

Heritability of Host Acceptance and Gallery Construction Behaviors of the Bark Beetle *Ips pini* (Coleoptera: Scolytidae)

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ABSTRACT We examined genetic variation in host selection behavior of a phloeophagous insect herbivore. Data from paternal families of the bark beetle *Ips pini* (Say) were used to estimate the heritability of host acceptance and gallery construction behaviors. Males are the host-selecting gender in this genus. Male beetles were assayed over three generations to determine whether they rejected or accepted host media amended with concentrations of alpha-pinene that simulated host tissue, and 10% from each group were selected for breeding lines. In a separate experiment, 10% of individuals constructing the shortest and 10% of individuals constructing the longest galleries in this medium were established in separate breeding lines. The results indicate high additive genetic variation with respect to both traits. On the basis of the results with full-sib breeding lines, we estimated heritability of host acceptance behavior (i.e., entry into simulated hosts) at 0.78 and heritability of gallery construction behavior at 0.64. The divergence between lines in host acceptance and gallery construction behaviors was associated with paternal performance and was symmetrical. This study demonstrates that the use of phytochemical cues to accept potential hosts has a heritable component in bark beetles. *I. pini* is a useful and convenient model for such studies.

KEY WORDS bark beetle, directional selection, heritability, host acceptance, plant-insect interactions

THE SUITABILITY OF POTENTIAL host plants encountered by insect herbivores varies widely because of genetic, environmental, and phenological factors (Fitt 1986, Singer and Parmesan 1993). Variability within plant populations has been proposed as a source of coevolutionary changes in host selection behavior, and of genetically based divergence among herbivore populations (Futuyma 1983, Rausher 1983, Jaenike and Holt 1991). Several studies have reported changes in ovipositional and feeding behaviors in response to changes in host plants (Harrison 1987, Thompson et al. 1990, Singer and Parmesan 1993). The potential of a population to undergo selective change is determined by the extent of phenotypic variation in behavior, the genetic basis to this variation, and the relationships between selection and fitness (Jaenike 1990, Via 1991, Carriere 1998). Genetic correlations between parental and offspring responses to different hosts have only been investigated in the past decade (Courtney and Hard 1990, Thompson et al. 1990, Via 1991), and heritable variation in acceptance of plants within a host species is even less understood.

Variation within host species can be especially challenging for stem-colonizing bark beetles (Coleoptera: Scolytidae), because of their requirement to kill trees

to reproduce, the corresponding ability of trees to kill beetles that fail in their attempts, and the requirement to locate new hosts each generation (Cates and Alexander 1982, Nebeker et al. 1993, Raffa and Smalley 1995). In addition, most conifer bark beetles deposit all or nearly all of their egg clutch within one host, and their progeny develop endophytically without the ability to seek a new host (Atkins 1966, Wood 1982). Adult bark beetles have short life spans, incur high risks of predation while outside trees, and experience decreased lipid reserves during host searching (Sahota et al. 1987), so they must make viable host selection decisions quickly. Discrimination between resistant and susceptible hosts is typically preferred following landing in tree-killing bark beetles (Rudinsky 1962, Hynum and Berryman 1980, Moeck et al. 1981). Field patterns suggest that the optimum host selection decision is density dependent, with colonization of healthy trees being more prevalent at high than low beetle densities (Safranyik et al. 1975, Wood 1982).

The possibility of a heritable component to bark beetle host acceptance behavior has been suggested but not explored, despite investigations with other insect feeding guilds (e.g., Diehl and Bush 1984, Futuyma and Peterson 1985, Prokopy et al. 1988, Singer and Parmesan 1993, Carriere 1998). However, there is evidence of a genetic basis for pheromone-mediated mating behavior in bark beetles (Birgersson et al. 1988, Berisford et al. 1990, Teale et al. 1994). In addition, isozyme variation has been associated with

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geographic region, host species, phloem thickness, and population phase (Stock and Amman 1980, Sturgeon and Robertson 1985, Sturgeon and Mitton 1986, Langor and Spence 1991).

