

# Incomplete barriers to mitochondrial gene flow between pheromone races of the North American pine engraver, *Ips pini* (Say) (Coleoptera: Scolytidae)

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The pine engraver *Ips pini* (Say) is known to include three pheromone races, but gene flow between these races has not been investigated. We used maternally inherited mitochondrial DNA (mtDNA) variation to infer gene flow between 22 widely distributed North American populations of *I. pini* for a total of 217 individuals, based on 354 bp of the cytochrome oxidase I gene. Gene flow was estimated cladistically as migrants per generation ( $Nm$ ) and as haplotype variation between populations ( $N_{st}$ ). Three distinct mtDNA haplotype lineages, generally corresponding to eastern (I), Rocky Mountain (II) and western (III) regions of North America, were resolved with a total of 34 distinct *I. pini* haplotypes. The distributions of these lineages were largely congruent with the geographical ranges of the 'New York', 'California' and 'Idaho–Montana' pheromone races. Only individuals with lineage I mtDNA were observed among eastern populations, whereas individuals with lineage II or III mtDNA predominated among western populations. Gene flow ( $Nm$  and  $N_{st}$ ) was generally moderate between all populations. However, the presence of lineage I mtDNA on the eastern side of western North America and the absence of lineage II and III mtDNA in eastern North America suggest directional gene flow from east to west. These results indicate that female-controlled assortative mating among pheromone races may disrupt gene flow between conspecifics, reflecting incomplete pre-mating barriers.

**Keywords:** bark beetles; mitochondrial DNA haplotype lineages; population structure; aggregation pheromone; directional gene flow; Pleistocene effects

## 1. INTRODUCTION

Pheromone races are populations within species that differentially produce or respond to chemically mediated mating cues. Such pheromone races present a contradiction. Correlated variation in the production of and response to race-specific pheromones should disrupt gene flow and promote lineage subdivision among conspecifics. Intuitively this situation should be rare or transient; however, there are many examples of race specificity in correlated pheromone variation among conspecific insects (Lanier & Burkholder 1974; Roelofs *et al.* 1985; Phelan 1992; Butlin 1995). To date, evidence supports the hypothesis that race-specific pheromones limit gene flow. Assortative mating occurs among conspecifics exhibiting pheromone variation (Butlin 1995) and genetic divergences between pheromone phenotypes have been documented (e.g. Sperling *et al.* 1996; Pornkulwat *et al.* 1998). Nonetheless, the effect of conspecific pheromone variation on historical

levels of gene flow has not been quantified for these insect examples.

The pine engraver *Ips pini* (Say), which occurs in North America as several pheromone races, provides a system for quantifying historical levels of gene flow and interpreting the patterns in the context of pheromone variation and biogeographical events. This beetle is an economically important pest of North American pine trees and has been the subject of considerable biological research (see Wood & Bright 1992; Bright & Skidmore 1997, for references). *Ips pini* ranges from the eastern to western coasts of North America and from Alaska to northern Mexico. The eastern and western populations of *I. pini* were originally described as separate species based on slight morphological differences, *I. pini* (Say 1826) in the east and *Ips oregonis* (Eichhoff 1869) in the west. However, their morphological characters intergrade in western Canada and the species were synonymized (Hopping 1964). Lanier (1972) added further evidence of conspecificity by recovery of viable offspring from no-choice breeding experiments between eastern (Ontario) and western (California and British Columbia) parents.

Pheromones and their role in aggregation behaviour have been investigated extensively in *I. pini* (e.g. Birch *et al.*

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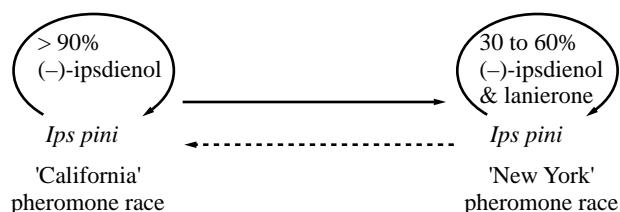


Figure 1. Attractive (solid arrows) and interruptive (dashed arrows) behavioural interactions between western and eastern *I. pini* pheromone races. The base of the arrow indicates the race producing the semiochemical blend, while the head of the arrow indicates the race responding to the semiochemical blend. The New York race of *I. pini* is partially attracted to the >90% (-)- ipsdienol produced by the California race, while the California race is interrupted by the 30–60% (-)- ipsdienol produced by the New York race. Lanierone is produced by both races, but has a much larger synergistic effect on the New York race.

