

THE
CANADIAN ENTOMOLOGIST



CAMBRIDGE
UNIVERSITY PRESS

**Testing for trade-offs between flight and reproduction in
the mountain pine beetle (Coleoptera: Curculionidae) on
two pine hosts**

Journal:	<i>The Canadian Entomologist</i>
Manuscript ID	TCE-ART-2018-002.R3
Manuscript Type:	Original Article
Date Submitted by the Author:	n/a
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Abstract:	Mountain pine beetle, <i>Dendroctonus ponderosae</i> Hopkins (Coleoptera: Curculionidae: Scolytinae) adults fly to disperse before host colonization. The effect of flight on reproduction was tested by comparing the number and quality of offspring from beetles flown on flight mills to that of unflown control beetles. Beetles reproduced in bolts of their native host, lodgepole pine (<i>Pinus contorta</i> Dougl. ex. Loud. var. <i>latifolia</i> (Pinaceae)), or a novel host, jack pine (<i>Pinus banksiana</i> Dougl. (Pinaceae)). Bolts infested by control beetles produced more offspring overall than bolts with flown beetles. The effect of pine species on the number of offspring produced per bolt varied by individual tree. Flown adults produced fewer offspring compared to control parents in all bolts in jack pine regardless of the tree, but tree-level variation was visible in lodgepole pine. An interaction between flight treatment and tree host affected beetle body condition. More offspring emerged from jack pine, but higher quality offspring emerged from lodgepole pine. The offspring sex ratio was female-biased regardless of parental flight treatment. This study reveals trade-offs between flight and reproduction in mountain pine beetle as measured at the level of the bolt.

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1 In Preparation for: *The Canadian Entomologist*

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4 Testing for trade-offs between flight and reproduction in the mountain pine beetle (Coleoptera:
5 Curculionidae) on two pine hosts

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15

16 **Abstract**

17 Mountain pine beetle, *Dendroctonus ponderosae* Hopkins (Coleoptera: Curculionidae:
18 Scolytinae) adults fly to disperse before host colonization. The effect of flight on reproduction
19 was tested by comparing the number and quality of offspring from beetles flown on flight mills
20 to that of unflown control beetles. Beetles reproduced in bolts of their native host, lodgepole pine
21 (*Pinus contorta* Dougl. ex. Loud. var. *latifolia* (Pinaceae)), or a novel host, jack pine (*Pinus*
22 *banksiana* Dougl. (Pinaceae)). Bolts infested by control beetles produced more offspring overall
23 than bolts with flown beetles. The effect of pine species on the number of offspring produced per
24 bolt varied by individual tree. Flown adults produced fewer offspring compared to control
25 parents in all bolts in jack pine regardless of the tree, but tree-level variation was visible in
26 lodgepole pine. An interaction between flight treatment and tree host affected beetle body
27 condition. More offspring emerged from jack pine, but higher quality offspring emerged from
28 lodgepole pine. The offspring sex ratio was female-biased regardless of parental flight treatment.
29 This study reveals trade-offs between flight and reproduction in mountain pine beetle as
30 measured at the level of the bolt.

31

32 Key words: flight, dispersal, offspring body condition, lodgepole pine, jack pine, bark beetle

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33 **Introduction**

34 Dispersal is a mechanism through which an organism can increase its fitness through movement
35 to a habitat better suited for reproduction (Bowler and Benton 2005). The process of dispersal
36 can be energetically costly (Zera and Harshman 2001, Harshman and Zera 2007, Zera 2009) and
37 may reduce the resources available for subsequent reproduction (Hanski *et al.* 2006). This is
38 particularly important for taxa that rely heavily on energy acquired as a juvenile to fuel adult
39 flight (Thomas 1988). Energy use during insect flight decreases subsequent reproductive output
40 through a reduction in the size or number of eggs in many species (Isaacs and Byrne 1998, Fox
41 and Czesak 2000, Elkin and Reid 2005, Gu *et al.* 2006, Zhang *et al.* 2009, Gibbs and Dyck 2010,
42 Guerra 201, Elliott and Evenden 2012, Steenman *et al.* 2013, Duthie *et al.* 2014). Compensation
43 of energy used in flight, however, can also occur by post-dispersal feeding (Niitepõld and Boggs
44 2015).

45 Bark and ambrosia beetles (Coleoptera: Curculionidae: Scolytinae) are interesting
46 taxa for studying physiological trade-offs between reproduction and flight, because adults
47 undergo an obligatory flight to locate suitable hosts for brood production (Wood 1982). Aerial
48 dispersal can occur over long distances through flight aided by wind (Jackson *et al.* 2008), or
49 over short distances through self-sustained flight (Robertson *et al.* 2007). Dispersal distance is
50 linked to both beetle physiology (Atkins 1966, Atkins 1969, Thompson and Bennett 1971, Jactel
51 1993, Williams and Robertson 2008, Chen *et al.* 2011, Evenden *et al.* 2014), and to the number
52 and distribution of suitable host trees on the landscape (Robertson *et al.* 2007).

53 The mountain pine beetle *Dendroctonus ponderosae* Hopkins (Coleoptera:
54 Curculionidae: Scolytinae), is a native bark beetle that colonizes pine species in western North
55 America. Its principal host is lodgepole pine (*Pinus contorta* Dougl. ex. Loud. var. *latifolia*).

