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Evolution, Vol. 52, No. 6. (Dec., 1998), pp. 1731-1743.

Evolution, 52(6), 1998, pp. 1731–1743

IS SPECIALIZATION A DEAD END? THE PHYLOGENY OF HOST USE IN *DENDROCTONUS* BARK BEETLES (SCOLYTIDAE)

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Abstract.—Ecological explanations for the prevalence of resource specialists are abundant, whereas phylogenetic evidence on their origins is scarce. In this paper, we provide a molecular phylogenetic study of the 19 specialist or generalist species in the bark beetle genus *Dendroctonus*, which collectively attack species in four different genera in the conifer family Pinaceae. Given substantial variation in diet breadth, we asked two general questions concerning the evolution of resource use in this group. How conservative is the evolution of host use in these insects? Does specialization tend to be derived (i.e., a "dead end")?

To answer these questions, we estimated the phylogeny of *Dendroctonus* using mitochondrial DNA sequences and mapped transitions in resource use on the resulting phylogeny estimate. The evolution of affiliations with *Pinus* and *Picea* hosts in *Dendroctonus* was conservative among beetle species (PTP test; $P < 0.012$), but there was no significant correspondence between the phylogeny of these beetles and the phylogeny among their Pinaceae hosts (among genera, $P = 0.28$; among *Pinus* species, $P = 0.82$).

Degree of specialization, as measured in the proportion of hosts used, was bimodally distributed with "generalist" species utilizing $\geq 60\%$ of the congeneric hosts within their range and six specialist species utilizing $\leq 40\%$ of the available hosts. Among the generalists, we found a strong correlation between the number of hosts encountered and the number of hosts utilized ($R = 0.97$, $P < 0.0001$), whereas there was no significant correlation among the specialists ($R = 0.27$, $P = 0.59$). The evolution of specialization in *Dendroctonus* proved highly labile—specialists arose from generalists at least six separate times (without reversal) all in derived positions, and closer examination of some specialists revealed instances where they appear to have lost particular host species from their diet. However, evidence from the ecological literature also suggests that several *Dendroctonus* generalists may have increased their range of host genera within the Pinaceae.

Key words.—Coevolution, Coleoptera, cytochrome oxidase I, *Dendroctonus*, insect-plant interactions, mitochondrial DNA, molecular systematics, phylogeny, phytophagous insects.

Received September 10, 1997. Accepted July 14, 1998.

Few questions in evolutionary ecology have been subject to such intense investigation as the question of why organisms specialize in resource use (Ehrlich and Raven 1964; Bush 1969; Fox and Morrow 1981; Bernays and Graham 1988; Futuyma and Moreno 1988; Mitter et al. 1988; Futuyma and McCafferty 1990; Jaenike 1990; Via 1990; Fox and Eisenbach 1992; Bernays and Chapman 1994; Dyer 1995). Because resource generalists should be less limited by resource availability, these should have an advantage over resource specialists, which may sometimes be extremely limited (Futuyma and Moreno 1988; Beissinger 1990; Mopper et al. 1995). Yet vast numbers of species, perhaps the majority of phytophagous insects, feed on only a fraction of available hosts (Futuyma and Moreno 1988; Mitter et al. 1988; Jaenike 1990; Thompson 1994). How can we explain this apparent contradiction?

The "jack of all trades, master of none" hypothesis is commonly used to explain the prevalence of specialists (Via 1984, 1986; Simms and Rausher 1989; Tienderen 1991; Schluter 1995; Robinson et al. 1996). Under this hypothesis, specialization evolves as a consequence of trade-offs in performance of organisms on different hosts, such that optimized use of one host limits performance on others (Simms and Rausher 1989; Futuyma and Moreno 1988; Jaenike 1990). Disparate results in the search for performance trade-offs has led to evaluation of other factors favoring specialization, such as freedom from natural enemies (Price et al. 1980; Bernays and Graham 1988). Regardless of the primary source of selection for specialization, however, long-term association with a particular host might eventually result in loss of genetic variation for the ability to use alternate hosts. Specialists might thus become irreversibly constrained on a particular resource or resources to the exclusion of others (Futuyma and Moreno 1988; Beissinger 1990; Via 1990; Siddall et al. 1993; Hougen-Eitzmann and Rausher 1994; Robinson et al. 1996). Because of this exclusionary use of more limited resources, specialization has been thought to result in a greater likelihood of extinction (Simpson 1953; Mayr 1963; Hansen 1980; Koch 1980).

The concept that specialization leads to an evolutionary dead end, first postulated by Cope (1896) as the "law of the unspecialized," has been a central idea in evolutionary biology (Huxley 1942; Mayr 1963; Rensch 1980). However, only a handful of studies address whether generalists tend to give rise to specialists, or, more directly, whether specialization tends to be derived. For example, the chrysomelid beetles in the genera *Oreina* and *Ophraella*, which feed on plants in the Apiaceae and/or Asteraceae, show little evidence that specialists tend to be derived (Futuyma et al. 1995; Dobler et al. 1996), although these studies were not designed to ask this question directly. However, trends toward evolution of increased specialization have been reported in lizards (Lo-

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sos 1992; Losos et al. 1994) and ectomycorrhizal fungi (Cullings et al. 1996), whereas generalists are derived in cowbirds (Lanyon 1992) and in anthidiine bees (Muller 1996). Although pertinent studies are accumulating, phylogenetic evidence bearing on evolutionary trends in resource specialization remains sparse and very few phylogenetic studies directly test for such trends (Futuyma and Moreno 1988; Farrell and Mitter 1993; Thompson 1994; Muller 1996). While shifts between generalist and specialist habits surely occur in both directions, it is not yet clear whether specialists are *more often* phylogenetically derived and dispersed (i.e., highly "tippy"; found at the tips of the phylogenetic tree) consistent with the notion that specialists more often go extinct.

Defining Specialists

An obvious challenge to phylogenetic study of the evolution of resource specialization in phytophagous insects is the long-standing problem of distinguishing "significant" differences in specialization among insect species in a clade. Usually, such distinctions between specialists and generalists draw on a combination of the number of plant species and higher taxa in their respective host lists (i.e., the "taxonomic disparity") to discover "gaps" in what otherwise might seem a continuum in breadth of resource use.

Because the plant taxonomic groups spanned by the species in different insects groups vary considerably, there is little consensus on categories of diet breadth. For example, monophagy has been defined as feeding on a single plant species (Bernays and Chapman 1994), several plant species within a genus (Stark 1982), or even plant species within a family (Muller 1996). However, because ecological studies of insect host use typically focus on closely related species or populations within species, the distinctions are often simply relative, and not meant to reflect some generally realized syndromes across insect groups. Nevertheless, it is difficult to gain an evolutionary perspective of diet breadth evolution without some agreement on the limits of variation.

The idea of "resource availability" has been important in the conceptual development of relative niche width (i.e., diet breadth; Colwell and Futuyma 1971). An obvious context is the simple geographic availability of resources (e.g., host plants) within the ranges of herbivore species, because a herbivore cannot use plant species it never encounters (Thompson 1994). Additionally, herbivores may have some historical propensity for feeding on related or chemically similar hosts (e.g., Futuyma et al. 1995; Dobler et al. 1996; Becerra 1997). For example, if herbivore X belongs to a clade that all feed on plant family Y, the available hosts for X might be most reasonably supposed to be those members of Y present in X's geographic range.

Therefore, an explicitly phylogenetic approach may aid in understanding the evolution of specialization in several important ways: (1) by helping define the suite of an herbivore's available hosts; (2) by allowing detection of host-use expansion or contraction in the diversification of a group; and (3) by identifying particular hosts gained or lost to particular insect species. In this paper, we use a phylogenetic approach to understand the evolution of resource specialization in a group of herbivorous insects, the bark beetle genus *Dendroctonus* (family Scolytidae). These insects show interspecific variation in the numbers and kinds of coniferous hosts they use. To address whether the host affiliations of these insects are conserved, apart from differences in diet breadth, we evaluate the frequency of shifts among host genera, on the grounds that these should represent the greatest disparity in plant traits (e.g., in secondary chemistry) in the collective range of hosts used by these herbivores. Recent phylogenetic studies of a variety of herbivorous insects suggest that insect host-use evolution is often quite conservative with respect to host taxon (i.e., plant family, tribe, or genus [Farrell and Mitter 1993]). Thus, some aspects of host-use evolution may be better predicted from recent phylogenetic history (and perhaps kinds of genetic variation) than by apparent ecological opportunity.

