et al*., 2011), but flight does not reduce glucose levels in male mountain pine beetles.

Glycogen content is marginally higher in flown mountain pine beetles compared to control beetles, but it does not differ between the sexes. Similarly, *D. armandi* does not use glycogen to fuel flight, as a considerable amount of stored glycogen was recovered in beetles after four days of flight assay (Chen *et al*., 2011). This is probably because stored carbohydrates are not efficient fuel sources for insect flight (Storey, 1985; Arrese &

Soulages, 2010). Glycogen is stored in adipocytes in the fat body along with triglycerides (Arrese & Soulages, 2010). Bark beetles may use lipids instead of glycogen to fuel flight, since the energy provided by lipid metabolism is greater than that of glycogen (Chen *et al*. 2011). Some bark beetles, however, appear to use glycogen to fuel long-distance migratory flight (Nêemec *et al*. 1993).

In the current study, the average lipid content of control female and male beetles was 304 ± 25µg and 102 ± 20µg respectively. Similar amount of lipids (female= 211.15µg, male= 175.14µg) were extracted from *D. armandi* using the same extraction method (Chen *et al*., 2011). The extracted lipid from mountain pine beetle using the vanillin-phosphoric acid reaction method is considerably less than the amount extracted using extraction with petroleum ether (Evenden *et al*., 2014, Lusebrink *et al*., 2013). In concurrence with our previous work (Evenden *et al*. 2014), mountain pine beetle use lipids to fuel the flight. Lipid is the main fuel used in flight of bark beetles, as beetles flown on flight mills have lower lipid content compared to un-flown control beetles in several species (Atkins, 1969; Evenden *et al*., 2014; William & Robertson, 2008; Thompson & Bennett, 1971). The energy level provided by lipids is much higher than carbohydrates (Chen *et al*., 2011), which may be why lipids are the major energy source used for flight in mountain pine beetle.

Females had more lipid than males in the current study and in previous work (Evenden *et al*. 2014). Higher lipid content of female mountain pine beetles may relate to their role as pioneers in the host colonization process (Pitman, 1968). Energy metabolism during flight is known to impact host finding and colonization behaviours (Atkins, 1966; Chubaty *et al*., 2009, 2014, Xu *et al*., 2016) and subsequent reproduction (Elkin & Reid, 2005) in bark beetles. Females need to be in good condition with adequate fat stores to successfully colonize well-defended hosts (Reid *et al*. 2017). The decline of fat reserves as a result of flight might influence mountain pine beetle tolerance to monoterpenes. Detoxification of

monoterpenes is costly; mountain pine beetles exposed to (R)-(+)-Limonene have reduced lipid content (Reid *et al*. 2017). Individual mountain pine beetles with low lipid reserves are less selective and accept poorer quality host trees sooner than do individuals with high-lipid reserves that are capable of continued dispersal (Chubaty *et al*., 2009, 2014; Latty & Reid, 2010). The reduction of adult feeding due to monoterpene tree defenses (Xu *et al*., 2016) can also affect gallery construction behaviour of incoming adults. Poor body condition after dispersal may contribute to beetle mortality. Individual traits such as body size, body condition, including fat content, and body mass positively correlate with beetle survivorship in monoterpene-rich environments (Chiu *et al*., 2017, Reid *et al*., 2017).

Fat use during flight may also affect host colonization through a direct influence on beetle pheromone communication. The biosynthetic pathways of pheromones occur in the fat body and the midgut. Starved beetles have lower expression of genes involved in pheromone production than fed beetles (Keeling *et al*., 2016, Nadeau *et al*., 2017, Song *et al*., 2014, Tittiger & Blomquist, 2016). It is yet to be determined if flight influences production or response to pheromone signals in the mountain pine beetle.

Protein is used as a fuel for flight only in male mountain pine beetles. In the spring, mountain pine beetle larvae have increased levels of nervous system proteins, which could be for preparation of adult activities such as dispersal by flight and the detection of semiochemicals (Bonnett *et al*., 2012). Both sexes of adult mountain pine beetles have functionally similar proteins in similar quantities prior to host colonization (Pitt *et al*., 2014). Early in the host colonization process, fed males have less protein involved in muscle and cellular structuring compared to females which may indicate that males access flight muscle tissues for energy during early host colonization (Pitt *et al*., 2014). Males may rely to a certain degree on proteins to provide energy for flight, because they have less lipid stores than females. Further studies are needed to explain how mountain pine beetles use proteins,

lipids and carbohydrates during the different stages of flight. These differences in energy metabolism during dispersal may influence subsequent reproduction (Elkin & Reid, 2005; Pitt *et al*., 2014) and might change host acceptance behaviours (Chubaty *et al*., 2009, 2014; Latty & Reid, 2010) of mountain pine beetle in its expanded range.