1980; Lanier *et al.* 1980; Miller *et al.* 1989, 1996, 1997; Teale *et al.* 1991; Seybold *et al.* 1992, 1995). Upon finding a suitable pine host, males bore through the outer bark and into the phloem to excavate a nuptial chamber. Pheromones are produced as males feed and other males and females are attracted to the host (Birch *et al.* 1980). Multiple females enter a nuptial chamber, mate and each female constructs an egg gallery. The brood completes its development under the bark. There are from one to five generations of this species in a year, depending on the locality and the length of the season (Furniss & Carolin 1992).

Specific differences in the production of and response to aggregation pheromones provide the basis for pheromone races of *I. pini*. Two pheromone components, lanierone and ipsdienol, comprise the aggregation attractant for *I. pini* and populations across North America vary in their production of and response to lanierone and to the enantiomeric blend of ipsdienol (figure 1). Generally, populations in eastern North America produce and respond optimally to between 30 and 60% (-)- ipsdienol ('New York' phenotype), whereas populations in western North America produce and respond to >90% (-)- ipsdienol ('California' phenotype) (Seybold *et al.* 1995). Lanierone is produced in small quantities (0.2% of the amount of ipsdienol) by male *I. pini* from New York and synergizes the attraction to ipsdienol (Teale *et al.* 1991). Lanierone has recently been discovered in a California population (Quilici *et al.* 1999); however, the magnitude of the synergistic effect of lanierone with ipsdienol is not as large for California *I. pini* as it is for New York or Wisconsin *I. pini* (Seybold *et al.* 1992; Miller *et al.* 1997).

The preference of female *I. pini* for the 'con-racial' pheromone phenotype may limit mating between eastern and western *I. pini* (Lanier *et al.* 1972; Piston & Lanier 1974; Teale *et al.* 1994). For example, the attraction of California male and female *I. pini* to >95% (-)- ipsdienol is interrupted by (+)- ipsdienol (Birch *et al.* 1980), which is produced in substantial amounts by eastern males. Assortative mating due to female preference for male pheromone (Teale *et al.* 1994) and the heritability of pheromone production and response (Hager & Teale 1995) have been demonstrated in New York.

Pheromone variation also occurs within western North America. Populations from south-eastern British Columbia, Idaho and Montana produce between 91 and 95% (-)- ipsdienol ('Idaho–Montana' pheromone phenotype), while other western populations produce >94% (-)- ipsdienol (Birch *et al.* 1980; Seybold *et al.* 1995). Based on pheromone and cuticular hydrocarbon data, Seybold *et al.* (1995) hypothesized that the region with the populations displaying the Idaho–Montana pheromone phenotype represents a zone of hybridization. A more extensive investigation of pheromone phenotypes in British Columbia revealed New York, California and intermediate pheromone phenotypes of different individuals within and between populations (Miller *et al.* 1996). Given the variation between individuals and the conjunction of the New York, California and Idaho–Montana zones defined above, most of British Columbia apparently represents an area of integration of eastern and western populations. Since the populations presumably interbreed in this area (Miller *et al.* 1996), the variability in pheromone production and response in British Columbia and Idaho–Montana suggests that gene flow has occurred in this area between the New York and California races.

Isolation of the eastern and western populations of *I. pini* during the Pleistocene has been hypothesized to be the dominant factor shaping the current distributions of the pheromone races (Seybold *et al.* 1992, 1995). These insects are entirely dependent on their pine hosts and the hypothesis of evolution of *I. pini* populations in segregated refugia is supported by the glacially impacted biogeographical histories of *Pinus banksiana* Lamb. in the east and *Pinus contorta latifolia* (Engelm. ex Wats) in the west (Critchfield 1985; Prentice *et al.* 1991).

The purpose of this study was to estimate the historical extent of gene flow between pheromone races of *I. pini* and to interpret gene flow in terms of biogeographical events. To do this, we assessed mitochondrial variation via phylogenetic analysis of individuals among populations that were previously determined to vary in aggregation pheromone production (Seybold *et al.* 1995).

