

Successful colonization, reproduction, and new generation emergence in live interior hybrid spruce *Picea engelmannii* × *glauca* by mountain pine beetle *Dendroctonus ponderosae*

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- Abstract**
- 1 Although mountain pine beetle *Dendroctonus ponderosae* Hopkins are able to utilize most available *Pinus* spp. as hosts, successful colonization and reproduction in other hosts within the Pinaceae is rare.
 - 2 We observed successful reproduction of mountain pine beetle and emergence of new generation adults from interior hybrid spruce *Picea engelmannii* × *glauca* and compared a number of parameters related to colonization and reproductive success in spruce with nearby lodgepole pine *Pinus contorta* infested by mountain pine beetle.
 - 3 The results obtained indicate that reduced competition in spruce allowed mountain pine beetle parents that survived the colonization process to produce more offspring per pair than in more heavily-infested nearby pine.
 - 4 We also conducted an experiment in which 20 spruce and 20 lodgepole pines were baited with the aggregation pheromone of mountain pine beetle. Nineteen pines (95%) and eight spruce (40%) were attacked by mountain pine beetle, with eight (40%) and three (15%) mass-attacked, respectively.
 - 5 Successful attacks on nonhost trees during extreme epidemics may be one mechanism by which host shifts and subsequent speciation events have occurred in *Dendroctonus* spp. bark beetles.

Keywords coevolution, *Dendroctonus ponderosae*, host shift, *Picea engelmannii* × *glauca*, sympatric speciation.

Introduction

The host range of the mountain pine beetle *Dendroctonus ponderosae* Hopkins encompasses at least 13 native pine (*Pinus* spp.) hosts, including lodgepole pine *Pinus contorta* Dougl. ex Loud., ponderosa pine *Pinus ponderosa* Dougl. ex Laws., white pine *Pinus monticola* Dougl. ex D. Don, sugar pine *Pinus lambertiana* Dougl., whitebark pine *Pinus albicaulis* Engelm., jack pine *Pinus banksiana* Lamb., Coulter pine *Pinus coulteri* D. Don, foxtail pine *Pinus balfouriana* Balf., limber pine *Pinus flexilis* James, piñon pine *Pinus edulis* Engelm., single leaf pine *Pinus monophylla* Torr. & Frem., Rocky Mountain bristlecone pine *Pinus aristata* Engelm., and Great Basin bristlecone pine *Pinus longaeva* D.K. Bailey

(Keen, 1952; Furniss & Carolin, 1977; Amman *et al.*, 1990). It also attacks and successfully reproduces in some introduced pine species, suggesting an ability of this insect to utilize most available *Pinus* spp. hosts. Mountain pine beetle are also known to attack non-*Pinus* members of the Pinaceae [e.g. Douglas-fir *Pseudotsuga menziesii* (Mirb.) Franco, true firs *Abies* spp., larches *Larix* spp, incense cedar *Calocedrus decurrens* and spruce *Picea* spp.] when such trees are directly adjacent to a mass-attacked pine (Amman *et al.*, 1990). However, brood production and emergence of a new generation in such typical nonhosts has rarely been observed (Furniss & Schenk, 1969; Amman *et al.*, 1990), except under artificial laboratory conditions with portions of trees, rather than standing, living trees (Safranyik & Linton, 1983).

Recently, there have been reports of mountain pine beetle successfully attacking, colonizing and reproducing in mature, live interior hybrid spruce *Picea engelmannii* × *glauca* in the

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central interior of British Columbia, Canada. We conducted a detailed analysis of gallery parameters and new adult emergence in infested spruce and pine. In the present study, we present the results of that analysis, and the results of a pheromone-baiting trial, in the context of avoidance of competition, coevolutionary theory, host shifts and incipient sympatric speciation events.