56 The mountain pine beetle has killed trees over an area of 18 million hectares during the most
57 recent outbreak, which began in the mid-1990s in western North America (Safranyik *et al.*
58 2010). During this outbreak, the range of mountain pine beetle reached the northern Rocky
59 Mountains in Canada due to favourable climatic factors and long-distance dispersal aided by the
60 wind (Jackson *et al.* 2008, de la Giroday *et al.* 2011, de la Giroday *et al.* 2012). In its expanded
61 range, the mountain pine beetle has successfully colonized a novel host, jack pine (*P. banksiana*
62 Lamb.) on the western edge of the boreal forest in Alberta (Cullingham *et al.* 2011). The
63 defensive chemical profile (Clark *et al.* 2014, Lusebrink *et al.* 2016) and the nutritional content
64 of jack pine are different from that of the historic host, lodgepole pine (Ishangulyyeva *et al.*
65 2016, Lusebrink *et al.* 2016). These differences between jack and lodgepole pine may alter the
66 colonization process (Erbilgin *et al.* 2014) and change the reproductive success of the mountain
67 pine beetle in its expanded range.

68 Like other bark beetles, adult mountain pine beetles feed on the natal host before
69 emergence (Elkin and Reid 2005) and may use stored energy during the obligatory flight period
70 before brood production. Evenden *et al.* (2014) found that lipids, at least in part, power beetle
71 flight, as measured on laboratory flight mills. The energy deficit that results from flight may be
72 partially offset by adult feeding during colonization of the new host (Elkin and Reid 2005).
73 Flight, however, could indirectly affect reproduction because activities required for successful
74 host colonization after flight are energetically costly (Reid *et al.* 2017). The male-produced
75 aggregation pheromone, *exo*-brevicommin, is synthesized in the fat body (Song *et al.* 2014), and
76 pheromone titre may be reduced in beetles with less fat following dispersal. Reduced fat reserves
77 may also directly reduce the reproductive potential and offspring fitness of mountain pine beetle.
78 Female mountain pine beetles with low energy reserves produce small eggs (Elkin and Reid

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79 2005), which may result in small offspring that are more susceptible to overwintering mortality
80 than large offspring (Lachowsky and Reid 2014). As differential overwintering mortality
81 between the sexually size-dimorphic mountain pine beetle contributes to the typical female-
82 biased adult sex ratio in this species (Lachowsky and Reid 2014), it is possible that maternal
83 energy used during flight could influence offspring sex ratio and impact population dynamics of
84 this species. Although previous rearing experiments in many pine species have found variable
85 effects of host characteristics on the reproductive output of mountain pine beetle (Amman 1982,
86 Langor 1989, Cerezke 1995, Cale *et al.* 2015, Esch *et al.* 2016, Lusebrink *et al.* 2016), none have
87 explored the effect of dispersal activity of adults on subsequent reproduction in different pine
88 hosts host.

89 Here we hypothesize that obligatory dispersal by flight for host colonization implicates a
90 trade-off with reproduction in the mountain pine beetle. We examine the influence of flight on
91 subsequent reproductive capacity and offspring fitness of mountain pine beetles in two pine
92 hosts. We predict energy use during flight will reduce the reproductive output of mountain pine
93 beetles. We further test the hypothesis that beetle physiological state influences reproductive
94 capacity differentially in different pine hosts. We predict that beetle condition will be more
95 important for reproduction in lodgepole pine than jack pine. Differences in defensive and
96 nutritional chemistry of the two hosts may interact with investment in reproduction to affect the
97 number and quality of the offspring.

98

99

Materials and methods

100 Beetles

101 Mountain pine beetle-infested lodgepole pine bolts (n=five/site) were obtained from three
102 sites near Grande Prairie, AB, Canada (55.1699°N; 118.7986°W) in October 2011. One ~50 cm
103 bolt was cut from each tree from 1 m above the soil surface. The cut ends of each bolt were
104 sealed with paraffin wax and housed at 5°C for 4 to 6 months to expose beetles to an overwinter
105 cold period. Uninfested lodgepole and jack pine bolts were obtained from Edson (53.5855°N;
106 116.4429°W) and Lac La Biche (54.7696°N; 111.9725°W), AB, respectively, in May 2012. Four
107 uninfested bolts (~70 cm long) (Table 1) were obtained from each of three trees at a single site
108 for each of the pine species. Bolts were transported to the laboratory at the University of Alberta
109 where the ends were sealed with paraffin wax before storage at 5°C until use.

110 Parental Beetle Flight Treatment

111 Infested bolts were removed from cold storage and placed at 24°C in separate 121 L bins
112 made of opaque plastic and fitted with glass emergence jars starting in April 2012. Removal of
113 bolts from cold storage was staggered to manage the number of beetles emerging at a given time.
114 The emergent adult beetles were separated by sex (Lyon 1958) and stored at 4°C in
115 microcentrifuge tubes (2 mL) with a piece of paper to provide a surface to which beetles could
116 cling (Evenden *et al.* 2014). Beetles were weighed to the nearest 0.0001 g (Mettler Toledo,
117 XS105, Columbus, OH) before flight. Age after emergence influences flight capability of the
118 mountain pine beetle (Evenden *et al.* 2014) and therefore beetles were flown 5 to 7 days post
119 emergence. Beetles were prepared for flight by attaching a tether to the pronotum (Evenden *et al.*
120 2014). Flight experiments were conducted in a controlled environmental chamber maintained at
121 24°C and a 16L:8D photoperiod (621 lux during the photophase). The tethered beetles were
122 attached to the mill for 23 h. The flight assay was initiated 4 h after the beginning of the light

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123 period. Males and females were flown on alternate days (n=3-15 beetles per day) to avoid
124 exposure to chemical cues from the opposite sex, which might affect flight behaviour. The
125 software (LabView, National Instruments Corporation, Austin, TX) output included number of
126 revolutions, longest single flight and flight duration. The flight distance and duration of flown
127 parent beetles subsequently introduced into the two pine hosts were compared using two-sample
128 t-tests (R v. 3.1.1 2014.07.10 (R Core Development Team 2014)).