## 2. MATERIAL AND METHODS

The DNA extraction, polymerase chain reaction (PCR) and DNA sequencing protocols followed Cognato & Sperling (2000). Ten specimens (except Rosebud Co., Montana,  $n=7$ ) were sampled from each of 22 localities (table 1). Fourteen out of the 22 localities were identical (nine) or within 80 km (five) of the populations previously surveyed for pheromone production (Seybold *et al.* 1995) (table 1). The British Columbia locality had males that were known to have the New York pheromone phenotype (M. Domingue and S. A. Teale, personal communication). Specimens were collected from naturally infested host material, except for the Wisconsin laboratory colony (table 1). The specimens were included in the samples without regard to sex. DNA was extracted from the thorax of each specimen. The remaining specimen parts were pinned, labelled with sex, locality and haplotype, and deposited at the University of California at Berkeley Essig Museum of Entomology. The pinned specimens were each numbered and corresponding numbers were assigned to each aqueous solution of DNA. These samples were stored at -20 °C and maintained by A.I.C.

Table 1. *Locality, host data and haplotypes for I. pini populations and associated North American geographical regions*

population symbol	locality, date and collector	host	haplotype (individuals)	geographical region of North America
NY1 <sup>b</sup>	New York: Suffolk Co. Smithtown. 25 July 1997. A. Cognato	<i>P. strobus</i>	A(1), B(1) and C(8)	eastern
NY2 <sup>a</sup>	New York: Onondaga Co. Syracuse. 18 October 1995. S. A. Teale	<i>P. resinosa</i>	C(7), D(2) and E(1)	eastern
RI	Rhode Island: Lincoln Co. Lincoln Woods State Park. 19 July 1997. A. Cognato	<i>P. strobus</i>	C(9) and H(1)	eastern
MD	Maryland: Baltimore Co. Towson. 31 July 1998. K. Galacatos	<i>P. strobus</i>	C(3), H(1), Y(1), Z(3) and AA(2)	eastern
WI <sup>a</sup>	Wisconsin: Dane Co. Madison. University of Wisconsin, laboratory colony collected originally from Sauk Co. B. H. Aukema and K. Raffa	<i>P. resinosa</i>	B(3), D(2), V(2) and FF(3)	eastern
MN	Minnesota: Clearwater Co. Itasca State Park, Preachers Grove. 6 August 1998. M. J. Bohne	<i>P. resinosa</i>	B(3), C(2), D(1), U(1), V(1), W(1) and X(1)	eastern
AB	Alberta: Calgary. 10 September 1994. J. H. Borden	<i>P. contorta latifolia</i>	J(2), K(6) and M(2)	north-western/western
BC <sup>a</sup>	British Columbia: Leadqueen Lake, near Brisco. 7–8 August 1998. M. Domingue	<i>P. contorta latifolia</i>	K(6), M(2) and H(2)	north-western/western
CO	Colorado: Larimer Co. Roosevelt National Forest, near Poudre Falls. 18 June 1997. A. Cognato and K. Galacatos	<i>P. contorta latifolia</i>	F(1), G(1) and K(8)	western
SD <sup>a</sup>	South Dakota: Pennington Co. 3 km south of Pactola Reservoir T1N, R5E, S14. 22 February 1990. R. Dorsett	<i>P. ponderosa</i>	K(3), Q(4), R(1), S(1) and HH(1)	western
MT1 <sup>a</sup>	Montana: Rosebud Co. 9.7 km south of Lame Deer. July 1993. S. J. Seybold	<i>P. ponderosa</i>	H(1), I(1), K(1), M(1), Q(1), R(1) and S(1)	western
MT2 <sup>b</sup>	Montana: Missoula Co. Hayes Creek. August 1998. D. Six	<i>P. ponderosa</i>	H(2), K(1), M(2), O(4) and T(1)	north-western/western
WA <sup>b</sup>	Washington: Kittitas Co. Roslyn. 30 June 1997. A. Cognato	<i>P. ponderosa</i>	H(4), K(4) and M(2)	north-western/western
WA2 <sup>a</sup>	Washington: Okanogan Co. 19.3 km north-west of Winthrop T36N, R20E, S13. 17 September 1989. S. J. Seybold	<i>P. ponderosa</i>	H(1), K(2), M(6) and GG(1)	north-western/western
OR <sup>b</sup>	Oregon: Klamath Co. near Crescent. 11 September 1998. D. W. Ross	<i>P. contorta murrayana</i>	H(4), K(1), L(2), M(1) and O(2)	north-western/western
CA1 <sup>a</sup>	California: Modoc Co. Benton Meadow T44N, R15E, S17. September 1992. S. J. Seybold	<i>P. contorta murrayana</i>	K(1), P(1), L(1), M(3) and O(4)	western
CA2 <sup>a</sup>	California: Lassen Co. 3 km north of Butte Lake. 7 November 1997. S. J. Seybold	<i>P. jeffreyi</i>	N(1), P(3), L(1), M(2) and O(3)	western
CA3	California: San Diego Co. Cleveland National Forest near Mount Laguna. 12 March 1998. A. Cognato and K. Galacatos	<i>P. ponderosa</i>	P(4) and O(6)	south-western/western
NV	Nevada: Clark Co. Toiyabe National Forest. Mount Charleston. 3 July 1998. A. Cognato and K. Galacatos	<i>P. ponderosa</i>	O(10)	south-western/western
AZ1	Arizona: Coconino Co. Kaibab National Forest, 18.6 km south of Jacob Lake. 3 July 1998. A. Cognato and K. Galacatos	<i>P. ponderosa</i>	Q(6), BB(1), CC(1), DD(1) and FF(1)	south-western/western
AZ2 <sup>b</sup>	Arizona: Greenlee Co. Apache National Forest, Rd 56. 3 September 1996. A. Cognato	<i>P. ponderosa</i>	Q(8), R(1) and S(1)	south-western/western
NM <sup>a</sup>	New Mexico: Otero Co. Lincoln National Forest. May 1994. A. Cognato	<i>P. ponderosa</i>	I(1), Q(5), S(3) and EE(1)	south-western/western