Next, to determine whether the diet breadth of *Dendroctonus* is conserved, we evaluate the rate of change in the *proportions* of available hosts used by these insects, using those aspects of host use that are strongly conserved to define the array of potential hosts. *Dendroctonus* species are restricted (with a few exceptions, discussed below) to one of four confamilial genera of conifers (Wood 1982), and the species vary markedly in how many species of host plants they use. The host affiliations and other aspects of the life histories of these insects are known in unusual detail, because these beetles are among the most damaging insects to coniferous forests in North America (Wood 1982). We will show that although affiliations with certain conifer genera seem highly conserved, there have been frequent constrictions of diet breadth, sometimes to subsets of hosts used by near relatives. Once the boundaries of specialization become clear, we then use a phylogenetic approach to determine, in at least this one group of insects, whether specialists tend to occupy phylogenetically derived and dispersed positions, as expected if specialization is a "dead end."

Natural History of Dendroctonus

The genus *Dendroctonus* belongs to the tribe Tomicini, subfamily Hylesinae, of the bark beetle family Scolytidae (Wood 1982, 1986). Tomicini is worldwide in its distribution, whereas *Dendroctonus* is mostly a New World group. *Dendroctonus* species are found from Guatemala to Alaska with two representatives in Eurasia. Although a phylogeny of the Tomicini is not yet available, Wood (1982) cites several morphological characters that suggest that the pine-feeding genera *Tomicus, Pseudohylesinus*, and *Hylurgus* are among the closest relatives of *Dendroctonus.*

Dendroctonus adults bore through the bark of standing or fallen trees and feed on the phloem within. Eggs are laid along the sides of tunnels, commonly called "galleries," excavated by the beetles, and the larvae feed outward into the phloem. This feeding habit is prevalent in most of the tribe, with the exception of several species of *Dendroctonus* that dig cavelike grooves in the phloem into which numerous eggs are deposited. After leaving the larval host, adult female *Dendroctonus* disperse to a suitable host. Once a host has been selected, females release sex pheromones (often termed "aggregation pheromones" because of the tendency for beetles to amass at high densities on the host) that attract members

TABLE 1. Distributions and host affiliations of non-*Pinus*-feeding *Dendroctonus* species. Host associations of the *Pinus* feeders are given in the Appendix. Asterisks indicate congeneric hosts available to the beetle species that are not reported hosts of those beetles.

Species	Hosts	Distributions
D. micans	*Picea abeis, P. stichensis, P. orientalis, P. ajanensis, P. breweriana, P. engelmanii, P. glauca, P. jezoensis, P. mariana, P. ovobata, P. omorika, P. pungens* (uncommon in *Pinus* spp., *Abies* spp., *Larix* spp.)	Eurasia
D. punctatus	*Picea glauca, P. rubens, P. sitchensis, P. engelmannii*, P. mariana**	N. United States and Canada
D. rufipennis	*Picea engelmannis, P. glauca, P. mariana, P. rubens, P. stichensii, P. breweriana**	N. United States and Canada
D. simplex	*Larix laricina*	N. United States and Canada
D. pseudotsugae	*Pseudotsuga menziesii, P. macrocarpa* (uncommon in *Larix occidentalis, L. laricina*)	W. United States and Canada

of both sexes to the tree (Borden 1986). Most *Dendroctonus* species kill their living host trees, although there are a few species (*D. valens, D. terebrans, D. parallelocollis, D. micans*, and *D. punctatus*) that do not necessarily kill their hosts.

Host-Plant Affiliations

The 19 species in the bark beetle genus *Dendroctonus* collectively encompass a wide range of resource specialization, including both extreme specialists (species restricted to a single host-plant species) and relative generalists (species able to feed on up to 20 plant species, sometimes in several genera: see Table 1; Appendix). Most species of *Dendroctonus* are restricted to feeding on host species in one particular genus of conifers. The majority of the species use plants in the genus *Pinus*, although *Dendroctonus punctatus, D. micans*, and *D. rufipennis* feed on *Picea*; *D. pseudotsugae* uses *Pseudotsuga*; and *D. simplex* is restricted to *Larix* (Wood and Bright 1992; Table 1). *Dendroctonus micans, D. valens*, and *D. pseudotsugae* have been recorded feeding on additional host genera, at least in outbreaks. (Outbreaks are defined as explosive rises in population densities in herbivorous insects that result in near decimation of large, contiguous host populations; see Barbosa and Schultz 1987.) It does not appear, however, that such periodic use of alternate hosts commonly supports normal development of offspring (Wainhouse and Beech-Garwood 1994).

An advantage of the *Dendroctonus* system is the unusually comprehensive information available on host use of these (and many other) bark beetles across their ranges. Because they are serious pests of timber, these beetles have been subject to intensive monitoring in coniferous forests in nearly every state and province for approximately 100 years (Wood 1982). This is important for confidence in inferences about relative diet breadth and host affiliations in two ways. First, the species that use few hosts have distributions and affiliations that overlap the distributions and affiliations of those that use many hosts (Appendix). Indeed, these apparent specialists are among the most serious and intensively studied pests (Wood 1982). Second, virtually all of the rarer pine conifer species are known hosts of bark beetle species (Wood 1982). Thus, it appears that neither the specialized beetles nor the rare hosts are inadequately sampled.

Another potential consideration in characterizing specialization of a species as a whole is the observation by Fox and Morrow (1981) that "generalist" species may actually comprise suites of populations specialized on particular hosts. Again, because of their economic importance, there are many reports that confirm the use of a variety of hosts in a single locale by *Dendroctonus* populations of several species (e.g., *D. ponderosae*: Sturgeon and Mitton 1986; *D. rufipennis*: Reynolds and Hard 1991, Holsten et al. 1995; *D. frontalis*: Cook and Hain 1987; and *D. micans*: Voolma 1980, Bejer 1984, Gregoire 1988).

METHODS

Collections

Many colleagues helped us identify regions of recent outbreaks of *Dendroctonus* where we were most likely to find beetles. Specimens were collected from dying and fallen trees by using a hatchet or a small ax to peel back the bark. The beetles were then removed from their gallery systems, either from within the bark or on the tree itself, and preserved in 100% ethanol or dried in vials of silica gel. A few species were collected with Lindgren pheromone traps (Phero-tech Inc., Delta, BC, Canada) and shipped to the authors in 100% ethanol. S. Wood provided dried museum specimens of one species, *D. vitei*, from the Brigham Young University entomology collections. Finally, specimens of several species were shipped by other researchers live in petri plates filled with wetted cloth or in small glass vials. All material was eventually stored at −20°C at the University of Colorado, Boulder (see Table 2).

Identification

Multiple individuals from each species were collected at each locale and a portion of each collection was mounted for identification. All specimens shipped to the authors arrived already identified by the contributors. Specimens from all samples were mounted and shipped to Donald E. Bright, Agriculture and Agri-Food Canada, Ottawa, for confirmation of identifications.

Preparation of DNA, PCR, and Sequencing

DNA was extracted from individual beetles crushed with a mortar and pestle in liquid nitrogen. After crushing, DNA was isolated and purified using procedures and materials from the QIAamp Tissue Kit (QIAGEN Inc., Chatsworth, CA). DNA was eluted from the QIAamp spin columns with 10 mM Tris-HCl pH 8.0 in volumes ranging from 0.1 to 0.5 ml depending upon the size of the beetle. We amplified a 1250

TABLE 2. Collecting sites from which populations of *Dendroctonus* species and *Pseudohylesinus n. nebulosus* were sampled. If multiple populations were collected for a species, the locale for each population is numbered in the table.