129 A random sample of beetles across the experiment served as control beetles. Control
130 beetles were initially tethered in the same manner as flown beetles, but the tether was then
131 removed and beetles were kept individually in a perforated microcentrifuge tube (2.0 mL), and
132 provided with a slip of paper to settle on. Control beetles were positioned in the same
133 environmental chamber that housed the flight mills during the flight period.

134 **Bolt Infestation and Offspring Rearing**

135 Beetles subjected to the flight treatment were removed from the tether immediately after
136 each flight period. Both flown and control adults were weighed and stored at 5°C for a day.
137 Control and flown beetles were introduced into separate uninfested lodgepole pine and jack pine
138 bolts (Table 1). Four bolts of each tree species received beetles from each treatment. Each
139 treatment included at least one bolt from three different trees of the same species. Phloem width
140 was measured for each bolt at three different locations. Pairs of male and female beetles were
141 introduced equidistantly (~10 cm apart) around the base of each bolt in microfuge tubes. A
142 female beetle was introduced, followed by a male beetle after the female had entered the bolt.
143 The number of pairs introduced per bolt (6-9 pairs) was based on the calculated surface area of
144 each bolt to control for phloem resource available per breeding pair (Table 1). Beetles flown on
145 the same day were distributed among different bolts during the introduction process. This

146 process was repeated three times between June-August 2012 until four bolts of each tree species
147 were infested with flown or control beetles. A total of 16 bolts were infested. Dead beetles or
148 beetles that did not enter the bolt within 48 h were replaced with beetles from the same
149 experimental treatment until pair establishment was successful. Infested bolts were kept for three
150 weeks at 24°C to allow for beetle mating, egg-laying and initial larval development of the
151 offspring. Bolts were then transferred to cold storage (5°C) for at least one month to provide
152 appropriate conditions for beetle development (Lusebrink *et al.* 2013).

153 Following a month of cold storage, bolts were handled in the same manner to rear out the
154 offspring beetles as described for the parental generation above. Offspring emergence began in
155 February 2013 and the offspring were counted and separated by sex. Pronotum width and body
156 length of the emergent offspring were measured using an ocular micrometer on a dissecting
157 microscope (6.3 X magnification) to the nearest 0.01 mm. Body size of individual beetles was
158 estimated by calculating the area of an ellipsoid (Knud Thompson Formula $S \approx 4\pi [apbp + apcp +$
159 $bpcp]1/p$) in which a=b=half the pronotum width, c=half the length of the beetle and p=1.6075
160 (Michon 2009, Xu et al. 2009). Beetles were weighed and stored at -20°C for subsequent fat
161 extraction. Following emergence, bolts were peeled to determine the number of beetle pairs that
162 successfully established breeding galleries and to measure the length of the parental galleries
163 (Table 1).

164

165 **Fat Extraction and Offspring Condition**

166 Adult offspring were dried for 24 h at 60°C in an oven and each was weighed to the
167 nearest 0.0001 g. Beetles were then placed in perforated microcentrifuge tubes (0.2 mL) and
168 submerged in petroleum ether (Fisher Chemical, Fair Lawn, NJ) in a Soxhlet apparatus (45/50

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169 Pyrex; Fisher, Canada). After 8 h of fat extraction, the beetles were dried again at 60°C for 24 h
170 and re-weighed. Individual fat content was determined by subtracting the dry weight after fat
171 extraction from the initial dry weight. Offspring condition was calculated using a body condition
172 residual index that controls for body size (Elkin and Reid 2005) by regressing offspring fat
173 content against its body size. The residuals of the regression were used to create the residual
174 index.

175

176 **Statistical analyses**

177 The mean phloem width of the two pine species were compared using a two-sample t-test.
178 The bolt diameter of bolts from moth species was compared using a general linear model. Flight
179 capacity (flight distance and duration) of the parent beetles introduced to the two pine hosts was
180 compared with two sample t- tests. The effect of pine host and adult flight treatment on gallery
181 length was analysed using a general-mixed effect model (Table 2).

182 We used generalized mixed-effect models (Bates *et al.* 2015) with gallery length/gallery
183 initiation date per bolt specified as a random variable and Poisson error distributions to analyse
184 the effect of parental flight treatment, pine species and the tree that bolts were obtained from on
185 the number of offspring produced per bolt (Table 2). The effect of parental flight treatment and
186 pine species on offspring sex ratio was tested using a χ^2 test. Average offspring body size per bolt
187 was compared using a general-mixed effect model (Bates *et al.* 2015) with flight treatment, pine
188 species, offspring sex and tree specified as fixed factors and gallery length/gallery initiation date
189 per bolt specified as a random effect (Table 2). Offspring condition was assessed using the
190 offspring body condition residual index. A general-mixed effects model compared the body-
191 condition residual indices of offspring produced by flown and control beetles in the two species

192 of pine in which gallery length/gallery initiation date per bolt specified as a random factor (Table
193 2). Model simplification was achieved using anova comparisons and by comparison of AIC
194 values. The models were checked for homogeneity of variance using Levene's test and for
195 overdispersion using one sample Kolmogorov-Smirnov test in DARMA package (Hartig 2018).
196 Model residuals were checked for normality using the Shapiro-Wilk test. The model fit was
197 checked using pseudo R^2 values. A Tukey post hoc test was conducted to test the separation of
198 means of each model (R v. 3.1.1 2014.07.10 (R Core Development Team 2014)).