<sup>a</sup>Population categorized for pheromone production.<sup>b</sup>Locality within 80.6 km of a population that was categorized for pheromone production (Seybold *et al.* 1995).

Mitochondrial cytochrome oxidase I DNA (mtDNA COI) from a total of 217 individuals was PCR amplified with primers CI-J-2183 (Simon *et al.* 1994) and CI-N-2611 (5'-GCA AAA ACT GCA CCT ATT GA) (Cognato & Sperling 1999) and then directly sequenced over 354 bp of the mtDNA COI gene. All *I. pini* haplotype sequences were submitted to GenBank (AF160 830–AF160 863). We chose an mtDNA genetic marker because, for most animals, it is maternally inherited (Mortiz *et al.* 1987) and, unlike nuclear genes, phylogenetic information in mtDNA sequence is less likely to be obscured by recombination. Furthermore, we chose to sequence a portion of the mtDNA COI gene for this study because Cognato & Sperling (1999) showed that it indicated high nucleotide divergence among three *I. pini* populations.

To assess the phylogenetic relationships of *I. pini* haplotypes, the mtDNA COI sequences were used in a parsimony analysis (PAUP\* 4.0b1; Swofford 1998) employing a heuristic search with ten random stepwise addition sequences. Branch support was represented as decay index values (Bremer 1994) and bootstrap values (Swofford 1998) calculated with 500 replicates. *Ips bonansea* (Hopkins), *Ips integer* (Eichhoff) and *Ips plastographus maritimus* Lanier were used as outgroups to polarize the *I. pini* haplotype phylogeny. These species represent the closest relatives to *I. pini*, as inferred from mitochondrial and nuclear sequence data (Cognato 1998).

Gene flow between geographical regions (table 1) was assessed by two measures. The cladistic method estimated the migration rate per generation ( $Nm$ ) (Slatkin & Maddison 1989; Maddison & Maddison 1992). The minimum number of migration events ( $s$ ) was determined by calculating the number of population locality changes within the mtDNA COI phylogeny. A computer simulation program was used (Slatkin & Maddison 1989) to estimate  $Nm$  and confidence limits (1000 replicates) given  $s$  and the number of individuals sampled for each population. HaploII (Lynch & Crease 1990) was used to estimate the haplotype variation between populations ( $N_{st}$ ) and calculate a test statistic for the population subdivision or structure ( $D$ ).

### 3. RESULTS

A total of 34 mtDNA COI haplotypes were found among the 217 *I. pini* individuals. The populations averaged 4.0 haplotypes and the Nevada and Montana (Rosebud Co.) individuals represented the haplotype diversity extremes. All ten individuals sampled from Nevada had the same haplotype, while each of seven individuals from Montana (Rosebud Co.) had a unique haplotype (table 1). Most haplotypes were represented in at least two populations, while unique haplotypes were found for ten populations. The uncorrected nucleotide sequence divergence among the 34 haplotypes ranged between 0.003 and 0.034 (mean = 0.020). Twenty-seven nucleotides differed among these haplotypes and these differences occurred at three first, one second and 24 third codon positions.

Phylogenetic analysis of the haplotypes without the outgroups resulted in five most-parsimonious trees. Haplotypes Q, R, BB, CC and EE were unresolved in the consensus of these trees (figure 2). Lack of phylogenetic information prevented resolution of other haplotypes in lineages I and II. Phylogenetic analysis with outgroups resulted in 323 most-parsimonious trees. The topology of this consensus tree was similar to the consensus of the five

trees found when the outgroups were excluded in the analysis. *Ips pini* haplotypes formed a monophyletic group exclusive of the outgroups; however, the *I. pini* clade was not rooted to a particular haplotype (figure 2).