Species	Localities sampled
D. adjunctus	(1) NM: Otero Co., Cloudcroft; (2) Mexico: Estado de Mexico
D. approximatus	CO: La Plata Co., Durango
D. armandi	China: Shaanxi Province
D. brevicomis	(1) CO: La Plata Co., Durango; (2) CA: Riverside Co., San Jacinto
D. frontalis	(1) TX: Angelina Co., Lufkin; (2) Mexico: Municipio Tancitaro, Est. de Michoacan
D. jeffreyi	(1) CA: Lassen Co., Susanville; (2) CA: Sierra Nevada Mt., Placerville
D. mexicanus	Mexico: Cerdo Cordo, Valle de Bravo, Est. de Mexico
D. micans	France: Les Estables, Departement de la Haute-Loire
D. murrayanae	Canada: Elkford, British Columbia
D. ponderosae	(1) CO: Boulder Co., Boulder; (2) CA: Lassen Co., Susanville
D. pseudotsugae	(1) CO: Jefferson Co., Deckers; (2) WY: Shoshone N.F
D. punctatus	Canada: Seventy Mile House, British Columbia
D. rhizophagus	Mexico: Municipio de Modena Chihuahua, Est. de Chihuahua
D. rufipennis	CO: Clear Creek Co., Empire
D. simplex	(1) Canada: Saskatchewan; (2) NY: Schenectady
D. terebrans	TX: Angelina Co., Lufkin
D. valens	(1) CO: La Plata Co., Durango; (2) CA: Lassen Co., Susanville
P. n. nebulosus	CO: Boulder Co., Boulder

base-pair fragment of the mtDNA gene Cytochrome Oxidase I (COI) using the polymerase chain reaction (PCR). Using 1 μl of extracted DNA, we amplified double-stranded DNA product under the following conditions: 10 pmole of each primer, 200 μM of each dNTP, 2.0 μM $MgCl_2$, 1X buffer provided by Promega (Madison, WI) and 1 unit of Taq DNA polymerase (Promega) in 50 μl total volume. Typical thermal cycling conditions were a 95°C denaturing step for 1 min followed by a 47°C annealing for 1 min and a 72°C extension for 2 min. This series of steps was repeated 35 times and ended in an indefinite 4°C refrigeration period until removal. For manual sequencing, 1 μl of the double-stranded product was then used to make single-stranded DNA with one of the primers from the double- stranded reaction. The reaction conditions were similar to the ones used to amplify double stranded product with the following changes: 30 pmole primer and 2 units Taq polymerase (Promega) were used in a total volume of 100 μl, and the thermal cycling conditions were changed to a 55°C annealing temperature and a total of 30 cycles. Both double-stranded and single-stranded PCR reactions were cleansed using QIAquick Spin PCR Purification columns (QIAGEN Inc., Chatsworth, CA) and eluted in 50 μl 10mM Tris-HCl pH 8.0. The single-stranded DNA was dried down for 2 hr in a vacuum centrifuge and brought up to 13 μl in 10 mM Tris. We sequenced 3 μl of the concentrated single-stranded DNA using Sequenase 2.0 (United States Biochemical, Cleveland, OH) following the protocol supplied with the kit. Double-stranded and single-stranded PCR reactions were carried out using the primers C1-J-1718 and C1-N-2962 (see Table 3). The sequencing primers were C1-N-2973, C1-N-2650, C1-N-2542, C1-N-2416, C1-N-2191, C1-N-2099, C1-J-2797, C1-J-2673, C1-J-2441, CI-J-2411, C1-J-1718, and C1-J-1863 (Table 3). Both strands were sequenced in all samples with the "J" strand sequenced on an automated Perkin-Elmer ABI 377 in the Molecular, Cellular, and Developmental Biology sequencing facility at the University of Colorado.

Sequence Analysis

Sequence data were entered, compiled and edited for analysis using the Sequencher 3.0 program (Gene Codes Corporation, Ann Arbor, MI). The alignment of the protein-coding genes was checked and compared with that of *Drosophila yakuba* (Clary and Wolstenholme 1985). Maximum-parsimony and bootstrap analyses were performed using PAUP* test, version 4.0d55. Decay analyses were performed using the program Autodecay 3.0 (Erikkson and Wilkström 1996). Maximum-parsimony analyses were performed using PAUP*

TABLE 3. Primers used for PCR and sequencing. The origins and names of the primers come from a compilation of primers designed from insects in Simon et al. (1994). This is true with the exception of a few, denoted by asterisks, that were designed exclusively for this project from *Dendroctonus* sequences, and primer "C1-N-2962" designed by S. Dobler and "C1-N-2542" designed by B. Farrell both for use with chrysomelid beetles. In the primer name, "C1" refers to cytochrome oxidase I, "N" and "J" refer to whether it was designed from the miNority or the maJority strand (see Simon et al. 1994, p. 687 for explanation), and the number indicates the position of the primers 3′ end when aligned with *Drosophila yakuba* (Clary and Wolstenholme 1985). Primer sequences read from 5′ to 3′ and standard (IUPAC) codes are used for degenerate sites.

Name	Sequence
C1-J-1718	GGA GGA TTT GGA AAT TGA TTA GTT CC
C1-J-1863*	GCY TCT AAT ATT KCT CAT GAA GG
C1-J-2411*	CTA TAA TTA TTG CAG TTC C
C1-J-2441	CCA ACA GGA ATT AAA ATT TTT AGA TGA TTA GC
C1-J-2673*	CAA TGA TTC CCT CTT TTT ACA GG
C1-J-2797	CCT CGA CGT TAT TCA GAT TAC C
C1-N-2099*	GTA GTG TTG ATA TTT CGG TC T
C1-N-2191	CCC GGT AAA ATT AAA ATA TAA ACT TC
C1-N-2416*	CAT CTA AAA ATT TTA CTT CC
C1-N-2542	GTA ATA TCA ATG GAT RAA TTA GC
C1-N-2650*	CAA TGA TTC CCT CTT TTT ACA GG
C1-N-2962	GG AAG TTC WGA ATA TCT ATG TTC WGC WGG WG

heuristic search function (Swofford 1993) with 100 random addition sequences. Although this algorithm does not guarantee finding the most parsimonious tree(s), the use of a large number of searches with a relatively small number of starting taxa (21) helped assure that a large portion of the tree space was searched (Maddison and Maddison 1992). For outgroups, we sequenced individuals of *Pseudohylesinus n. nebulosus*, a species from a closely related genus within the same subfamily (Hylesinae) and tribe (Tomicini) as *Dendroctonus* (Wood 1982) and obtained from Genbank the following additional sequences from two other scolytid genera representing the two subfamilies of Scolytidae: *Tomicus minor* (Hylesinae: Tomicini; Genbank Accession No. U82583) and *Orthotomicus erosus* (Scolytinae: Ipini; Genbank Accession No. U82236).

Analysis of Character Evolution

Character mappings of the transitions between specialists and generalists and between different genera of host plants were performed using MacClade 3.0 (Maddison and Maddison 1992). For these analyses, we rooted the trees with the single outgroup *Tomicus* because our analysis indicated it to be the most closely related genus to *Dendroctonus* (Fig. 1), although rooting with any of the outgroups gave the same answers. All *Tomicus* species feed on *Pinus*, and most appear to share a generalist habit (Wood 1982), but we also used the most basal *Dendroctonus* species, *D. armandi* (Fig. 1), as an outgroup in the analysis of diet breadth evolution because diet breadth appeared to evolve quite rapidly.