199 Results

200 The mean phloem width was similar between jack (0.74 ± 0.05 mm) and lodgepole pine
201 (0.81 ± 0.06 mm) bolts used for introduction of the flown and control parent beetles ($t_{13} = -0.85$,
202 $P=0.41$), but lodgepole pine bolts had a larger diameter on average (27.9 ± 1.36 cm) compared to
203 jack pine bolts (23.97 ± 1.15 cm) ($F_{1,12}=9.41$, $P=0.01$) (Table1). Average gallery length did not
204 differ between the two host tree species ($F_{1,12}=0.01$, $P=0.92$) and was not affected by adult flight
205 treatment ($F_{1,12}=1.09$, $P=0.36$) (Table1).

206 Pre-flight weight was similar for beetles subjected to the flight treatment (10.68 ± 0.30
207 mg) and those used as unflown control beetles (10.34 ± 0.31 mg) ($t_{155} = 0.67$, $P = 0.22$). The
208 flight capacity of the beetles ($n=77$) that were subsequently introduced to the two pine hosts was
209 similar between host species (flight duration: $F_{1,74}=0.31$, $P=0.58$; flight distance: $F_{1,74}=0.38$,
210 $P=0.54$), but females flew longer ($F_{1,74}=8.64$, $P=0.0044$) and further ($F_{1,74}=12.22$, $P=0.0008$)
211 than males. The average (\pm SE) flight duration of beetles was 3.20 ± 0.36 h and the average
212 flight distance was 5.61 ± 0.35 km. As expected, flown beetles lost significantly more weight
213 (1.2 ± 0.04 mg) during the bioassay compared to unflown control beetles (0.78 ± 0.003 mg)

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214 ($t_{181}=58.367$, $P<0.0001$). A total of 122 beetle pairs were introduced to the bolts. Ninety-two
215 pairs accepted the hosts, but only 80 pairs were successful in establishing galleries (Table 1). The
216 gallery length results should be interpreted with caution, as gallery construction by adult beetles
217 may not have been completed before the bolts were moved to 5°C.

218 There was an interaction between parental flight treatment and pine species that affected
219 the total number of offspring produced per bolt ($\chi^2_1=5.4416$, $P=0.0197$) (Fig. 1). Control beetles
220 produced more offspring than flown beetles in both pine hosts (Tukey post hoc test, $P<0.05$), but
221 more offspring emerged from jack than lodgepole pine ($\chi^2_1=14.8712$, $P=0.001$). There was a
222 significant interaction of flight treatment and tree that affected the total number of offspring
223 ($\chi^2_4=11.3744$, $P=0.0227$). In lodgepole pine, flown parents produced fewer offspring than control
224 parents in only one experimental bolt. In jack pine, flown parents produced fewer offspring than
225 control parents in all trees (Tukey post hoc test, $P<0.05$). An interaction of flight treatment and
226 tree affected the number of female offspring ($\chi^2_4=11.5055$, $P=0.0422$). Flown parents produced
227 fewer female offspring than control beetles in one of the trees in both hosts. More female
228 offspring emerged from jack pine than lodgepole pine ($\chi^2_1=14.81$, $P<0.0001$). An interaction
229 effect between flight treatment and tree influenced the number of male offspring ($\chi^2_2=29.7817$,
230 $P<0.0001$). Flown parents produced fewer male offspring than control parents in two jack pine
231 trees and one lodgepole pine tree (Tukey post hoc test, $P<0.05$). The sex ratio of offspring of
232 flown adults in lodgepole (1.93:1, female: male) was marginally more female biased than the sex
233 ratio of offspring of control beetles in lodgepole pine (1.3:1) ($\chi^2_1=3.44$, $P=0.05$). The sex ratio
234 did not differ by parental flight treatment ($\chi^2_1=1.8$, $P=0.17$) for offspring reared in jack pine
235 (flown: 1.87: 1; control: 1.5: 1, female:male).

236 Parental flight treatment did not influence offspring body size ($\chi^2_1=0.19947$, $P=0.1579$).
237 The pine species offspring were reared in also did not influence body size ($\chi^2_1=0.5348$,
238 $P=0.4646$). There was no effect of individual tree on the body size of offspring ($\chi^2_1=0.4090$,
239 $P=0.9817$). As would be expected, female offspring were larger than males ($\chi^2_1=303.9$, $P<$
240 0.0001). An interaction between pine species and flight treatment influenced the body condition
241 residual index of offspring ($F_{1,16}=5.2787$, $P=0.0472$). Offspring from flown adults had a slightly
242 lower body condition index compared to the offspring from control parents in lodgepole pine
243 (Tukey post hoc test, $P<0.05$) (Fig. 2). Control parents produced offspring with a slightly higher
244 body condition index in lodgepole pine than in jack pine. The body condition residual index did
245 not differ between male and female beetles ($F_{1,16}=1.2864$, $P=0.2745$) (Fig. 2).