Materials and methods

Analysis of mountain pine beetle-infested spruce and pine

On 10 April 2006, a landowner living near Prince George, British Columbia, Canada contacted us regarding two interior hybrid spruce on his property (53°56'25"N, 122°56'30"W), which he suspected were infested with mountain pine beetle. His property, and that of an adjacent landowner, had a mixed stand of mature lodgepole pine and interior hybrid spruce. The lodgepole pine in the surrounding area was in the midst of a heavy outbreak of mountain pine beetle. The spruce in the area, including the attacked spruce, had medium site indices (i.e. they were not unduly stressed by nutrient or moisture imbalances). Upon inspection, we found that parent adults and mid-instar larvae in the two spruce were probably mountain pine beetle. On 6 June 2006, we affixed four emergence traps to each of the two spruce and two emergence traps on two nearby mountain pine beetle-infested lodgepole pines, of similar diameter. Emergence traps were constructed from black, 32 per square inch mesh high density polyethylene screening (Synthetic Industries, Gainesville, Georgia) fitted with a collection jar, the lid of which was attached to the top of an inverted plastic soda pop bottle, which in turn had been secured to the mesh with staples and hot glue. In all cases, the traps were attached to the trees with staples around their perimeters to minimize insect escape from the traps. The collecting jars were filled with approximately 10 mL of a 50% propylene glycol:water mix. The emergence traps were emptied weekly until September, when the flight period of the insect was over. Voucher specimens of emerged beetles were identified as *D. ponderosae* Hopkins (R. Duncan¹, personal communication.) and were deposited at the Canadian Forest Service, Pacific Forestry Centre (Victoria, British Columbia) insect collection.

After maturation of the brood and complete emergence, the four trees were felled on 2 October 2006. Their heights and diameters at breast height (d.b.h.), respectively, were: spruce 1 (S1) = 14.96 m, 19.4 cm; spruce 2 (S2) = 22.86 m, 25.0 cm; pine 1 (P1) = 19.51 m, 22.3 cm; pine 2 (P2) = 19.69 m, 27 cm. The trees were bucked into bolts in the range 31–93 cm long and transported to the laboratory. In the laboratory, the precise position and coverage of each trap was marked on the bolts to facilitate calculation of surface area of individual traps. Lengths of bolts, positions in the bole of each tree and bolt diameters were measured and recorded.

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All mountain pine beetle parental galleries and associated larval galleries were counted and the lengths of all parental galleries were also recorded up to a height of 3.67 m (P1), 3.69 m (P2), 4.04 m (S1) and 5.25 m (S2) above the ground. Pupal chambers were counted, but results are not reported because, potentially due to the nature of the wood or bark anatomy in spruce compared with pine, they were generally not visible in spruce. The pronotum width of all emerged adults were measured to the nearest 0.1 mm using a Nikon SMZ-1 microscope (Nikon, Japan) with a × 10 ocular fitted with an ocular scale. The magnification was calibrated using a stage micrometer so that each division on the ocular scale equaled 0.1 mm. Pronotum width was chosen as it is a fixed measure regardless of beetle condition.