Permutation tests (Faith and Cranston 1991) were performed using the PTP utility in PAUP*. In our utilization of the PTP utility, we randomized the taxa (i.e., their associated ecological character states) 10,000 times while holding the tree topology constant (as a constraint tree). This application of the PTP test produces a distribution of the frequencies of the number of steps (i.e., the tree length) of the randomly permuted character to test whether the observed tree length is significantly shorter (i.e., more correlated with phylogeny) than expected under a random model (Maddison and Slatkin 1991). We also tentatively introduce another utilization of the PTP utility that tests whether particular character states are in significantly derived/dispersed positions (i.e., are tippy) on the phylogeny (Farrell et al., unpubl. ms.). Specifically, we test whether the specialists (i.e., state 1) collectively

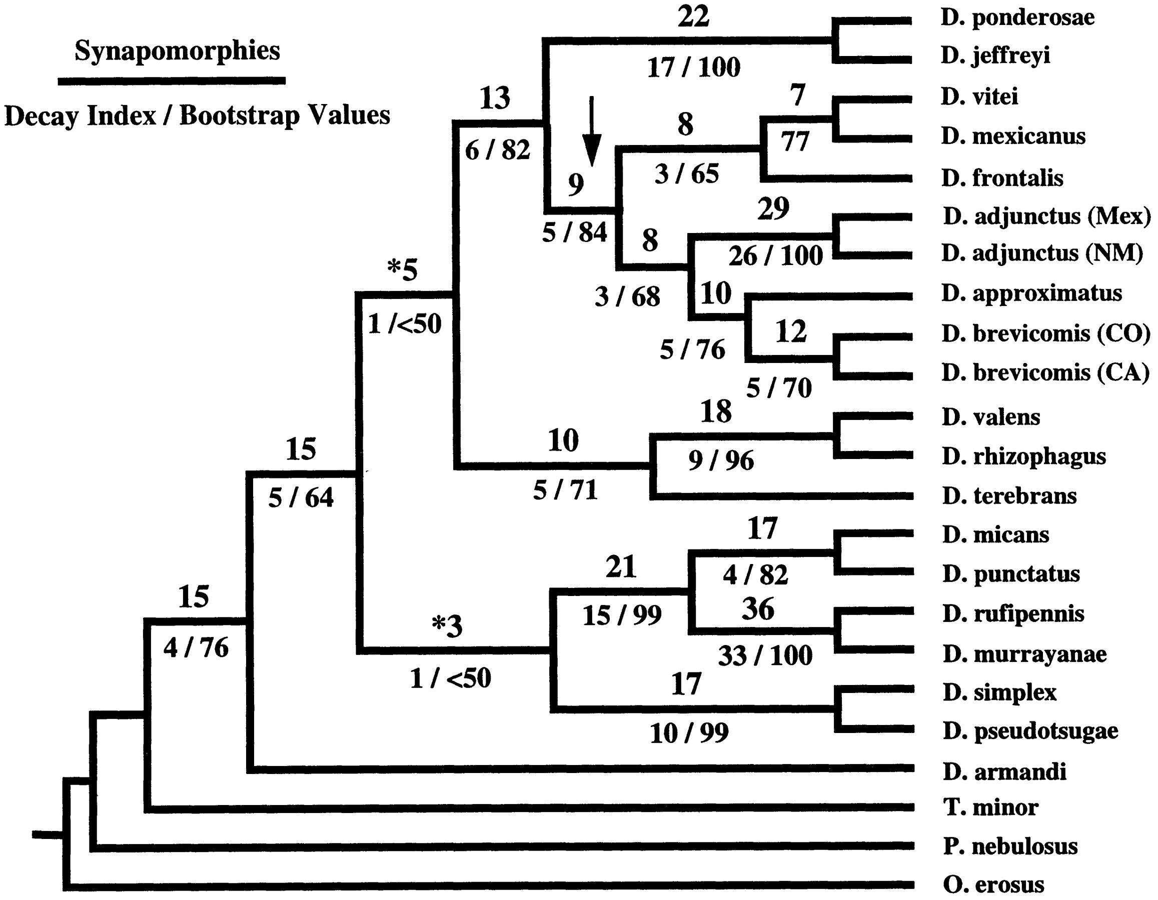

FIG. 1. Single most-parsimonious tree for the relationships among 18 of 19 species of *Dendroctonus* based on information from the cytochrome oxidase I mitochondrial gene. Numbers of synapomorphies are given above the branches. Decay indices and bootstrap values (calculated without *D. vitei*, which has much missing data, see Methods) are presented below the branches. Asterisks denote clades with relatively weak support (bootstrap proportions < 0.50). The arrow indicates the alternative placement of *D. frontalis* when *D. vitei* is included in the analysis.

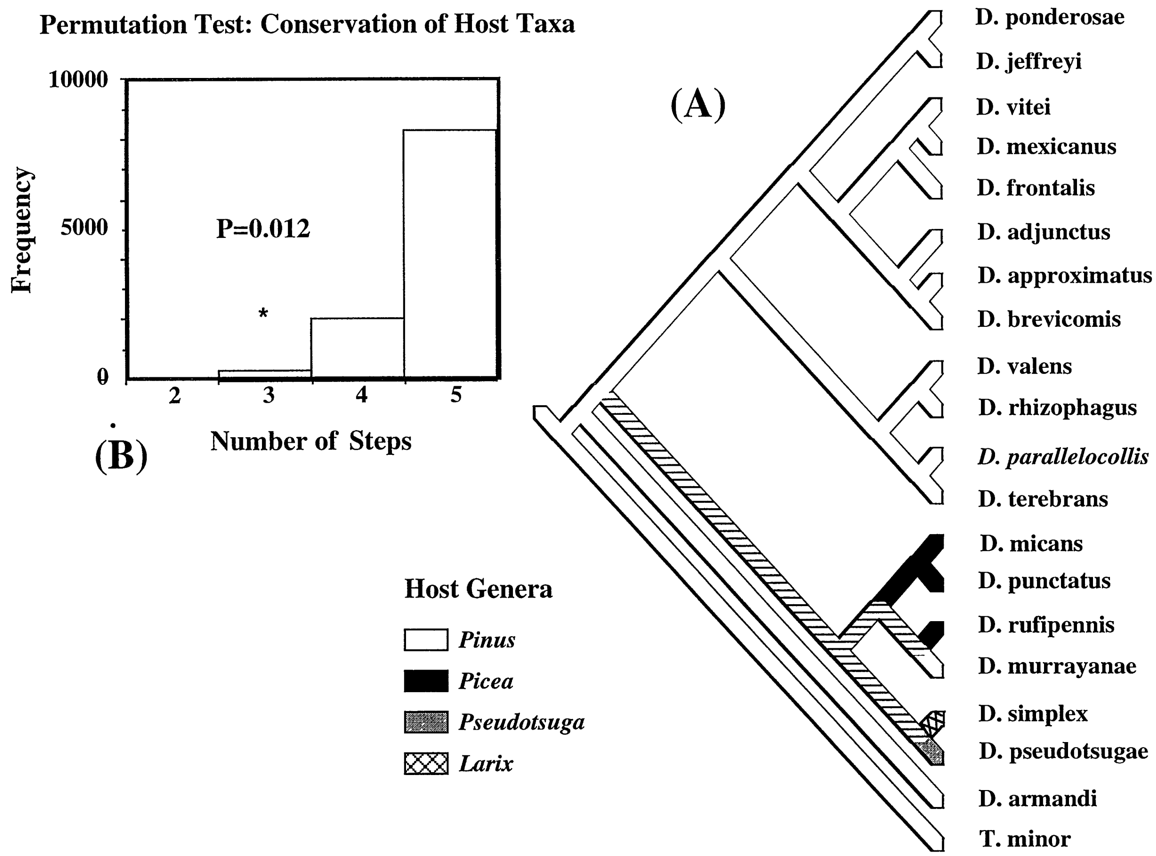

FIG. 2. (A) Phylogeny estimate of all 19 *Dendroctonus* species mapped with most-parsimonious transitions between host genera of Pinaceae. The name of the one beetle species, *D. parallelocollis*, for which we do not have sequence data is in italics. Because the phylogeny estimate in Figure 1 corresponds very closely to Wood's (1982) groupings of *Dendroctonus* species, we place this taxon according to Wood (1982) for completeness. Its inclusion does not change the significance of any of the tests. The arrow indicates the alternative position of *D. frontalis* when *D. vitei* (which has missing data) is included in the analysis. The alternative placement of *D. frontalis* does not affect any of the test results or interpretations. (B) Histogram detailing the results of a permutation tail probability test randomizing the character host genera across beetle species, holding the tree topology constant ($P = 0.012$).

occupy positions that are a surprisingly large number of nodes from the root of the tree. To accomplish this, the character state changes were fixed to be "irreversible down" (1→0) going from specialist to generalist. This procedure counts a step for each node that lies between an origin of a specialist (1) and the root by requiring a separate origin for each instance of a generalist (0) that arises from such intervening nodes. Therefore, the more derived and dispersed (tippy) the specialists are, the more steps will be added to the overall tree length. We then permuted the observed states across the tree 10,000 times, producing a frequency distribution for evaluation of whether the observed number of steps (under irreversibility) is *significantly longer* than randomly generated tree lengths.