246 Discussion

247 This study reveals an impact of flight on subsequent reproduction in the mountain pine
248 beetle. Beetles subjected to a flight treatment before inoculation into bolts produced fewer
249 offspring per bolt than control beetles. Individual tree effects, however, influenced the offspring
250 number produced by flown adults. Flown adults produced fewer offspring than control beetles
251 only in one lodgepole pine tree. In jack pine, flown adults produced fewer offspring than control
252 beetles in all three trees. It is not known, however, if offspring produced per female is affected
253 by flight treatment. In previous studies, reduced body condition induced by starvation of
254 mountain pine beetle adults did not impact the number of eggs laid by females (Elkin and Reid
255 2005). This suggests that flown beetles are able to compensate, at least in part, for lost energy
256 through feeding in the newly colonized tree (Elkin and Reid 2005). Female mountain pine
257 beetles can allocate resources to somatic condition or reproductive investment but this allocation
258 process is independent of beetle condition (Elkin and Reid 2005). In the few studies that directly

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259 test for an effect of flight on reproduction in other bark and ambrosia beetles, evidence for trade-
260 offs is equivocal (Biedermann *et al.* 2011, Fraser *et al.* 2014). Trade-offs between flight and
261 reproduction, however, is evident in other insects (Isaacs and Byrne 1998, Zhang *et al.* 2009,
262 Gibbs and Dyck 2010, Elliott and Evenden 2012, Duthie *et al.* 2014). Future studies on
263 reproductive trade-offs in the mountain pine beetle would benefit from assessment of offspring
264 production per female in live tree hosts (Esch *et al.* 2016).

265 The number of offspring produced per bolt was also influenced by the species of bolt that
266 beetles were reared in. More offspring emerged from jack than lodgepole pine. Studies to date
267 are highly variable with regard to host effects on reproductive output of mountain pine beetle.
268 Similar numbers of offspring per mated pair of adults emerge from bolts of lodgepole, jack and
269 red pine (*P. resinosa* Aiton) (Cale *et al.* 2015). Although establishment of egg galleries is greater
270 in lodgepole pine than whitebark pine (*P. albicaulis* Engelmann) bolts, both hosts are equally
271 suitable for brood production in terms of offspring number and offspring fat content (Esch *et al.*
272 2016). Naturally infested bolts of limber pine (*P. flexilis* James) produce more larvae with larger
273 body size compared to offspring produced in bolts of lodgepole pine, on a per bolt basis
274 (Cerezke 1995). Beetles had higher fecundity and produced more eggs in living stands of limber
275 pine compared to lodgepole pine (Langor 1989). Artificially infested lodgepole pine bolts
276 produced fewer offspring with smaller females as compared to four other pine host species
277 (Amman 1982). Similarly, beetles inoculated into lodgepole pine bolts in our study produced
278 fewer offspring compared to jack pine. Although brood production increases with the phloem
279 thickness (Amman 1986), phloem width, did not vary with host species in the current study.

280 Mountain pine beetles that emerge from jack pine contain higher fat reserves than those
281 from lodgepole pine (Lusebrink *et al.* 2016). Nutritional quality may differ between the two pine

282 hosts and affect the success of developing brood. Jack pine contains a higher concentration of
283 fatty acids compared to lodgepole pine (Ishangulyyeva *et al.* 2016). Reproductive success of the
284 ambrosia beetle *Pityophthorus lautus* Eichhoff (Coleoptera: Platypodidae) correlates with phloem
285 nitrogen and carbohydrate levels (Kirkendall 1983). Host defensive chemistry may also impact
286 brood development but further studies on mountain pine beetle reproduction in jack pine in
287 nature are required as jack pine monoterpene composition varies geographically with climatic
288 conditions, which may influence host susceptibility (Taft *et al.* 2015). The trees for each pine
289 species tested in the current study were selected from the same stand and differences in host
290 suitability between species revealed here may simply reflect stand and not pine species
291 differences.

292 Offspring body size was not affected by parental flight treatment. The reduction in fat
293 reserves during flight (Evenden *et al.* 2014) may be compensated for by feeding in the newly
294 colonized host (Elkin and Reid 2005). A significant interaction between flight treatment and pine
295 species influenced offspring body condition in this study. Offspring of control mountain pine
296 beetles have better body condition than offspring from flown beetles when reared in lodgepole
297 pine, but flight did not affect the offspring body condition in jack pine. The effect of flight on
298 offspring condition may vary with the host species. Control beetles produced offspring with
299 slightly higher body condition when reared in lodgepole pine than jack pine, suggesting that a
300 maternal effect may render offspring better able to resist the defenses produced by lodgepole
301 pine. Lodgepole pine produces higher levels of defensive monoterpenes than does jack pine
302 (Clark *et al.* 2014, Lusebrink *et al.* 2016). Beetles in good condition can survive higher
303 concentrations of monoterpenes compared to beetles in poor body condition (Reid and Prucell
304 2011, Manning and Reid 2013, Reid *et al.* 2017). Nutritional quality of the two hosts may also

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305 affect offspring condition. Total phloem nitrogen content is higher in lodgepole than jack pine
306 (Lusebrink *et al.* 2016), while jack pine contains higher concentrations of fatty acids compared to
307 lodgepole pine (Ishangulyyeva *et al.* 2016). The body condition residual index should, however,
308 be interpreted with caution because it may not predict energy stores of individual beetles (Kelly
309 *et al.* 2014).