Paired spruce and pine pheromone-baiting trial

A pheromone baiting experiment was conducted in 2007 in a mixed lodgepole pine and interior hybrid spruce stand infested with mountain pine beetle, northwest of Prince George, British Columbia, Canada at 54°22'19"N, 122°59'32"W. The experiment consisted of 20 replicates of two treatments each: an unattacked baited pine and an unattacked baited spruce. Specifically, a single, unattacked lodgepole pine was chosen a short distance from an unattacked spruce selected to minimize size differences. The mean ± SE d.b.h. was 16.9 ± 0.52 cm for the pines and 17.3 ± 0.48 cm for the spruce. There was no significant difference (*t*-test, *P* = 0.7) in d.b.h. between the two treatments. The mean distance (± 1 S.E.) between the two trees within each replicate was 11.3 ± 0.63 m, with trees in different replicates separated by at least 25 m. Each tree in each replicate was baited with a tree bait for mountain pine beetle (0.3 mg/day *exo*-brevicomin and 1.5 mg/day *trans*-verbenol, both at 20 °C; Pherotech International Inc., Delta, Canada) on the north side of the bole at highest reach. Baits were affixed to trees on 27 June 2007 and the trees were assessed for presence of attack and attack density on 24 and 31 July and 7 and 15 August 2007. Assessments began when mountain pine beetle began emerging from ten nearby lodgepole pines in two plots, which were being monitored in an unrelated emergence trial. Attack densities were estimated by counting the number of entrance holes (pitch tubes and/or holes with frass) in two 20 × 40 cm rectangles (Huber & Borden, 2001): one on each of the east and west sides of each tree, at approximately eye-level (1.7–1.9 m above ground). Assessment rectangles were not permanent. The entire bole was visually inspected, as well, for evidence of attack because no attacks were present in the assessment rectangles on some lightly attacked trees. A tree was classified as 'attacked' if at least one attack was observed on its bole or 'mass-attacked' if the estimated attack density was greater than 30 attacks/m² (Raffa & Berryman, 1983; Huber & Borden, 2001) (i.e. a total of five attacks in the two assessed rectangles). Attack density assessments were conducted until after mountain pine beetle had finished emerging from the trees in the nearby, unrelated emergence trial. The mass-attacked spruce were felled on 17 November 2007 and were brought back to the laboratory for assessment of colonization success.

Statistical analysis

Measurements of mean attack density, gallery length, larval gallery per cm parental gallery and emergence per gallery start in the pine and spruce colonized by mountain pine beetle were analysed in a linear mixed effects model. Host species (pine versus spruce) was included as a fixed effect, whereas bolts or traps within the randomly selected trees were incorporated as appropriately nested random effects. Measurements of larval gallery per unit area or parental gallery as a function of height on the tree (a continuous variable) and species (a factor) were analysed using analysis of covariance, again in a mixed effects framework using the same random effects. Measurements of emergence per gallery start as a function of gallery start density and species were also analysed using analysis of covariance (ANCOVA) in a similar mixed effects framework. In ANCOVA models, nonsignificant fixed effects were removed from a full model containing the covariate, factor, and their interaction using a backward elimination procedure and setting $\alpha = 0.05$.

In the pheromone baiting trial using paired spruce and pine, the measurements of attack density and proportion of trees attacked and mass-attacked were analysed using ANCOVA in a linear mixed effects framework. Fixed effects included time fit as a covariate, and species incorporated as a time by species interaction, to allow species responses to change through time (i.e. different slopes). No main effect of species was included because the response variables must start from zero for each species and thus may not have different intercepts. The covariate, time, was natural-log transformed as the response appeared asymptotic. The random effect included the treatment pairs within the stand.

In all analyses, transformations of the response variable (usually square root) were used where necessary to fulfill assumptions of homoscedasticity and normality of errors. Assumptions were checked by visual inspection of residual and normal-scores plots. Specific outliers were tested via examination of their marginal effect after a Bonferroni correction when fit as a dummy variable. This correction was implemented conservatively using the full number of points in the data set as the basis for correction.

Results

Analysis of mountain pine beetle-infested spruce and pine

The mean \pm SE attack densities in the two naturally-attacked pines and two naturally-attacked spruce were 129.29 ± 0.69 attacks/m² and 76.19 ± 27.10 attacks/m², respectively (Fig. 1A). These means were significantly different ($F_{1,26} = 19.26$, $P = 0.0002$). Similarly, mean gallery lengths differed significantly ($F_{1,442} = 7.74$, $P = 0.005$), and were 22.28 ± 0.51 cm and 20.79 ± 0.74 cm in pines and spruce, respectively (Fig. 1B). The mean number of larval galleries per cm of parental gallery was 1.28 ± 0.029 in the pines and 1.73 ± 0.16 in the spruce (Fig. 1C). These means were signi-