Tests of the hypothesis that host affiliations are correlated with beetle phylogeny took two forms: (1) association with each of four conifer genera; and (2) association with *Pinus* and *Picea* (i.e., separately, as states 0 and 1) versus the other conifer genera, *Pseudotsuga* and *Larix*, which were grouped as a single character state (i.e., state 2). This procedure was followed for two reasons. First, *Pseudotsuga* and *Larix* are close relatives, and *D. pseudotsugae* has been found to feed occasionally on *Larix*, although it is not a common host. Second, the use of these two genera is autapomorphic and can fit the phylogeny estimate no better or worse than observed.

Note that for all the following tests, we added to the phylogeny *D. parallelocollis*, which we have not sequenced. For completeness we included this species according to S. Wood's (1982) placement (Fig. 2) because all other aspects of the phylogeny estimate reported here show close correspondence to Wood's morphologically based groupings.

To examine patterns in the evolution of specialization in *Dendroctonus*, we first determined whether there were distinct categories of specialization that would allow parsimonious reconstruction of ancestral states. Because the use of host genera seems strongly conserved in this group, we tallied the number of species within host genera (as opposed to all Pinaceae or all conifers) within the geographic range of each beetle species, judging these to comprise the set of potentially available hosts. We excluded those very few conifer species that were unused by any *Dendroctonus* species. We then plotted the frequencies of *Dendroctonus* species in incremental proportions of available hosts used (from zero to one) for statistical evaluation of variation in apparent diet breadth. Finally, we assessed whether specialists tended to be in derived positions, both graphically in MacClade 3.0 and statistically with the altered PTP test described above. For these analyses, we left *D. simplex* and *D. pseudotsugae* undetermined because, although they use all the congeners in their ranges, there are only one and two host species available, respectively (Table 1).

Statistical analyses were performed using SAS/Insight (Statistical Analysis Software, Raleigh, NC) on a Sun Sparc 20/712 computer at the University of Colorado. Analyses of the correspondence of beetle and host phylogeny were performed with TreeMap 1.0 (Page 1994). This program maximizes cospeciation events by permitting host shifts, duplication, or extinctions and then compares the observed number of cospeciation events to a distribution produced by randomizing the tree topology of the hosts or herbivores. For these tests, we used the *Pinus* phylogeny estimate of Krupkin et al. (1996) and the relationships among the Pinaceae genera from Price et al. (1987). For evaluation of correlation of beetle host use with host range, we used *Pinus* species distributions in Critchfield and Little (1966) and estimated the total geographic area of distribution by weighing paper tracings of these distributions on a fine-scale balance adjusting for scale following Sturgeon and Mitton (1982). Using the weight of a traced known area (the state of Colorado), the distribution weights were then converted to square kilometers (Appendix). Finally, information on *Dendroctonus* distributions and host use in the Scolytidae came from Wood (1982) and Wood and Bright (1992).

RESULTS

Sampling of Mitochondrial DNA Sequences

We obtained a 1075-bp region of COI from 30 individuals representing 18 species of *Dendroctonus* and an outgroup. Nucleotide composition was 37% A, 30% T, 17% G and 15% C. For 12 of the species, we gathered sequence data from individuals of more than one population (Table 2). In the majority of cases, individuals of the same species from separate populations differed less than 1% at the sequence level in COI. In these cases, with so few differences, we made consensus sequence from the populations to reduce the search time of the phylogenetic analyses (e.g., if a base position was A in one individual and T in another, we left it as A/T). However, in two cases individuals of the same species demonstrated more pronounced sequence differentiation. Two populations of *D. adjunctus*, one from New Mexico and one from Mexico (Est. de Mexico), differed in 2.9% of their positions in COI and two populations of *D. brevicomis*, one from California and one from Colorado, differed in 8.9% of their positions in COI. We treated these populations as distinct taxa in the phylogenetic analysis (Fig. 1). Finally, because the DNA sample of *D. vitei* (prepped from a museum specimen) yielded small amounts of DNA, we were only able to PCR amplify and sequence short regions on either end of COI for this species. Therefore, we performed analyses with and without this taxon (see below; for phylogenetic analysis with missing data see Wiens and Reeder 1995). The nucleotide sequences have been deposited in Genbank under accession numbers AF067985–AF068005.

Phylogenetic Analysis and Tree Robustness

Phylogenetic searches with maximum parsimony yielded a single most-parsimonious tree (length = 1541 steps, consistency index = 0.46; Fig. 1). Bootstrap values were high ($> 70\%$) for most of the nodes in the tree with the majority of the values exceeding 80% (Fig. 1). Because of the considerable (nearly 600 bp or 60%) missing sequence data from *D. vitei*, we performed phylogenetic analyses with and without this taxon. When *D. vitei* is excluded, *D. mexicanus* and *D. frontalis* form a group with a 65% bootstrap percentage. When *D. vitei* is included, *D. vitei* groups with *D. mexicanus* and *D. frontalis* moves to the site of the arrow in Figure 1. However, because the missing data in *D. vitei* apparently disrupts the otherwise robust association between *D. frontalis* and *D. mexicanus* we include the grouping (*D. frontalis* (*D. vitei, D. mexicanus*)) in the phylogeny estimate (Fig. 1). We note that these three species were also treated as a group of very closely related species by Wood (1982).

Host-Plant Affiliation: Genera

The common ancestor of *Dendroctonus* appears to have been associated with the genus *Pinus* (Fig. 2A). There have been as few as one to as many as four shifts from *Pinus* species onto other coniferous hosts. However, given that *D. pseudotsugae* and *D. simplex* feed on the closely related *Pseudotsuga* and *Larix*, we suggest that there was only one switch to either (or both) of these genera, and that a single shift to *Picea* was followed by reversal in *D. murrayanae*. Association with each of the four host genera showed only weak overall correlation with beetle phylogeny (PTP test; $P = 0.18$), whereas association with *Pinus* and *Picea* vs. association with other conifers (*Larix* and *Pseudotsuga*) revealed a much stronger correlation (PTP test; $P = 0.012$, Fig. 2B). To determine whether use of *Pinus* species is correlated with beetle phylogeny (i.e., related species of *Dendroctonus* use similar sets of host species), we used the PTP test to randomize the observed associations with *Pinus* species across the phylogeny, but did not find a significant correlation ($P = 0.1$). However, when we excluded *D. valens*, which is unusually broad in both diet and distribution (see Fig. 4, Appendix), we found a significant correlation (PTP test, $P < 0.02$). (*Dendroctonus valens* utilizes almost three times as many hosts [i.e., 22; average = 6 ± 5.2] as the average *Pinus*-feeding *Dendroctonus*.) Thus, related *Dendroctonus* do tend to feed on similar sets of host species. However, randomization tests using TreeMap 1.0 (Page 1994) did not reveal significant correspondence between the phylogenies of *Pinus*-feeding *Dendroctonus* and either *Pinus* species ($P = 0.28$) or the four host genera ($P = 0.82$).

Patterns of Specialization

Figure 3 shows a histogram of the proportion of hosts used by *Dendroctonus* species. The distribution is distinctly nonnormal (Kolmogorov-Smirnov goodness-of-fit test, $P < 0.0001$) and bimodal ($g^2 = -1.33$). The gap in the distribution showed that the proportion of hosts used was not a continuum. Rather, the bimodal distribution suggested a dichotomy: specialists utilize $\leq 40\%$ of the available hosts,

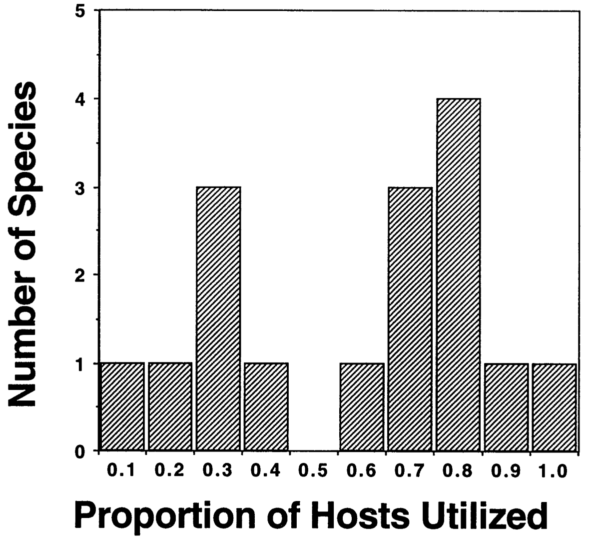

FIG. 3. Histogram of the proportions of hosts used by species of *Dendroctonus*. Proportions of hosts used are given on the x-axis and the number of *Dendroctonus* species is shown on the y-axis. The distribution is significantly nonnormal (Kolmogorov-Smirnov goodness-of-fit test, $P < 0.0001$) and bimodal ($g^2 = -1.33$).