Three major monophyletic mtDNA lineages were supported by bootstrap values greater than 50% (figure 2). These lineages generally corresponded to the eastern (I), Rocky Mountain (II) and western (III) regions of North America (figure 3). No general associations between host tree and mtDNA haplotypes or mtDNA haplotype lineages were observed, particularly with the individuals collected from *Pinus contorta* and *P. ponderosa* Laws. (figure 3). However, the mitochondrial lineages are associated with the generalized boundaries of the pheromone phenotypes (figure 3). Lineage I is associated with the distribution of the New York phenotype, representing 94% of the individuals collected within the range of the New York phenotype. A population from British Columbia known to have the New York pheromone phenotype was composed of individuals with all three haplotype lineages. Lineage III is generally associated with the distribution of the California phenotype, representing 77% of the individuals collected within the range of the California phenotype. All three lineages are more evenly associated with the distribution of the Idaho–Montana phenotype (I = 30%, II = 18% and III = 52%).

The variation in gene flow was estimated with  $Nm$  and  $N_{st}$ . An  $Nm$  of 1.0 is theoretically sufficient to maintain genetic homogeneity among populations (Wright 1931). In general, gene flow was moderate for all populations and regions (table 2). Gene flow was highest between the western and north-western populations, while it was lowest between the south-western populations. The population structure ( $D$ ) was significant for the combined, western and south-western populations, while it was not significant for the eastern and north-western populations (table 2). These results are reflected in the haplotype phylogeny (figure 2) because the consensus of the five trees was mostly resolved.

### 4. DISCUSSION

The geographical pattern of *I. pini* haplotypes described by our analysis has been undoubtedly influenced by biogeographical events. Based on the presence of pheromone races, Seybold *et al.* (1992, 1995) hypothesized that glacial movement during the Pleistocene resulted in southerly host refugia that separated *I. pini* into eastern and western populations. The post-glacial reunification of these populations occurred in north-western North America and secondary contact may explain the current complexity of the pheromone systems. Alternatively, the complexity may have resulted from the migration of individuals from other refugia (e.g. Beringia or intra- or interglacial refugia) (Critchfield 1985; Pielou 1991; Seybold *et al.* 1992, 1995). These biogeographical patterns are similar to those of many other North American taxa (e.g. Pielou 1991; Hewitt 1996; Avise *et al.* 1998).

Seybold *et al.* (1992) also proposed human-mediated translocation of *I. pini* in host material (e.g. barked timbers or firewood) to explain the presence of the New York pheromone phenotype in British Columbia. In our analysis, the presence of lineage I haplotypes in western