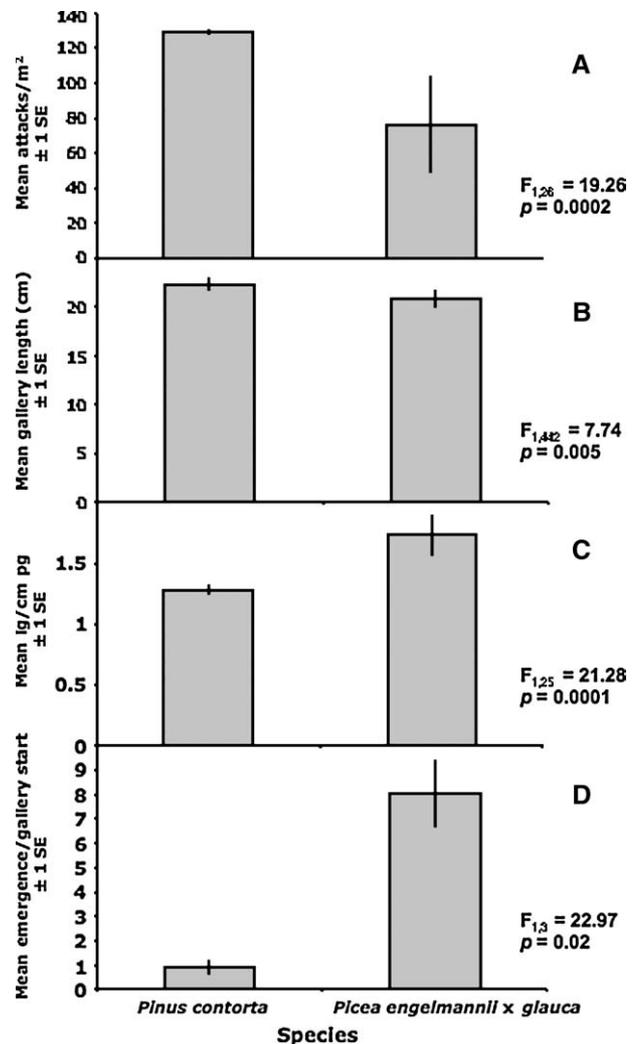


Figure 1 (A) Mountain pine beetle mean \pm SE attack densities of mountain pine beetle in lodgepole pines *Pinus contorta* and interior hybrid spruce *Picea engelmannii* x *glauca*. (B) Mean \pm SE parental gallery lengths in both hosts. (C) Mean \pm SE number of larval galleries (lg) per cm of parental gallery (pg) in both hosts. (D) Mean \pm SE emergence per gallery start in both hosts.

ficantly different ($F_{1,25} = 21.28$, $P = 0.0001$). Finally, the mean emergence of new generation adults per gallery start also differed significantly ($F_{1,3} = 22.97$, $P = 0.02$) in the pines (0.90 ± 0.27 new adults) and spruce (8.03 ± 1.35 new adults) (Fig. 1D).

The number of larval galleries/m² decreased slightly for the pines as height increased ($y = [69.09 + 0.0086(x)]^2$) but, in spruce, over the distance measured, the larval gallery density increased with height ($y = [24.37 + 0.12(x)]^2$) (Fig. 2A). Larval gallery density at different heights in the trees differed within each species ($F_{1,23} = 12.00$, $P = 0.002$). The number of larval galleries per parental gallery increased with height up the bole for both species but there was no significant difference between the tree species ($F_{1,22} = 1.19$, $P = 0.29$) (Fig. 2B). The top-most spruce bolt of specimen S2