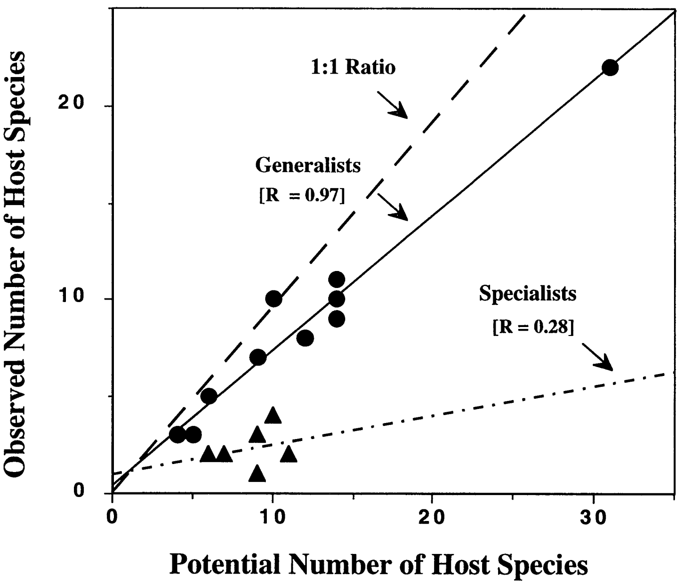

FIG. 4. Correlation between the number of host species available and the number of host species used over *Dendroctonus* species distributions, using the classification of generalists and specialists indicated in Figure 3. We counted the number of host species available to each beetle species as all the conifer species that were of the genus to which each beetle species is restricted (see Fig. 2). The correlation is significant among generalist species, indicated by the circles ($R = 0.97$, $P < 0.0001$; $R = 0.87$, $P < 0.001$ with the extreme value, representing *D. valens*, removed). There was no significant correlation between potentially available hosts and observed hosts among specialists ($R = 0.27$, $P = 0.59$).

whereas generalists utilize ≥ 60% of the available hosts. Using this classification of habits, a Spearman-Rank correlation between the potential (i.e., congeneric) host-plant species encountered by the generalist species and the host-plants actually used is highly significant ($R = 0.97$, $P < 0.0001$; Fig. 4), whereas that for the specialists is not ($R = 0.27$, $P = 0.59$; Fig. 4). Although we recognize that these comparisons effectively treat species as independent observations (Felsenstein 1985), this approach is reasonable because diet breadth does not seem correlated with phylogeny in *Dendroctonus* (Fig. 5A).

Once diet breadth was categorized into the two distinct habits (specialist and generalist), we mapped transitions in diet breadth along the phylogenetic tree. The generalist strategy appears to be the ancestral habit and has given rise to much more specialized habits at least six different times without reversal (Fig. 5A). In regards to the phylogenetic conservatism of specialization, a permutation test failed to reject the null hypothesis that diet breadth is uncorrelated with phylogeny (PTP test; $P = 0.64$). We also discovered that specialists tended to be derived (tippy) and dispersed across the phylogeny (PTP test [under irreversibility]; $P < 0.04$). Additionally, we examined trends in the evolution of specialization by mapping the proportion of hosts used as a continuous character on the phylogenetic tree and found an overall, but not invariable, decrease in the proportion of hosts used toward the tips of the phylogenetic tree (Fig. 5B).

Because our results seemed to indicate a dichotomy in diet breadth, we tested whether the six *Dendroctonus* specialists feed on *Pinus* species that are underutilized by other scolytid bark beetles or that are more or less widely distributed than average. We found *Dendroctonus* pine-feeding specialists tend to feed on *Pinus* that are attacked by significantly more bark beetle species (Wilcoxon ranked sums test; $P < 0.0001$; Appendix), but that the *Pinus* hosts of specialists are neither more nor less widely distributed than *Pinus* species used by generalists (Wilcoxon paired sample test, $P < 0.4$; Appendix).

DISCUSSION

The most apparent pattern in the evolution of resource use in *Dendroctonus* is the conserved affiliations with the genera *Pinus* and *Picea* through most speciation events (Fig. 2). The most recent common ancestor of *Dendroctonus* species appears to have used the genus *Pinus*, and there have been few shifts from *Pinus* to other host genera within the gymnosperm family Pinaceae (Table 1; Fig. 2). Therefore, there would seem to be barriers to colonization of new host genera that are rarely surmounted, even though members of all the host genera are available within the ranges of nearly every *Dendroctonus* species. The conserved association with *Pinus* is sufficiently pronounced that related species even tend to use the same *Pinus* species (if host use by *D. valens*, an unusually polyphagous and wide-ranging species, is discounted), but not so conservative that *Dendroctonus* phylogeny reflects *Pinus* phylogeny. Thus, the evolution of host affiliation in these beetles is typical of most insect herbivore groups, which exhibit moderately strong levels of conservatism with respect to host taxa used (Farrell and Mitter 1993).

Although we have characterized each species of *Dendroctonus* as feeding on plants belonging to only one host genus, some little-understood exceptions to this pattern occur during outbreaks of three species, namely *D. micans, D. pseudot-*

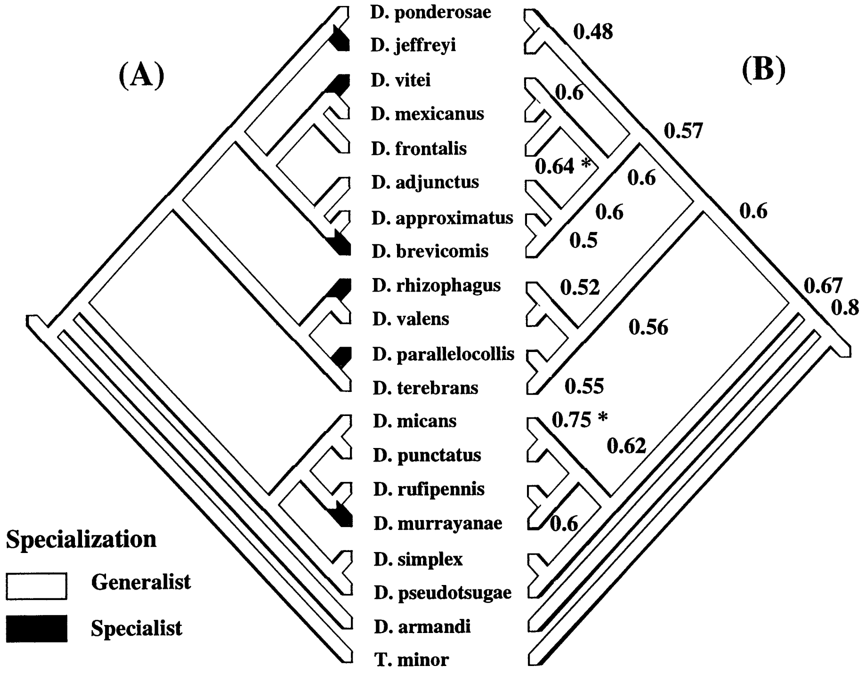

FIG. 5. (A) Most-parsimonious reconstruction of the transitions between (dichotomously defined) generalists and specialists in *Dendroctonus* (see Results; Figs. 3, 4). Specialized habits arose six different times in the genus and are not reversed. (B) Ancestral state reconstructions of the proportions of hosts utilized by *Dendroctonus* using MacClade 3.0 procedure that minimized the sum of squared changes. There is a gradual decrease in the proportions of hosts utilized moving from the base to the tips of the phylogeny, except in a few instances (noted by asterisks) where the proportion of host use increases. The nodes containing specialists all decrease.

sugae, and *D. valens* (Table 1). Investigations of *D. micans*, for example, showed that the majority of the normally *Picea*-feeding *D. micans* larvae reared on *Larix, Pseudotsuga*, and *Abies* (all reported hosts of *D. micans*) did not survive to maturity, whereas larvae survived best on *Picea* and moderately well on *Pinus*, the ancestral host in *Dendroctonus* (Wainhouse and Beech-Garwood 1994). Studies of performance on alternate hosts in these other two outbreaking species have not yet been undertaken. Nevertheless, given that shifts among these host genera have occurred in *Dendroctonus* phylogenesis and that outbreaks are sometimes accompanied by unusual use of conifer genera that serve as hosts to other *Dendroctonus* species, outbreaks do seem a potential mechanism of host shift in these insects. For example, the shift to *Pinus* in *D. murrayanae* from an apparently *Picea*-feeding immediate ancestor may have been facilitated during such a population surge in the common ancestor with *D. rufipennis*, a notoriously outbreaking species (see Fig. 2). The permanence of such shifts presumably depends on the persistence and localization of alternate hosts, which may in turn depend on characteristics of population structure (Futuyma 1987).