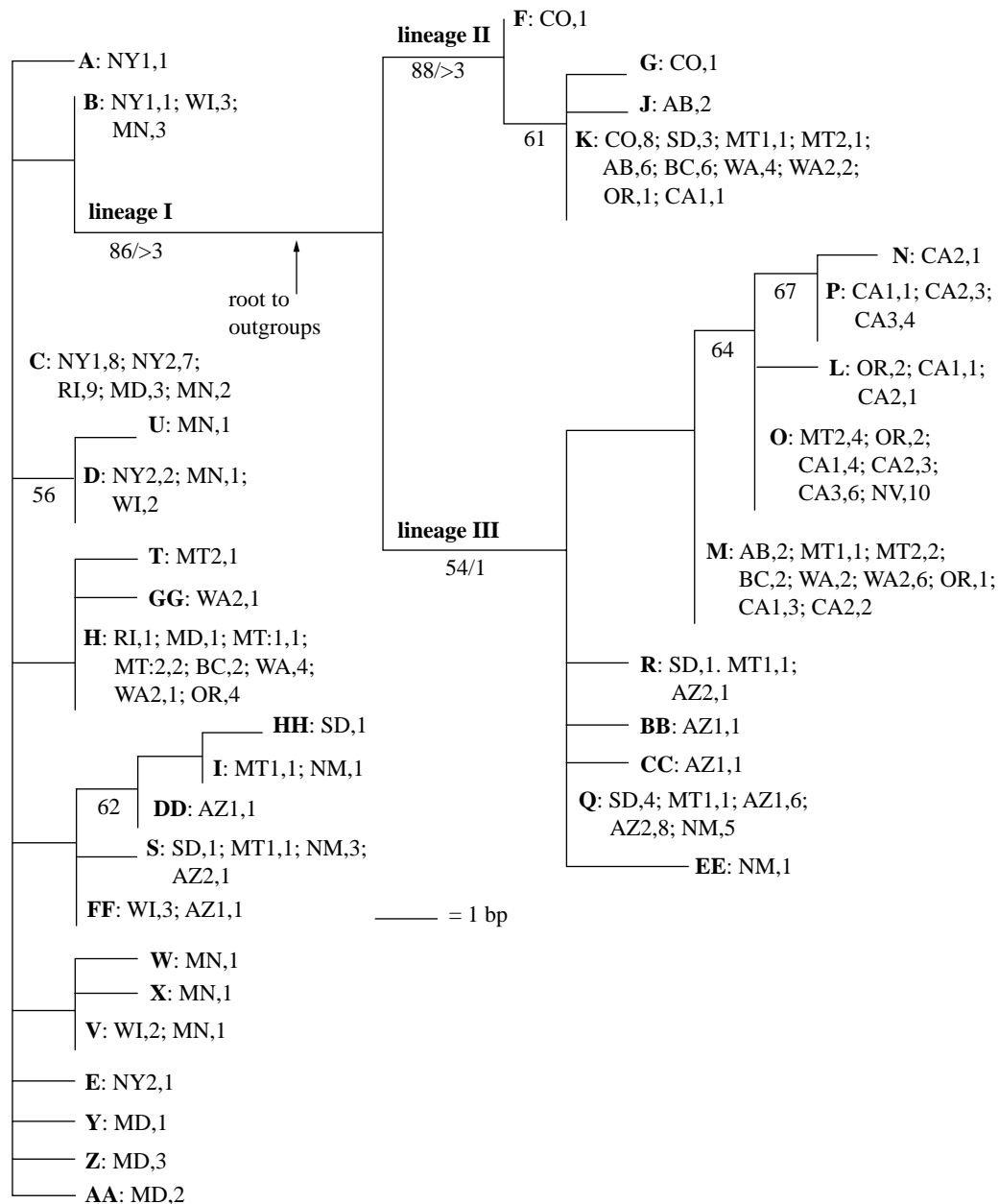


Figure 2. Strict consensus of five most parsimonious unrooted trees of 34 *I. pini* mtDNA COI haplotypes. The notation follows haplotype, population and number of individuals. Numbers at branch nodes indicate bootstrap-decay index values. Only bootstrap values > 50% and decay indices for the major haplotype lineages (lineages I, II and III) are shown. CI = 0.61, RI = 0.39 and RC = 0.63.

North America could also be explained by single or multiple human introductions of eastern populations; however, we find this hypothesis less likely. First, lineage I haplotypes are widely distributed in the west (figure 3). Second, concerted human movements between eastern and western North America are very recent (*ca.* 100 years), suggesting that there is an extremely low probability that human movement of beetles played a significant role in establishing the current geographical patterns of *I. pini* haplotypes. This is particularly true of the multiple occurrences of lineage I haplotypes in the southwest, where beetle populations are dependent on 'islands' of high elevation pine hosts that occur scattered and isolated in this arid region.

The geographical analysis of haplotypes (figures 2 and 3) and estimations of gene flow between the populations

(table 2) indicated that it is highly probable that the former hypothesis (*i.e.* Pleistocene events) largely determined the current population structure of *I. pini*. Assuming 2.3% sequence divergence per million years (Brower 1994), the maximum nucleotide divergence among haplotypes (0.034) suggests that the major lineages of *I. pini* mtDNA haplotypes diverged *ca.* 1.5 million years ago. This divergence corresponds approximately with the beginning of the Pleistocene (Hewitt 1996). However, the rate at which the mtDNA genome evolves in various insect and other animal taxa may be quite variable (Langor & Sperling 1997; Strauss 1999), so calibration of sequence divergence specific to *I. pini* is needed to confirm this assumption.