The pine engraver, *Ips pini* (Say), colonizes red pine, *Pinus resinosa* Aiton, jack pine, *Pinus banksiana* Lambert, and white pine, *Pinus strobus* L. within the Great Lakes region (Schenk and Benjamin 1969, Klepzig et al. 1991). The predominant allelochemicals in these trees are monoterpenes, of which alpha-pinene is the most abundant. Alpha-pinene accounts for 78, 75, and 70%, of the phloem monoterpenes in these species, respectively (Erbilgin et al. 2001). Male *I. pini* make host acceptance decisions (Schenk and Benjamin 1969). As they bore into a tree, they emit aggregation pheromones that attract both genders. Males excavate a gallery and are joined by one to three females each. Each female deposits eggs along a single ovipositional gallery (Schenk and Benjamin 1969). Offspring feed as larvae on the phloem tissue, pupate, and emerge as adults \approx 3-5 wk from initial host acceptance by the male.

I. pini preferentially colonize recently killed and stressed trees (Wood 1982, Amman and Safranyik 1985, Klepzig et al. 1991). This range of host acceptance behavior is determined in part by response to host monoterpenes, which are generally lower in stressed trees (Wallin and Raffa 1999, 2000). Host acceptance behavior is at least partially modulated by nonheritable factors, including density of male *I. pini* on the surface of the substrate, age, and experience (Wallin and Raffa 2002a, 2002b). Our objectives were to test whether host acceptance and gallery construction by *I. pini* would respond to directional selection under laboratory conditions. If so, this indicates a substantial level of genetic variation within the population, which could be maintained through pheromone-mediated mate selection behavior. Shifts in population structure mediated by host selection may feed back into population density because of the direct relationship between gallery length and number of eggs deposited by the female.

Materials and Methods

Beetle Population. *I. pini* from a laboratory culture were tested during 1999. A colony consisting of \approx 1,500 pairs was initiated in 1995 by sampling from several populations across south-central Wisconsin. To minimize inbreeding, we replenished the colony five to seven times per year with several hundred field-caught beetles. The colony was maintained by introducing 10 groups of one male and three females into a 0.3 m \times 10-20 cm section of red pine. Each colonized log was placed in a metal container with two clear glass emergence jars securely attached to the wall. Culture conditions were held at 12L:12D h, 24°C, and 80% RH. Progeny develop to adults after \approx 30 d under these conditions. Adult progeny were collected daily from the jars at 0700 h and randomly assigned for either experimental use or for colony maintenance.

Bioassay to Measure Host Acceptance and Gallery Construction Behaviors. We used a laboratory assay in which denatured phloem is amended with a defined concentration of alpha-pinene; then the proportion of beetles that enter this medium and their subsequent gallery construction behavior are recorded. This assay has proved applicable to five species of three genera of bark beetles, *Ips*, *Dendroctonus*, and *Hylastes* (Klepzig et al. 1996; Wallin and Raffa 2000, 2002a, 2002b). It also corresponds to natural conditions in that:

- (1) beetle entrance and gallery construction decrease with increasing concentrations of monoterpenes (Wallin and Raffa 2000, 2002a), as does entry into live trees (Smith 1965, 1975);
- (2) beetles are less likely to enter phloem amended with extracts from induced reaction than constitutive tissue (Klepzig et al. 1996), and conifer defense and survival in the field are often correlated with the rate and extent of monoterpene accumulation in localized induced reactions (Cook and Hain 1986, Christiansen 1985, Raffa 1991);
- (3) beetles are more likely to enter phloem amended with extracts of induced reaction stem tissue from root-diseased trees than from healthy trees (Klepzig et al. 1996) and are likewise more likely to colonize the former category in the field (Klepzig et al. 1991, Erbilgin et al. 2001).