Long evolutionary associations such as those between *Dendroctonus* and *Pinus* seem common in phytophagous insects (Futuyma and McCafferty 1990; Farrell and Mitter 1993; Funk and Futuyma 1995; Dobler et al. 1996; Becerra 1997; Farrell 1998). Long-conserved associations, maintained over broad ranges, might argue more for limitations on genetic variation in physiological and behavioral traits for use of different host genera, whereas associations with particular host congeners would seem more likely to be maintained by stabilizing selection. Consistent with this conjecture are experiments by Futuyma et al. (1995) on the composite-feeding *Ophraella* beetles. The several *Ophraella* species investigated show a lack of genetic variation for feeding on many of the confamilial plants used by closely related species, whereas many *Ophraella* species show geographic variation in use of congeneric host species.

Specialization in Dendroctonus

Over 70% (i.e., 14 of 19) of *Dendroctonus* species are apparent generalists, able to use a majority (≥ 60%) of the pine or spruce hosts in their respective ranges. The distribution among *Dendroctonus* species of the trait "generalist" may be entirely ascribed to common ancestry (i.e., there is no convergence), suggesting there is homology in diet breadth, per se, as generalists parasitize both *Pinus* and *Picea*. Although the generalist habit is apparently plesiomorphic and is certainly widespread in *Dendroctonus*, each of the six instances of specialization are independently derived, namely in *D. brevicomis, D. jeffreyi, D. murrayanae, D. rhizophagus, D. parallelocollis*, and *D. vitei*. Specialization is not just a by-product of geographic range; the range of the pine-feeding *D. brevicomis*, for example, extends over much of the western United States and overlaps the distributions of 11 different pine species, yet *D. brevicomis* feeds on only two pines: *Pinus ponderosa* and *P. coulteri*. Indeed, narrow range may even preclude estimates of relative specialization. Thus two species that have been previously classified as specialists (*D. pseudotsugae* and *D. simplex*; Wood 1982; Critchfield and Little 1966) because they use only one or two host species, use all the geographically available species in their respective host genera *Pseudotsuga* and *Larix*. Indeed, *D. simplex* and *D. pseudotsugae* present a seeming paradox of this approach to characterizing specialization. These two species use all the hosts "available" in their ranges, yet they use fewer hosts than some of the specialists we identify. Nevertheless, the importance of considering resource availability in measuring variation in "niche breadth" seems unequivocal (Colwell and Futuyma 1971).

Evolution of Specialization

The invariable occurrence of specialists in the youngest (i.e., most derived) positions (Fig. 5A) is consistent with the idea that specialists either have high extinction rates or that the trait "specialization" is ephemeral and new specialist species either eventually broaden their diets or disappear. The strong differentiation in mtDNA within *D. brevicomis* (on different hosts in different places) suggests a role for geography in fostering specialization. Similarly, allozyme studies revealed fixed allelic differences between populations of *D. pseudotsugae* from the coastal race of Douglas-fir in Oregon and from the inland race of Douglas-fir in Idaho (Stock et al. 1979), whereas host-associated allozyme polymorphism has been reported among samples of *D. ponderosae* from lodgepole, limber, and ponderosa pine in Colorado (Sturgeon and Mitton 1986). It appears that populations of these beetles may evolve local specialization concomitant with isolation, perhaps rather rapidly. Although such local differentiates may often be lost to extinction, specialist species might accumulate if differentiation is sometimes preserved by reproductive isolation (Mayr 1954; Futuyma 1987). Supportive of this conjecture is the observation that at least some specialists use subsets of the hosts of generalist ancestors (Fig. 6). While it appears that specialists tend to be in derived positions in *Dendroctonus*, there are surely many examples to the contrary (Thompson 1994). More phylogenetic work on this question clearly needs to be done to assess whether the patterns seen in *Dendroctonus* are common in herbivorous insects and other organisms.

Although we found no evidence for parallel phylogenesis between the phylogeny of the beetles and their hosts, *Pinus* species use is correlated with *Dendroctonus* phylogeny (PTP test; $P < 0.02$). In other words, there is some phylogenetic conservatism in the use of particular host species, such that related beetles overlap in their associations. This permitted parsimony-reconstruction of ancestral associations of several *Dendroctonus* specialists (*D. jeffreyi, D. brevicomis*, and *D. vitei*), which revealed particular *Pinus* species that were dropped from their diets that were used by their more generalized ancestors (Fig. 6). Another corollary of such persistent affiliations would be that retention of ancestral host species should result either in extinction or reinforcement/character displacement between nearest relatives, if these become sympatric. In fact, populations of the sister-species *D. mexicanus* and *D. frontalis* in Mexico (Zuniga et al. 1995) and Honduras (Wood 1982) and of *D. approximatus* and *D. brevicomus* in Colorado (S. Kelley, unpubl. obs.) are known to coexist in the same individual trees. Laboratory crosses of the first pair of species (which differ in chromosome number) produce inviable hybrids (Zuniga et al. 1995), whereas each species of the latter pair uses different parts of the trees (Wood 1982; S. Kelley, unpubl. obs.).

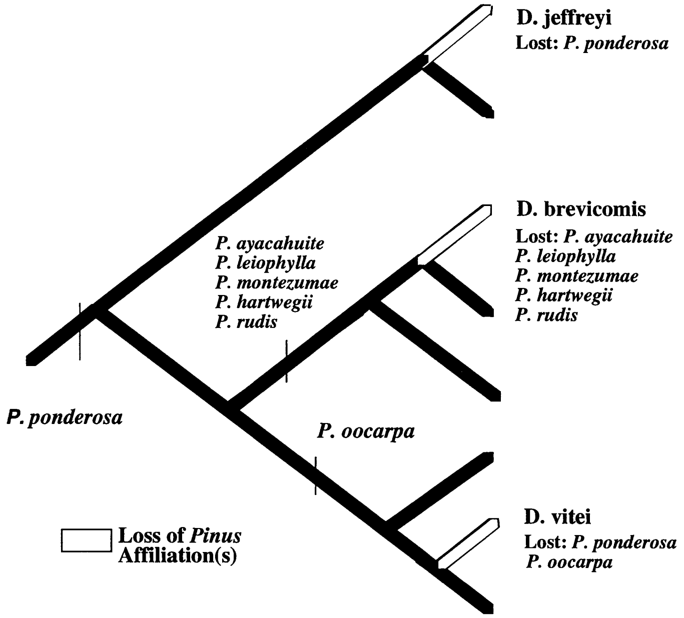

FIG. 6. Reconstructions (under ACCTRAN optimization in PAUP) of affiliations of hosts shared among the closest relatives of three specialist *Dendroctonus* species. Most of these species also use unique hosts (see Appendix). *Dendroctonus jeffreyi* appears to have dropped ancestral affiliations with *P. ponderosa*, one of the most widespread pine species, whereas *D. brevicomis* apparently lost affiliations with five *Pinus* species of much narrower distributions. *Dendroctonus vitei* also seems to have lost three ancestral *Pinus* species. The other three *Dendroctonus* specialists either show no geographic (and hence no host-range) overlap with their near-relatives (*D. rhizophagus, D. parallelocollis*) or use a different host genus (*D. murrayanae*).