310 The sex ratio of the offspring produced by both flown and unflown control beetles was
311 female-biased. The offspring of control beetles emerged in 1.3:1 female:male ratio from
312 lodgepole pine which was marginally lower than the 1.93:1 female:male ratio that emerged from
313 lodgepole pine infested with flown beetles. The flight treatment of parents did not influence the
314 sex ratio of offspring reared in jack pine as a 1.5:1 and 1.87:1 female: male ratio emerged from
315 bolts infested by control and flown parents, respectively. The sex ratio of offspring from flown
316 beetles is similar to the sex ratio of emergent mountain pine beetle recorded from naturally
317 infested trees (Reid 1958, Safranyik 1976, Amman 1984, Amman and Bartos 1991, Lachowsky
318 and Reid 2014). The female-biased sex ratio in natural conditions is most likely due to male
319 winter mortality during development (Lachowsky and Reid 2014, James *et al.* 2016). Additional
320 mechanisms such as body lipid content may contribute to the sex-ratio bias (Lachowsky and
321 Reid 2014) as cold tolerance depends on lipid content in bark beetles (Lombardero *et al.* 2000).
322 Both pre-flight (Reid and Purcell 2011, Graf *et al.* 2012) and post-flight (Evenden *et al.* 2014)
323 adult males have lower absolute and relative amounts of fat compared to females. Male larvae
324 may also have less fat, which would make them less tolerant to cold temperatures. The cold
325 conditions that mountain pine beetle offspring were subjected to in the current study (5°C),
326 however, would not be expected to induce much mortality, which may be why the sex-ratios
327 observed in our study were not as strongly female-biased as those typically observed in nature.

328 The slight difference in offspring sex ratios produced by control and flown adult beetles in
329 lodgepole pine may be related to body condition of the offspring. Flown beetles with less fat than
330 control beetles (Evenden *et al.* 2014) may produce smaller offspring (Elkin and Reid 2005).

331 Our results indicate that mountain pine beetles have physiological trade-offs between
332 flight and reproduction in terms offspring produced per bolt and offspring condition. This
333 suggests that prolonged adult dispersal may decrease beetle fitness despite the possibility of
334 locating a higher quality host, or unrelated mates (Chubaty *et al.* 2009). Further studies with
335 more trees from different stands are needed to assess the effects of flight on offspring fitness in
336 different host species. Pioneer female mountain pine beetles that fly long distances in search of
337 suitable hosts produce fewer offspring than non-pioneering females that join the aggregation
338 later in the dispersal period (Latty and Reid 2009). Beetles with a moderate level of energy,
339 however, are more likely to pioneer while beetles with smallest and greatest energy reserves
340 avoid pioneering (Latty and Reid 2010). It is not clear from this study whether the trade-off
341 between flight and reproduction exists on a per capita basis.

342 This, and other studies (Erbilgin *et al.* 2014, Lusebrink *et al.* 2016), show that the novel
343 jack pine host suitable for mountain pine beetle brood production using artificially infested bolts,
344 although offspring condition is better in the native lodgepole pine host. Studies that use naturally
345 infested pine bolts (Cerezke 1995) and live standing trees (Cullingham *et al.* 2011), also show
346 that jack pine is a suitable host for brood production. The physiological trade-offs between flight
347 and reproduction appear to vary with host species. Future research will be needed to understand
348 how the effect of energy use during flight on subsequent reproduction can be influenced by
349 environmental factors and affect the host colonization pattern in the expanding range of the
350 mountain pine beetle in the boreal forest.

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Acknowledgements

351
352 We thank Devin Letourneau of Alberta Agriculture and Forestry for bolt collection and
353 Caitlin Reich for carrying out the flight bioassay. This research was supported by a grant to
354 Maya Evenden from the Natural Science and Engineering Research Council of Canada (grant no.
355 NET GP 434810-12) to the TRIA Network, with contributions from Alberta Agriculture and
356 Forestry, fRI Research, Manitoba Conservation and Water Stewardship, Natural Resources
357 Canada - Canadian Forest Service, Northwest Territories Environment and Natural Resources,
358 Ontario Ministry of Natural Resources and Forestry, Saskatchewan Ministry of Environment,
359 West Fraser and Weyerhaeuser. Research presented in this manuscript was conducted in
360 accordance with all applicable laws and rules set forth by provincial and federal governments
361 and the University of Alberta and all necessary permits were held when the research was
362 conducted.