Host entrance behavior has been shown to occur within 15 min and to remain consistent over a 24-h period (Wallin and Raffa 1999). This assay is identical to the one we used to test the above-mentioned nonheritable host selection behaviors (Wallin and Raffa 2002a, 2002b).

Red pine phloem was freeze-dried, ground through a mill (0.5-mm screen), and autoclaved to remove any residual monoterpenes (Wallin and Raffa 2000). The ground phloem was mixed in boiling distilled water with Batch-agar (Difco, Detroit, MI), poured into a 9-cm plastic petri dish, and dried in a fume hood for 24 h (Klepzig et al. 1996, Wallin and Raffa 2000). We dissolved 3.4 mg/gm racemic α -pinene in pentane and applied to the surface of the medium. Previous assays showed this concentration causes 50% repellency with equivalent responses to (+) and (-) α -pinene (Wallin and Raffa 2000). A 9-cm disk of acetate transparency film was placed over the amended medium to prevent volatilization. One beetle was introduced into each arena, and the specific conditions are varied to assay either host acceptance or gallery construction behaviors (see below). The arenas were randomly arranged in stacks of five, and stacks were randomly assigned to positions in environmental chambers (24°C; dark; 80% RH).

Heritability of Host Acceptance Behavior. A disk of medium was removed from the 9-cm base of the petri dish, placed in the 11-cm petri dish lid, and amended with alpha-pinene or pentane control. This provided a 1-cm space in which beetles could move. 200 male *I. pini* were individually placed on the centers of the

transparency disks in each assay arena. Because *I. pini* are phototropic, we observed each arena under red light (to which they do not respond) for 15 min. During this time, each beetle could either remain outside or enter the medium by initiating gallery construction behavior, and each was scored as accepting or rejecting the medium accordingly.

Two separate breeding lines were established using beetles that accepted or rejected the media. Fifty males per breeding treatment were selected, paired with one randomly selected virgin female each, and established singly on a log section, using the method of Teale et al. (1994). We longitudinally quartered 0.3-m red pine log sections and applied a thin layer of paraffin to the ends and exposed sides. Females were introduced into the log sections 24-h after the males. Each colonized log was placed bark side up on sand in a plastic shoebox, which had one clear glass emergence jar securely attached to its wall. The boxes were held at 12L:12D h, 24°C, and 80% RH. Adult progeny were collected from the jars, bark surface, and sand daily at 0700 h, and sorted according to gender.

Host acceptance behaviors of 2-6 sons per father were assayed as described above. Sons from their respective lines were paired with one sister each, resulting in full-sib breeding lines. The 100 families were kept separate throughout the experiment. This process was repeated for three generations. To ensure that the modified rearing technique did not contribute to potential differences in host selection and gallery construction behaviors, an additional 50 males were chosen randomly and paired singly with virgin females to establish a baseline control. Males from the baseline control were tested in each generation and randomly paired with virgin females from the laboratory colony.

Heritability of Gallery Construction Behavior. The gallery construction assay was similar except that the medium was kept in the base of the petri dish, a hole was drilled through its side, and a beetle was gently inserted into the medium, head first. The hole and petri dish were sealed with paraffin wax, and gallery lengths were quantified at 24 h using a map measurer (PECO, Jackson, MS).

Of the beetles assayed, the 100 males that constructed the shortest and the 100 males that constructed the longest galleries were selected as the parents for the first generation of the two breeding treatments. In each of the two subsequent generations, males were assayed for gallery construction behavior, selected for short and long gallery breeding lines, and paired with a randomly chosen sister. An additional 50 males were randomly chosen and paired singly with virgin females to establish a baseline control. Families were kept separate throughout the experiment.