There is a link between bark beetle energy content and dispersal capacity. The flight distance achieved by mountain pine beetle negatively correlates with lipid content after flight (Evenden *et al*., 2014). Dispersal from the natal host is further for individuals in physiologically good condition with high energy levels, and these individuals are more efficient in energy consumption (Kautz *et al*., 2014). In *D. armandi*, total flight distance and time spent flying decrease with a reduction in body lipid content but these flight parameters are not related to glucose or glycogen content (Chen *et al*., 2011). *Dendroctonus pseudotsugae* with high fat reserves fly further and longer than beetles with low initial fat reserves (Williams & Robertson, 2008). The lipid content of male and female *D. frontalis* after flight is negatively correlated with beetle flight duration and distance (Hodges & Barras, 1974, Kinn *et al*., 1994).

Mountain pine beetles will experience new hosts and environmental conditions with range expansion into the boreal forest of Canada (Cullingham *et al*., 2011), which may change energy metabolism during flight. The dispersal period of mountain pine beetle is shorter and later in northern compared to southern regions in Alberta, Canada (Bleiker & Hezewijk, 2016). Variation in flight phenology may alter beetle energy use during flight and beetle establishment in novel habitats. Mountain pine beetle larval survival increases when the fatty acid composition of the host tree is compatible with that of the larvae (Ishangulyyeva *et al*., 2016). This indicates that an incompatibility of fatty acids in the diet may influence mating, fertility and survival of the insect. Reduction of certain types of fatty acids during dispersal may influence the fatty acid compatibility with the novel host and

affect adult reproduction in the new host. Reduction of energy resources following starvation increases the bacterial diversity in *D. armandi* (Hu *et al*., 2016), which may increase nitrogen fixation in low nutritional bark environments and influence fitness and reproduction.

This study reveals the total energy budget of mountain pine beetle and the energy reserves used to power flight. Mountain pine beetle primarily uses lipids to fuel flight but carbohydrates are also used and protein is a flight substrate in males. Energy metabolism during flight under natural conditions may differ from the controlled conditions used in this study. Future studies should consider energy use during flight in natural habitats as habitat quality and environmental variables may change the flight capacity and energy expenditure of beetles. Knowledge of beetle energy metabolism during flight is important for modelling of beetle dispersal because flight capacity and propensity may differ with energetic condition. Beetles in physiologically good condition may be better competitors and better able to bear the cost of dispersal.

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applicable laws and rules set forth by provincial and federal governments and the University of Alberta and all necessary permits were held when the research was conducted.

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Table 1: ~~Statistical~~ Generalized mixed effect models used in analysis of total energy budget and metabolite content of mountain pine beetles. Each model includes dependent variables, fixed factors, random factors and the interaction between fixed factors used in each test. The symbol * indicates interactions between fixed factors and symbol + indicates no interactions between fixed factors. Gamma distribution was used in each model.

Response variable	**Model**	**Fixed effects**	**Random effects**	**Data family**
Metabolite weight/ total body weight	1	metabolite type*flight treatment* sex	bolt/ individual beetle	Gamma
Total protein content	2	flight treatment* sex	bolt	Gamma
Male protein content	3	flight treatment	bolt	Gamma
Female protein content	4	flight treatment	bolt	Gamma
Total glucose content	5	flight treatment * sex	bolt	Gamma
Female glucose content	6	flight treatment	bolt	Gamma
Male glucose content	7	flight treatment	bolt	Gamma
Gamma Total trehalose content	8	flight treatment+ sex	bolt	Gamma
Total glycogen content	9	flight treatment+ sex	bolt	Gamma
Total lipid content	10	flight treatment+ sex	bolt	Gamma
~~**Experiment**~~		~~**Models**~~		
	~~*General linear model*~~			