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For Peer Review

577

578 Table 1:

Bolt number	Host	Flight treatment	Surface Area of the bolt (m ²)	Bolt length (cm)	Bolt Diameter (cm)	Number of pairs introduced	Number of pair entrees	Number of galleries	Average gallery length \pm SE (cm)
1	Lodgepole pine	Flown	0.71	66.5	28.01	9	7	6	31.65 \pm 3.2
2			0.77	70	29.12	9	7	2	48.40 \pm 5.6
3			0.71	69	27.37	8	7	4	25.07 \pm 10.6
4			No data	No data	No data	6	3	3	43.60 \pm 2.2
5		Control	0.76	68	29.60	9	7	7	43.03 \pm 6.8
6			0.73	71	27.53	8	4	4	44.17 \pm 13.6
7			0.65	68	25.78	8	6	4	51.08 \pm 4.2
8			No data	No data	No data	8	5	5	36.83 \pm 5.2
9	Jack pine	Flown	0.63	76	22.91	7	6	4	19.62 \pm 6.2
10			0.50	70	20.05	7	6	6	55.48 \pm 4.7
11			0.67	70	25.78	8	6	6	35.08 \pm 9.1
12			No data	No data	No data	6	3	3	24.83 \pm 14.9
13		Control	0.66	70	25.46	8	6	5	41.60 \pm 5.2
14			0.73	71	21.96	7	6	6	45.57 \pm 10.7
15			0.56	69	27.69	7	7	7	50.86 \pm 5.2
16			No data	No data	No data	7	6	6	29.87 \pm 7.6