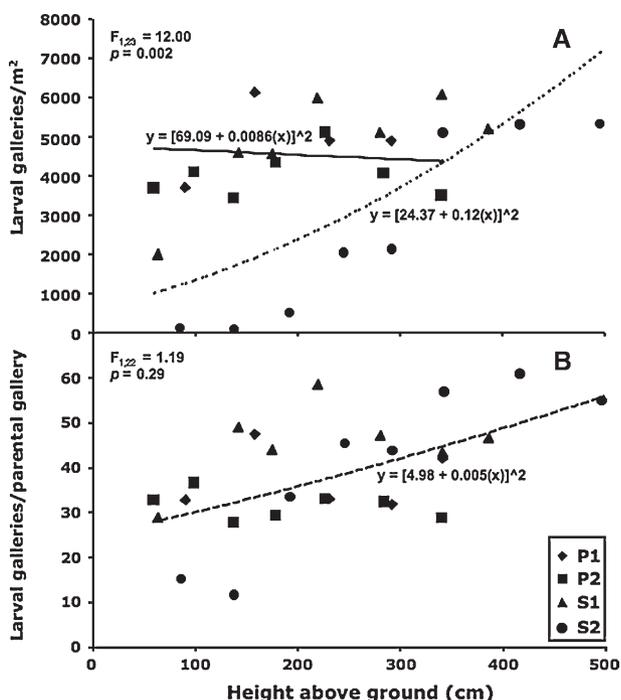


Figure 2 (A) Mountain pine beetle larval gallery densities at different heights above the ground along the bole for lodgepole pine (solid line) and interior hybrid spruce (dashed line). (B) number of larval galleries per parental gallery at different height above the ground for both species combined (dashed line). In each case, P1 and P2 or S1 and S2 refer to pine and spruce specimens, respectively.

was removed from analysis because it was an extreme outlier (193 larval galleries/parental gallery).

The number of new generation emerged adults captured in the 12 emergence traps on both species showed a negative relationship with the number of parental gallery starts under the traps when all specimens of both species were considered ($y = [4.12 + 0.018(x)]^2$) (Fig. 3). There was no significant difference between tree species. There was also no difference in the pronotum width of mountain pine beetle that had emerged from spruce (1.91 ± 0.16 mm; $n = 225$) compared with those that had emerged from pines (1.90 ± 0.18 mm; $n = 31$).

Paired spruce and pine pheromone-baiting trial

The number of attacks/m² increased after the time of baiting for both pines and spruce (Fig. 4A); the rates of increase in attack density over time differed significantly between the two species ($F_{1,218} = 331.58$, $P < 0.001$) (Fig. 4A). The proportion of trees with at least one attack also increased after the time of baiting for both species (Fig. 4B); the rates of increase in proportion of attacked trees were significantly greater in pine than spruce ($F_{1,218} = 153.51$, $P < 0.001$). Similarly, the proportion of mass-attacked trees increased over time after baiting for both species (Fig. 4C); the rates of increase in the proportion of attacked trees were significantly greater in pine than in spruce ($F_{1,218} = 962.69$, $P < 0.001$). Of

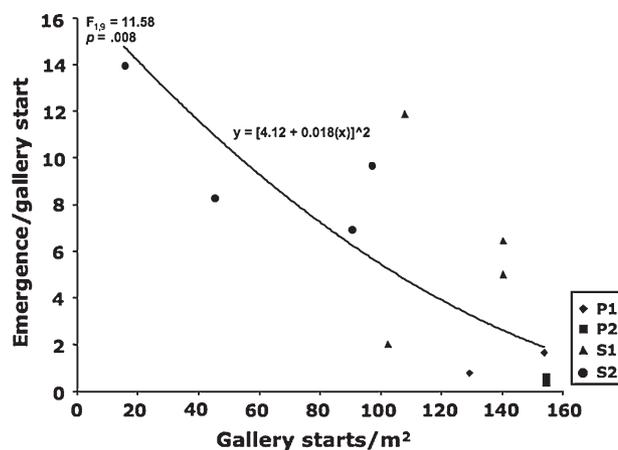


Figure 3 The effect of competition (gallery starts per m²) on reproductive success (emergence per gallery start) of mountain pine beetle in lodgepole pine (P1 and P2) and interior hybrid spruce (S1 and S2).

the attacked pines (95% of the baited trees), all but one (90%) were also mass-attacked, whereas, of the attacked spruce (35% of the baited trees), only three (15%) were mass-attacked. Mountain pine beetle had successfully colonized two of the three spruce, as demonstrated by construction of long parental galleries, but had been pitched out of the third. No larval galleries were visible in the two mass-attacked spruce with parental galleries, but the colonizing adults were still alive at the time of phloem dissection. Larval galleries were present in nearby baited pines.