Statistical Analyses. The percent entry and average gallery length were compared within groups among generations by analysis of variance (ANOVA). The number of generations under selection was considered the treatment. Host acceptance and gallery construction behaviors after three generations of selection were analyzed using one-way ANOVA, in which

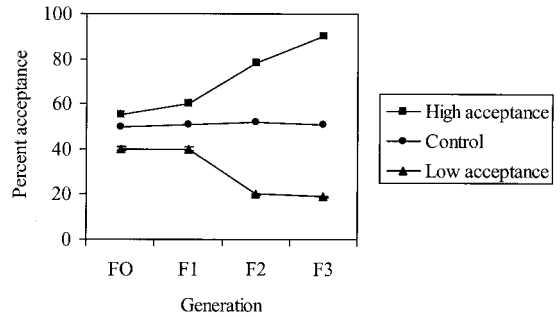


Fig. 1. Percent entry into media (% ± SE) in base population of *Ips pini* and in the first three generations of selection. Solid line represents selection for higher entry. Triangles represent selection for lower entry. $n = 100$.

the source of variation was breeding line (SAS Institute 1996). Symmetry of response was analyzed using orthogonal contrasts. The response to selection was computed using full-sibling correlations and son-father regressions for entry and gallery construction behaviors. Divergence of breeding lines was calculated by subtracting response of each treatment group at each generation (Falconer and Mackay 1996).

Results

Heritability of Host Acceptance Behavior. Directional selection resulted in symmetrical divergence in host acceptance behavior in response to simulated host plants by the F₂ generation (Fig. 1). In the breeding line that was selected for acceptance, the percentage of beetles that entered increased from 59 ± 7 (mean ± SD) in the base population to 90 ± 11 by the third generation ($n = 100$; $df = 1, 98$; $F = 255.25$; $P < 0.0001$) (Fig. 1). This represented >4 SD. In the line selected for low acceptance, the percentage of beetles that entered dropped from 50 ± 6 (mean ± SD) in the base population to 25 ± 3 by the second generation and was 9% by the third generation ($n = 100$; $df = 1, 98$; $F = 209.88$; $P < 0.0001$) (Fig. 1). This represented 4 SD from the base population. Control lines did not differ from those in the first generation after three generations ($n = 50$; $df = 48$; $t = 0.9$; NS) and remained consistent at 51 ± 5 (mean ± SD).

We used a weighted-regression procedure described in Falconer and Mackay (1996) for the regression of mean son acceptance on father acceptance. We used the reciprocal of the number of male offspring tested per male as weights because of the unequal number of males tested per family. This resulted in a slope of $b = 0.39$ ($P < 0.005$, 95% CI: $0.1 < b < 0.6$). Because this is a regression of offspring on one parent, the estimate of heritability (h^2) is $2b = 0.78$ (Falconer and Mackay 1996).

Heritability of Gallery Construction Behavior. Directional selection on gallery lengths also resulted in symmetrical divergence by the third generation (Fig. 2). There were significant differences between F₂ and F₃ male gallery lengths within the long gallery breed-