Although haplotype and pheromone variation occurs between the populations, the range of *I. pini* is presently

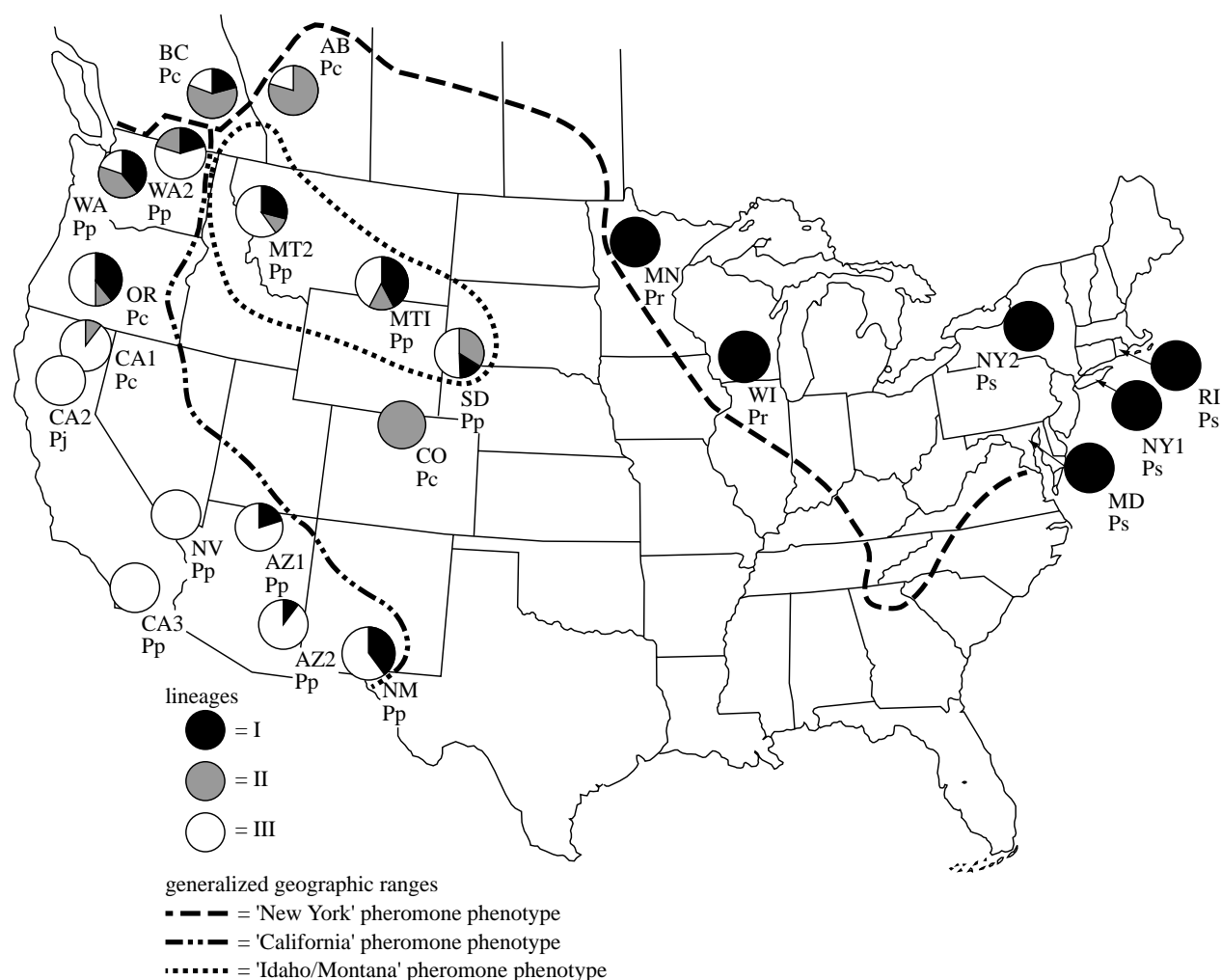


Figure 3. Geographical distribution of *I. pini* haplotypes in North America. The pie graphs show the proportion of individuals per major haplotype lineage. Letters below pie graphs indicate population locality abbreviations and the host tree from which *I. pini* adults were collected (Ps = *Pinus strobus*, Pr = *P. resinosa*, Pc = *P. contorta*, Pp = *P. ponderosa* and Pj = *P. jeffreyi*). The centre of the pie chart approximates the collection locality. The dashed lines indicate the generalized boundaries of the distribution of the pheromone races suggested by Seybold *et al.* (1995). Pheromone race designations for populations from the localities AB and CO have not been determined.