Discussion

The results obtained in the present study demonstrate that mountain pine beetle can successfully mass-attack and reproduce within mature, standing, live interior hybrid spruce *P. engelmannii* × *glauca*. It is unlikely, however, that the phenomenon that we report (i.e. the successful colonization, reproduction, and emergence of a new generation of mountain pine beetle in hybrid spruce) will result in a permanent host shift by a portion of the mountain pine beetle population. However, our observations may provide insight into mechanisms of past host shifts and speciation events in *Dendroctonus* spp. Although it is possible that the natural attacks reported in the present study were merely random, it should be noted that these are not isolated incidents because we have subsequently encountered a substantial number of additional successfully attacked spruce in central British Columbia.

The host shifts and subsequent speciation events suggested by Kelley and Farrell (1998) imply that at least some of these were geographically sympatric in nature. Although allopatric speciation and its causes are accepted and quite well-understood (Bush, 1975) among a variety of animals, including the Scolytinae (Jordal *et al.*, 2006), the same cannot be said about sympatric speciation. However, there is currently a higher degree of acceptance of the likely reality

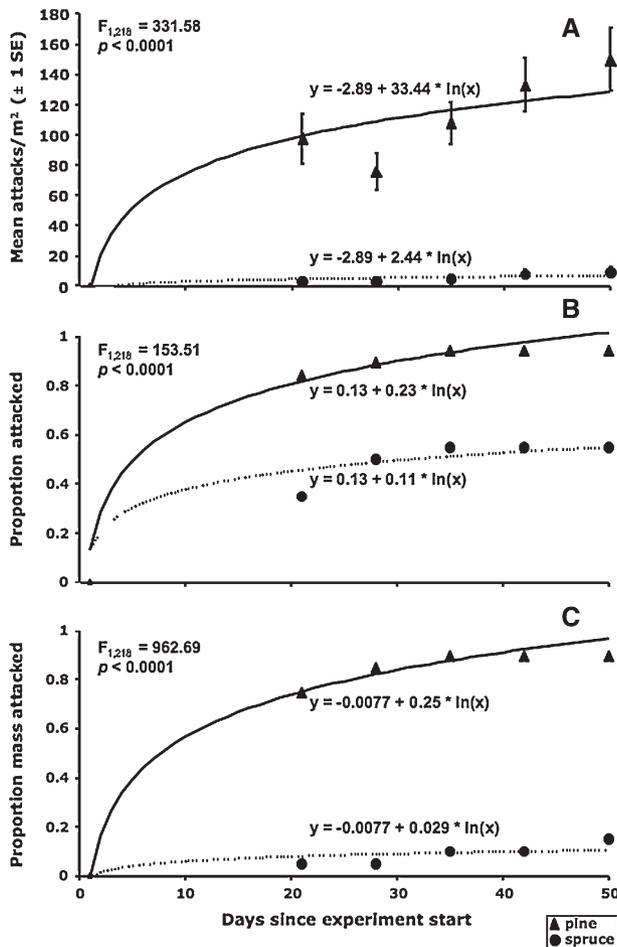


Figure 4 (A) Mountain pine beetle mean \pm SE attack densities on pheromone-baited lodgepole pines (solid line) and interior hybrid spruce (dashed line) at each assessment date after baiting. (B) Proportion of attacked pines and spruce at each assessment after baiting. (C) Proportion of mass-attacked pines and spruce at each assessment after baiting ($n = 20$).

of sympatric speciation (Via, 2001) due to past observations and current research on dramatic examples of that phenomenon (Bush, 1969; Knerer & Atwood, 1973; Jiggins & Bridle, 2004).