~~Total energy budget~~	~~M1= (metabolite weight as a fraction of body weight ~ metabolite type*flight treatment* sex, family= Gamma)~~
	~~*Generalized mixes effects models*~~
~~Metabolite content~~	~~M2= (protein ~ flight treatment* sex, random= Bolt, family= Gamma)~~
	~~M3= (male protein content ~ flight treatment, random= Bolt, family= Gamma)~~
	~~M4= (female protein content ~ flight treatment, random= Bolt, family= Gamma)~~
	~~M5= (glucose ~ flight treatment* sex, random= Bolt, family= Gamma)~~
	~~M6= (female glucose content ~ flight treatment, random= Bolt, family= Gamma)~~
	~~M7= (male glucose content ~ flight treatment, random= Bolt, family= Gamma)~~
	~~M8= (trehalose ~ flight treatment+ sex, random= Bolt, family= Gamma)~~
	~~M9= (glycogen ~ flight treatment+ sex, random= Bolt, family= Gamma)~~
	~~M10= (lipid ~ flight treatment+ sex, random= Bolt, family= Gamma)~~

568 Table 2: Statistical results of biochemical assay of mountain pine beetles. Symbol * indicates interactions between fixed factors.

Dependent variables	**Model**	**Independent variables**	**Statistical results**
Total energy content	M1	metabolite type*flight treatment* sex	χ^2F=3855231.55, df=2, -p <0.001
Protein	M2	Flight treatment* sex	χ^2=7.5003, df=1, p=0.0062
	M3	Flight treatment (male only)	χ^2=6.951, df=1, p=0.0083
	M4	Flight treatment (female only)	χ^2=0.4864, df=1, p=0.4855
Glucose	M5	Flight treatment * sex	χ^2=9.2923, df=1, p=0.0023
	M6	Sex	χ^2=0.3030, df=1, p=0.5820
	M7	Flight treatment (female only)	χ2=34.604, df=1, p= 4.037e-09
Trehalose	M8	Sex	χ^2=0.3166, df=1, p=0.5731
		Flight treatment	χ^2=17.8609, df=1, p=2.38e-05
Glycogen	M9	Sex	χ^2=2.9071, df=1, -p=0.0882
		Flight treatment	χ^2=3.6654, df=1, p=0.0555
Lipid	M10	Sex	χ^2=11.0554, df=1, -p=0.0008
		Flight treatment	χ^2=9.0054, df=1, p=0.0026

570 **Table and Figure legends**

571 **Table 1:** Statistical models used in analysis of total energy budget and metabolite content of
572 mountain pine beetles. Each model includes dependent variables, fixed factors, random factors
573 and the interaction between fixed factors used in each test. The symbol * indicates interactions
574 between fixed factors and symbol + indicates no interactions between fixed factors. Gamma
575 distribution was used in each model.

576 **Table 2:** Statistical results of biochemical assay of mountain pine beetles. Symbol * indicates
577 interactions between fixed factors.

578 **Figure 1:** Successive steps used for extraction of proteins, soluble carbohydrates, glycogen
579 and lipids from individual mountain pine beetles. Reagents used for each extraction are given
580 in parentheses. Method adapted from Foray *et al*., (2012).

581 **Figure 2:** The proportional metabolite content of flown and un-flown (control) female and
582 male mountain pine beetles. Proteins, lipids and carbohydrates were extracted from each
583 individual beetle sample using a colorimetric method. Data were analyzed using a generalized
584 mixed effects model. In the box plots, the lower and upper ends of the boxes represent the 25th
585 percentile and 75th percentile respectively. The black line within the box marks the median.
586 The whiskers are the two lines outside the box that extend to the highest and lowest
587 observations. The open circles represent the outliers.

588 **Figure 3:** Mean protein content as a proportion of individual body weight, determined for
589 female and male mountain pine beetle (n=20-32) under different flight conditions. Mountain
590 pine beetles were flown on flight mills for 23 h. The proteins were extracted from flown and
591 un-flown (control) beetles using a Bradford assay. Data were analyzed using a generalized
592 mixed effects model. Raw data are plotted.

593 **Figure 4:** Carbohydrates as a proportion of individual body weight, determined for female and
594 male mountain pine beetle (n=20-32) under different flight conditions. Mountain pine beetles

595 were flown on flight mills for 23 h. The glucose, trehalose and glycogen were extracted from
596 flown and un-flown beetles using a Hot anthrone reaction. Data were analyzed using
597 generalized mixed effects models for each carbohydrate. Raw data are plotted.

598 **Figure 5:** Mean lipid content as a proportion of individual body weight, determined on female
599 and male mountain pine beetle (n=20-32) under different flight conditions. Mountain pine
600 beetles were flown on flight mills for 23 h. The lipids were extracted from flown and un-flown
601 (control) beetles using a Vanillin assay. Data were analyzed using generalized mixed effects
602 models. Raw data are plotted.