Table 2. Populations, haplotypes and measures of gene flow and population structure for regions of *I. pini* populations

region	number of populations	haplotypes	$Nm^a$ (95% CI)	$N_{st}^b$ (95% CI)	$D^b$	gene flow, genetic structure inferences
all populations	22	34	0.5 (0.3–0.7)	0.530 (0.289–0.771)	18.51 ( $p < 0.01$ )	moderate, structure
east	6	14	0.5 (0.1–0.9)	0.094 (0.000–0.831)	0.0628 (n.s.)	moderate-high, no structure
west	16	22	0.7 (0.3–1.0)	0.348 (0.076–0.620)	6.30 ( $p < 0.05$ )	moderate-high, structure
north-west	6	8	0.8 (0.3–1.9)	0.140 (0.000–0.328)	2.12 (n.s.)	high, no structure
south-west	5	11	0.3 (0.0–0.7)	0.417 (0.050–0.784)	4.94 ( $p < 0.05$ )	moderate-low, structure

<sup>a</sup>Slatkin & Maddison (1989).

<sup>b</sup>Lynch & Crease (1990).

uninterrupted in northern North America (Hopping 1964) and gene flow is moderate between all populations; approximately one individual per two generations migrates between adjacent populations (table 2). The highest rate of gene flow is observed between the

populations within the north-west, which supports the hypothesis that the Idaho–Montana pheromone phenotypes and the mixed representation of all three haplotype lineages in this region are the result of integration of eastern and western *I. pini*. This could be interpreted as a

reunification (at some point during the Holocene) of formerly isolated populations. Gene flow between the other geographical regions ranges from moderate to high, except for the south-west where it is lower, perhaps due to the isolated nature of the habitat as noted above.

There is both mtDNA haplotype and pheromone phenotypic evidence for directional introgression and gene flow in North American populations of *I. pini*. For example, lineage I is present in the west, while lineages II and III have not introgressed into the eastern populations (figure 3). Furthermore, lineage I haplotype H and the closely related T and GG are only present in northern portions of western North America (table 1 and figure 2). This suggests that haplotypes of eastern descent have introgressed into populations in the north-west. Directional introgression of mtDNA haplotypes has been observed in other animal populations (Hewitt 1996). In addition, asymmetrical gene flow from east to west has also been documented in the Pacific north-west in *Limnopus* water striders (Sperling & Spence 1991).

This directional phenomenon in *I. pini* can potentially be explained by maternal inheritance of mtDNA and assortative mating observed for the New York *I. pini* pheromone race (Teale *et al.* 1994). Western females are attracted to males that produce large, relative amounts of (–)– ipsdienol and their attraction is interrupted by moderate, relative amounts of (+)– ipsdienol (Birch *et al.* 1980). Thus, in zones of integration individual western females would be relatively less attracted to eastern males of the New York pheromone phenotype because of the presence of (+)– ipsdienol (figure 1). The mating frequency between western females and eastern males appears to have been low enough to have prevented the introgression of western haplotypes into eastern populations.

Conversely, eastern females are attracted to moderate, relative amounts of (+)– ipsdienol (Teale *et al.* 1994). They would be attracted to individual western males that have been shown to produce greater amounts of (+)– ipsdienol than most of their counterparts in the population (Miller *et al.* 1996). However, their attraction significantly increases with the addition of lanierone (Teale *et al.* 1991; Miller *et al.* 1997). This response of eastern females to western males may provide the directional impetus to gene flow, resulting in the observed geographical pattern of mtDNA lineages (figure 3). Thus, mating between eastern females and western males could explain the introgression of haplotype H and the slightly higher percentage of (+)– ipsdienol in north-western populations.

The lineage I haplotypes observed among individuals in the south-west (figures 2 and 3) may also be explained by directional gene flow. The vegetation patterns during the last glacial maximum suggest geographical proximity of eastern and western pine species in southern refugia (Betancourt *et al.* 1990; Prentice *et al.* 1991), thus allowing lineage I haplotypes to introgress into the south-west. The presence of haplotypes I and S in Montana (Rosebud Co.) and S and HH in South Dakota suggest that these populations may have been or are presently continuous with those in the south-west. Alternatively, the presence of lineage I haplotypes in the west may be a remnant of ancestral polymorphism, with subsequent differential

lineage sorting in different regions (Avice 1994). However, we consider the latter hypothesis to be less likely because lineage I haplotypes tend to occur on the eastern side of western North America, consistent with a hypothesis of introgression from the east.

Thus, even though the pheromone races are generally associated with mtDNA haplotype lineages, complete lineage divergence has not occurred for *I. pini*. There is indirect evidence of gene flow between eastern and western individuals and the association between pheromone race and mtDNA haplotype lineage appears to break down in the integration zone in north-western North America. Incomplete pre-mating barriers appear to have allowed directional gene flow in *I. pini*, sufficient to prevent complete genetic divergence of the pheromone races.

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As this paper exceeds the maximum length normally permitted, the authors have agreed to contribute to production costs.