For example, in a well-studied host shift and likely speciation event, the apple maggot, *Rhagoletis pomonella*, has diverged into two host-races, or possibly two incipient species (Bush, 1969; Jiggins & Bridle, 2004), with partial reproductive isolation in one contiguous geographical area. The putative sympatric speciation event appears to have been driven by available hosts, with a host shift of a part of the fly population from their normal hawthorn, *Crataegus* spp., host to introduced apple, *Malus* spp., in which the fruits on the two plants are available to reproducing fly populations at different times (Bush, 1969). Other genetic and behavioural mechanisms may also be factors in this phenomenon (Jiggins & Bridle, 2004; Linn *et al.*, 2004). Thus, this likely incipient speciation event is geographically sympatric at the same time as being temporally allopatric.

It is not clear whether the ability of some mountain pine beetle to successfully reproduce in spruce hosts is indicative of the early stages of a host shift-driven sympatric speciation event, although previous studies have noted this possibility (Furniss & Schenk, 1969). A number of factors related to the insect's overall biological and ecological characteristics make it at least plausible. Contiguous populations of mountain pine beetle range from southern California to central British Columbia and east to the Black Hills of South Dakota (Keen, 1952; Furniss & Carolin, 1977; Amman *et al.*, 1990). Across this vast geographic range, mountain pine beetle utilize at least 13 *Pinus* spp. hosts (Amman, *et al.*, 1990). Although the different hosts are probably quite similar in terms of chemical defences (von Rudloff & Holst, 1968; Hunt & von Rudloff 1977; von Rudloff & Lapp, 1987), there are differences in quality and quantity of secondary metabolites among host species and even between populations of the same host species (Forrest, 1980). As such, it is likely that a coevolutionary mosaic exists across the range of this insect, with interactions between mountain pine beetle and its various hosts in some regions causing specific changes in the ability of the beetles to resist certain tree defences (Raffa & Berryman, 1987; Thompson, 1999). Because mountain pine beetle populations interacting with different hosts or host populations are contiguous, gene flow should allow resistance alleles to spread beyond local coevolutionary 'hotspots', and potentially pre-adapt some mountain pine beetle populations to chemical defences of novel hosts (Thompson, 1999). Spruce and pine are both in the Pinaceae and, as such, there is a substantial amount of overlap in terms of the quality, if not also the quantity, of resin secondary metabolites. Thus, because the terpenoid secondary metabolite defensive secretions of some *Pinus* spp. hosts (Huber *et al.*, 2004) of mountain pine beetle are at least similar in quality to those of interior hybrid spruce (von Rudloff & Holst, 1968; Hunt & von Rudloff, 1977; von Rudloff & Lapp, 1987; Pureswaran *et al.*, 2004), the possibility of pre-adaptation of some mountain pine beetle populations to some phenotypes of spruce exists. Some mountain pine beetle may then utilize spruce when they grow in close proximity to normal hosts, as is the case in central British Columbia in mixed stands of lodgepole pine and interior hybrid spruce. The fact that other extant *Dendroctonus* spp. that do not use pines appear to derive from pine-infesting ancestors (Kelley & Farrell, 1998), and vice versa in the case of *D. murrayanae*, lends support to this possibility, implying that there may be factors relating to adaptation to *Pinus* spp. defences that allows survival in other Pinaceae, at least in low-vigour trees (Safranyik & Linton, 1983).