579

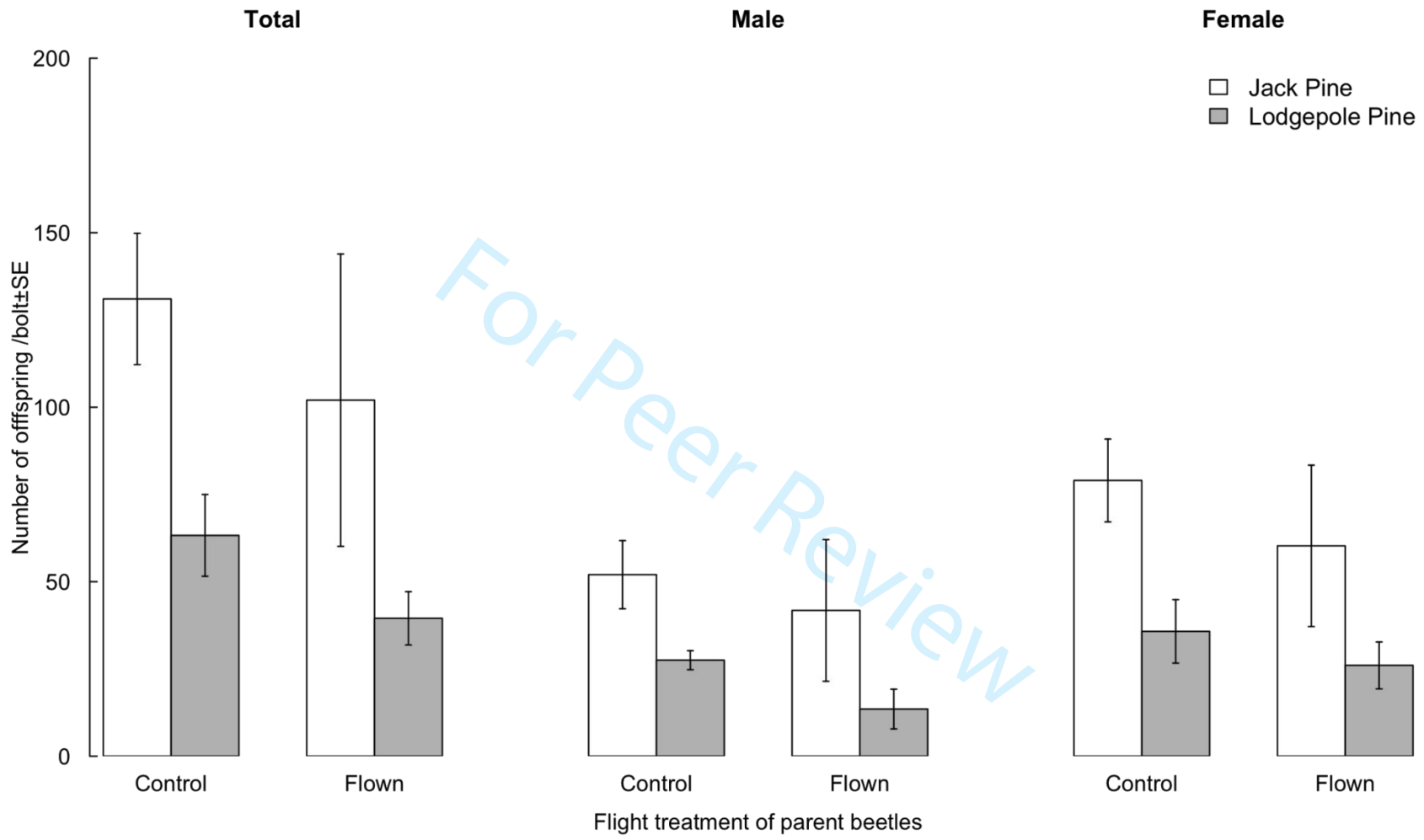
580

581

582 Table 2:

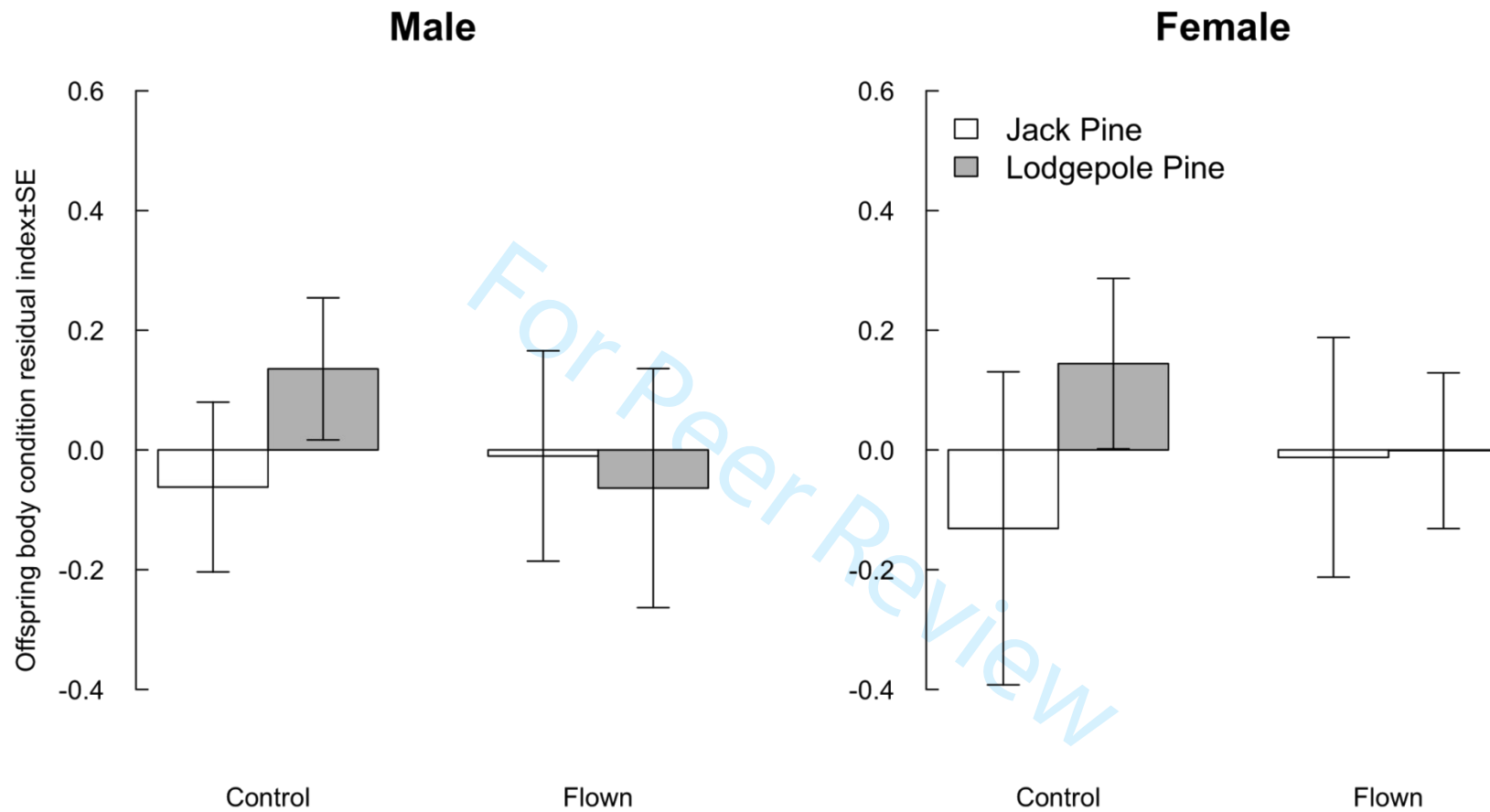
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Dependent variables	Fixed factors	Random factor
Gallery length	Flight treatment x host	Pine bolt
Total number of offspring per bolt	Flight treatment x host x tree	Average gallery length/Gallery initiation date per bolt
Number of female offspring per bolt	Flight treatment x host x tree	Average gallery length/Gallery initiation date per bolt
Number of male offspring per bolt	Flight treatment x host x tree	Average gallery length/Gallery initiation date per bolt
Offspring body size per bolt	Flight treatment x offspring sex x host	Average gallery length/Gallery initiation date per bolt
Body condition residual index	Flight treatment x offspring sex x host	Average gallery length/Gallery initiation date per bolt



583 Figure 1
584

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585

586 Figure 2:

587

Table captions

588 **Table 1:** Characteristics of lodgepole and jack pine bolts infested with control and flown
589 mountain pine beetles. Beetle establishment characteristics include the number of beetle pairs
590 introduced and those that successfully entered each bolt. The number of resulting galleries per
591 infested bolt and the average gallery length for each bolt is presented.

592

593 **Table 2:** Summary of the mixed-effect models used for analyses of average gallery length, total,
594 female and male offspring number, offspring body size and body condition residual index of
595 mountain pine beetles. Each model tests for effects of fixed factors and the interaction between
596 fixed factors with gallery initiation date and average gallery length per bolt specified as the
597 random factors in each case.

598

599

Figure Captions

600 **Figure 1:** Average number of offspring produced per bolt produced by flown and control parental
601 mountain pine beetles subsequently introduced to lodgepole pine and jack pine for reproduction.
602 Six to nine pairs of flown and control adult beetles were introduced to individual lodgepole pine
603 (n=4 per treatment) and jack pine (n=4 per treatment) bolts. Emergent male, female and total
604 number of offspring were counted (n=1405).

605 **Figure 2:** Body condition residual indices of male and female mountain pine beetle offspring
606 produced by flown and control parental beetles in lodgepole and jack pine (n=1384). Six to nine
607 pairs of parental beetles were introduced to lodgepole pine (n=4 per treatment) and jack pine
608 (n=4 per treatment) bolts.