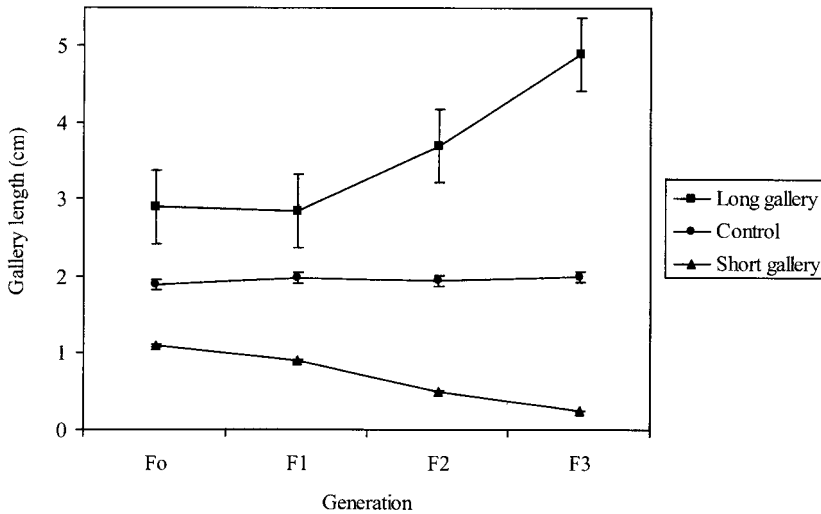


Fig. 2. Gallery lengths constructed in media (mean \pm SE) in base population of *Ips pini* and in the first three generations of selection are shown. Solid line represents selection for longer gallery lengths. Triangles represent selection for shorter gallery lengths. $n = 100$.

ing line but not within the short gallery breeding line (Fig. 2). In the long gallery breeding line, gallery lengths were approximately twice as long by the third generation than the controls. On average, beetles in the long breeding line constructed galleries that were 3 cm longer as the control by the third generation (Fig. 2; $n = 100$; $df = 1, 98$; $F = 19.8$; $P < 0.01$). Beetles in the short breeding line constructed galleries that were slightly shorter than the control line (Fig. 2; $n = 100$; $df = 1, 98$; $F = 14.2$; $P < 0.05$). Control lines did not differ from those in the first generation after three generations ($n = 50$; $df = 48$; $t = 0.6$; NS) and remained consistent at 1.9 (± 0.22) cm.

The regression of mean son gallery length on father gallery length resulted in a slope of $b = 0.32$ ($P < 0.001$, 95%CI: $0.2 < b < 0.5$). Therefore the estimated heritability of gallery construction behavior is 0.64.

Discussion

These results demonstrate that there is substantial phenotypic variation in host acceptance and gallery construction behaviors in *I. pini*, and that this variation has a strong genetic component. There was a continuous response to selection for both increased and decreased acceptance of, and for increased gallery lengths in, simulated host tissue over three generations. Gallery lengths showed no indication of having reached an upper limit after three generations of selection. Observations of heritable host acceptance behavior suggest it is a quantitative character in *I. pini* with considerable additive genetic variance. Our results are consistent with studies on heritable variation in host-selection behavior of other insect herbivores, which suggests that such variation is common for ovipositional preference (Singer 1982, reviewed in Via 1991, Jaenike and Holt 1991, Lu and Logan 1995).

Because only one sex of *I. pini* can be assayed for host selection behavior, maternal effects cannot be separated from additive genetic variance (Via 1986, Falconer and Mackay 1996). However, using host-selecting gender reduces potential biases introduced by maternal effects, as endophytic habitat they choose determines the environment. Future experiments using half-siblings or backcrosses can account for potential maternal effects. If maternal and paternal contributions were equal within the population, our heritability estimates would be reduced (Falconer and Mackay 1996), but still differ significantly from each other. Because bark beetles breed communally, heritability estimates could also include some environmental effects. However, we attempted to minimize this factor by rearing pairs in individual log sections.

A high genetic contribution to these two traits could contribute to behavioral differences seen within populations and between population phases of bark beetles. For example, individuals expressing either type of acceptance behavior could be favored, depending on environmental conditions, and high heritability permits rapid change of a population as environmental factors change. Observations of bark beetles in the field suggest that population density and tree vigor may comprise important environmental conditions that affect factors of host selection behaviors (Safranik et al. 1975), and optimal behaviors may vary under different conditions. Host selection behaviors likewise may exert feedback on population density by affecting the range of host use and intraspecific competition. For example, host selection and subsequent reproduction in a host of low nutritive quality (dead or dying host) may primarily reflect efficiency of food utilization, whereas these parameters within a host of higher nutritive quality more strongly reflect competition. Understanding the genetic correlations of such

characters in different environments can help address how environments select for and maintain genetic variation within populations. Our results suggest that optimal management practices may differ depending on beetle population phase, and that genetic markers assist in the detection of possible outbreaks.

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