The semiochemical-driven aggregation behaviour of mountain pine beetle could be a factor that helps to drive host-shifts due to pre-adaptation, particularly under the extreme population levels seen during epidemics, such as the current one in central British Columbia. If a sub-population of mountain pine beetle in an area was pre-adapted to survive at least the constitutive defences of spruce, and if some members of that population were to initiate aggregations on spruce, the aggregation pheromone of pioneering insects that survive the constitutive defences of spruce may draw in

further conspecifics. Such a scenario is consistent with the results of our synthetic baiting trials that induced mass-attacks in pine and spruce (Fig. 4). Survival and increased reproductive success of pre-adapted mountain pine beetle, along with any symbiotic fungi able to survive in the novel host, would preserve and spread any associated resistance alleles. Our results indicate that mountain pine beetles that do survive spruce colonization, and that are able to reproduce, benefit in terms of reproductive output from reduced competition in spruce compared with nearby pines (Figs 1A, 3). The reduced competition resulted in a reduced cost of parental gallery construction (i.e. shorter galleries) in spruce compared with pines (Fig. 1B). In addition, more larval galleries were present per unit length of parental gallery (Fig. 1C), augmenting the significantly higher brood production per gallery (Fig. 1D), probably because the egg-laying females in spruce are not required to escape perceived heavy competition. The fact that there was no significant difference in the size of individual beetles from the two host species suggests that there are no obvious negative fitness consequences to development in spruce (Leather & Awmack, 2002).

It is apparent from our observations, as well as those of Safranyik and Linton (1983), that *Picea* spp. are potentially suitable hosts for mountain pine beetle (i.e. mountain pine beetle can successfully reproduce in these trees). Live spruce are generally not susceptible to attack, however, presumably due to induced defences, which may either repel the initial attackers outright, or prevent optimal production of aggregation pheromones. Thus, genotypes of mountain pine beetle prone to attack spruce are probably rare. Dead spruce are rarely available for these insects because other bark beetles rapidly colonize any available breeding resource (Werner & Holsten, 1984), effectively excluding mountain pine beetle (Hanski, 1987). During outbreaks, however, rare genotypes may become sufficiently common that an effective expansion of the host range is possible, similar to the shift to a predominance of 'aggressive' genotypes of *D. rufipennis* during epidemics (Wallin & Raffa, 2004). When such attacks occur on susceptible live spruce (i.e. trees with poor induced defences), successful brood production may occur. Both our observations of the naturally-attacked spruce and our baiting experiment indicate that most mature spruce are probably not susceptible to mass-attack in the absence of stress. In the former case, an additional seven spruce trees had been attacked on the same property but beetles were pitched out before mass aggregation could occur and, in our baited experiment, only three of 20 baited trees were mass-attacked. Furthermore, although live adults were still present in their galleries, larval galleries were not present in those trees during our winter assessment. If conditions are conducive to an increase in the availability of susceptible spruce over a longer time, such as variable temperature and precipitation conditions that may occur due to global climate change, a host switch may be sustained, and speciation could conceivably occur.

In the latter case, however, the conditions that may allow for an incipient host shift are also generally very transient. That is, when the mountain pine beetle population is very high and the number of available suitable hosts is low, the

population cannot be sustained and generally drops to a very low level within a year. Thus, if our observations are indicative of what may happen in a host shift and speciation event in *Dendroctonus* spp., it is unlikely that it will carry on to completion in this instance. In that regard, it should also be noted that, when available host pines are present, mountain pine beetle do not indiscriminately attack nonhost spruce, even when in the presence of a strong positive stimulus, such as an aggregation pheromone bait (Fig. 4). However, bark beetles and conifers have been interacting for many millions of years, and host shifts have probably been a driving force in past speciation events in *Dendroctonus* spp. (Kelley & Farrell, 1998) and other insects (Jiggins & Bridle, 2004). Thus, our observations may present a viable explanation of a potential mechanism for such shifts because mass-attacks on spruce can be induced under some conditions (Fig. 4C).

Further work on comparisons of secondary metabolite profiles in the various *Pinus* spp. hosts of mountain pine beetle and in *Picea* spp., changes in the composition of vectored symbiotic fungi in the different hosts, the role of heritability and learning in mountain pine beetle host preferences, and effects of mortality agents such as natural enemies in different hosts, are required for a deeper understanding of this phenomenon and of the potential insight that it provides into bark beetle host usage patterns and evolutionary history.

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