Advantages of Specialization

Among the commonly believed advantages of specialization on particular host plants are freedom from competitors and natural enemies and enhanced physiological efficiency in converting resources into offspring (for reviews of this literature see Futuyma and Moreno 1988; Jaenike 1990). The sizable literature on bark beetle host use assembled in Wood and Bright (1992) provides a rough and indirect assessment of the first two of these hypotheses. Although other bark beetle species would seem to be the very closest competitors of *Dendroctonus* species, we found that *Dendroctonus* pine-feeding specialists tend to feed on *Pinus* species that are associated with *significantly more* bark beetle species (Wilcoxon ranked sums test; $P < 0.0001$; Appendix). Contrary to expectation under an enemy-free space hypothesis (Price et al. 1980; Bernays and Chapman 1994), there is also no evidence that *Dendroctonus* specialists have fewer predators or parasitoids than generalists (Dahlsten 1982).

Although it might also be supposed that specialists should prefer more widespread (and hence more easily found) hosts, or, conversely, that specialization might be favored on hosts that are narrow in distribution, neither of these possibilities seems to be true. The *Pinus* species used by *Dendroctonus* specialists are neither more nor less widespread than *Pinus* used by generalists (Wilcoxon paired sample test, $P < 0.4$). However, the fact that there are significantly more scolytid beetle species known to feed on the hosts of the specialists suggests that these hosts might be generally better resources. Nevertheless, the possibility remains that the initial fitness advantages of specialization responsible for fixation of the trait are ephemeral and that irreversibility has a genetic, rather than an ecological, explanation.

An Evolutionary Concept of Specialization

The phylogeny of host use in *Dendroctonus* suggests a general strategy to characterize specialization using a phylogenetic approach. As mentioned previously, most species are restricted to a single genus of host but use a majority of the congeneric species encountered in their respective ranges. More often than not, these species seem to inherit both affiliations with certain genera as well as the ability to use a fairly broad array of congeners. Although we have characterized these species as relative generalists, these would seem to correspond to the oligophagous habits typical of many plant-feeding insect groups (Bernays and Chapman 1994). However, the six specialists depart strongly from this pattern toward a more "monophagous" habit (although, strictly speaking, monophagy would mean feeding on only a single host species). Finally, the three outbreaking species that (occasionally) use multiple host genera may be departures in the opposite direction, that is, toward polyphagy. If these prove able to sustain use of multiple hosts through successive generations, their polyphagous habits also may reflect fundamental differences in several aspects of host use. Given these patterns, we suggest that evidence on the phylogeny of resource use may permit discovery of which aspects of resource use are conservative and which are labile. Therefore, phylogenetic analysis allows detection of fundamental departures in strategy within particular groups and thus presents a way in which to define degrees of specialization in an evolutionary context. Such an approach may lend additional focus to ecological and genetic study of particular species and provide a common basis for comparative study across groups.

ACKNOWLEDGMENTS

We wish to thank the following colleagues for sending critical samples of *Dendroctonus* and directions to productive field locales: J. C. Gregoire, M. Furniss, Z. Jiaxi, G. Zuniga Bermudez, G. Sanchez-Martinez, D. Langor, D. Linton, W. Schaupp Jr., D. Grosman, D. Six, B. Bentz, M. Ramos, D. Schultz, and A. Cognato. Special thanks to D. Leatherman for providing specimens, locales, and field assistance and to D. Bright for encouragement and for confirming identifications. We are grateful to J. Mitton and R. Ramey for comments, advice, and technical support on this project. A. de Queiroz provided invaluable assistance with phylogenetic inference, computing, and helpful reviews of the manuscript; and M. Grant provided assistance and advice on statistical procedures. For advice, discussion, and constructive criticism we thank M. D. Bowers, S. Dobler, B. Kreiser, B. Latta, R. Lewontin, Y. Linhart, E. Mayr, G. Morse, B. Normark, D. Olmstead, M. Peterson, and N. Pierce. For critical review of this manuscript, we thank J. Jaenike, O. Pellmyr, and an anonymous reviewer. Finally, in particular, we thank D. Futuyma for discussion that led to the formulation of this project. We gratefully acknowledge the support for this project that was provided by the National Science Foundation (DEB 9306667, DEB 962376) and the U.S.D.A. Competitive Grants Program. We are also thankful for financial support from the Graduate School and the Department of E.P.O. Biology at the University of Colorado, and the Theodore Roosevelt Memorial Fund at the American Museum of Natural History, New York.

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APPENDIX

Host species use among the *Pinus*-feeding *Dendroctonus.* The "P" (potential host) column indicates *Pinus* species (fed on by other *Dendroctonus*) encountered by the beetle species over its range. The "O" (observed host) column indicates *Pinus* species fed on by the beetle species. The approximate range ($\times 10^3$ km^2) of the *Pinus* species is given under the "Range" column and the number of Scolytidae known to utilize those host species is given in the "# Comp." (number of potential competitors) column.

***Dendroctonus* species**	**RANGE**	**#COMP**	*D. terebrans*		*D. valens*		*D. rhizophagus*		*D. murrayanae*		*D. parallelocollis*		*D. approximatus*		*D. adjunctus*		*D. jeffreyi*		*D. ponderosae*		*D. brevicomis*		*D. frontalis*		*D. mexicanus*		*D. vitei*	
***Pinus* species**			P	O	P	O	P	O	P	O	P	O	P	O	P	O	P	O	P	O	P	O	P	O	P	O	P	O
P. monticola	605	6			X	X			X								X		X	X	X							
P. strobus	3,405	8	X	X	X	X			X	X							X						X	X				
P. lambertiana	150	6			X	X											X		X	X	X							
P. flexilis	524	8			X				X						X		X		X	X	X							
P. strobiformis	100	3			X		X				X		X						X	X	X		X					
P. ayacahuite	108	5									X		X	X	X	X									X	X	X	
P. monophylla	415	5			X				X								X		X	X	X							
P. edulis	705	6			X	X			X				X		X				X	X	X		X					
P. balfouriana	4,504	3			X												X		X	X	X							
P. leiophylla	257	10			X	X	X				X	X	X	X	X	X					X				X	X		
P. resinosa	1,425	5			X	X																						
P. palustris	664	2	X	X																			X	X				
P. taeda	1,098	6	X	X	X																		X	X				
P. echinata	1,493	7	X	X	X	X																	X	X				
P. glabra	367	1	X																				X	X				
P. serotina	432	1	X	X																								
P. rigida	702	1	X	X	X	X																	X	X				
P. elliottii	288	3	X	X																			X					
P. ponderosa	2,160	25			X	X	X		X		X		X	X	X	X	X		X	X	X	X	X	X	X	X		
P. jeffreyi	450	10			X	X											X	X	X									
P. engelmannii	246	8			X		X	X			X		X	X									X	X				
P. montezumae	156	9									X	X	X	X	X	X									X	X	X	
P. durangensis	108	5					X	X			X																	
P. hartwegii	143	4			X	X					X		X	X	X	X											X	
P. pseudostrobus	240	8			X	X							X		X	X									X	X	X	X
P. teocote	170	4					X						X	X											X	X		
P. lawsonii	29	1			X	X							X		X										X	X		
P. sabiniana	68	1			X	X													X									
P. coulteri	28	4			X	X													X	X	X	X						
P. contorta	3,132	18			X	X			X	X									X	X								
P. banksiana	3,154	5			X				X	X																		
P. virginiana	800	5	X		X	X																	X	X				
P. radiata	78	5			X	X													X									
P. muricata	7	4			X																							
P. oocarpa	205	5			X	X	X				X	X	X		X								X	X	X	X	X	
P. murrayana	7	1			X	X																						
P. sylvestris	?	1			X	X																						
P. tenufolia	240	3			X	X					X	X			X	X									X	X		
P. rudis	143	3											X	X	X	X									X	X	X	X
P. chihuahuana	256	2											X	X														
P. albicaulis	726	2			X				X								X		X	X	X							
		TOTALS	9	7	31	22	7	2	9	3	10	4	14	9	12	8	9	1	14	11	11	2	13	10	10	10	6	2