

Biosystematics of *Ips mexicanus* and *Ips plastographus* (Coleoptera: Scolytidae) and Their Fungal Symbionts¹

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Ips mexicanus (Hopk.) (*concinus* group of S.L. Wood 1982) and *I. plastographus* (*plastographus* group) share common coniferous hosts throughout western North America (S.L. Wood 1982). In California their distribution in the coastal pines (*Pinus radiata*, *P. muricata*, *P. contorta contorta*, and *P. contorta bolanderi*) and in the high elevation *P. contorta murrayana* of the Sierra Nevada Mountains (and Cascade Mountains) is especially interesting because of their wide separation by the Sacramento and San Joaquin Valleys. This separation is 2-400 km from Redding in the north to Bakersfield in the south, a latitudinal distance of about 1200 km. Thus these populations have been noninterbreeding populations for thousands of years. Furthermore, these coastal and montane populations probably come together where *P. contorta* and *P. contorta murrayana* or *P. c. contorta* hybridize. Native Monterey pine (*Pinus radiata*) is now represented by only three small separated coastal populations. Other hosts of *I. mexicanus* and *I. plastographus* occur on small islands off the southern California coast and into the mountains of Mexico (Bright and Stark 1973, S.L. Wood 1982, Seybold and others 1992a).

On the basis of morphological criteria and reduced hatching in the F₁, Lanier (1970b) defined subspecies of *I. plastographus*: *I. plastographus maritimus* Lanier for the coastal form and *I. plastographus plastographus* (Leconte) for the montane form. In limited cross-mating studies, he showed that both populations were interfertile at the F₁ and at the parental backcrosses. Similar limited studies were conducted with the cohabiting *I. mexicanus* (Lanier 1967). However, in recent studies of the distribution of ipsenol (2-methyl-6-methylene-7-octen-4-ol) and ipsdienol (2-methyl-6-methylene-2,7-octadien-4-ol), pheromone components in *Ips* spp., Seybold and others (1992b) found both components in montane *I. mexicanus* but only ipsdienol in coastal *I. mexicanus*. The enantiomeric composition of ipsdienol is ~90 percent (-) for both populations. Although the status of these compounds as pheromone components for these species has not been established, they have been shown to be pheromone components in all species studied to date (D.L. Wood 1982). Nevertheless, the presence of ipsenol in only the montane population offers considerable opportunity to investigate the genetics of these two populations.

Very little is known about the fungal symbionts of these species. Recently Parmeter and others (unpublished data) have isolated unidentified species in the genera *Ophistoma*, *Leptographium*, and *Graphium* from *I. maritimus* and coastal populations of *I. mexicanus*. These fungi were inoculated into living trees. Through dye studies (Parmeter and others 1989) some isolates were shown to interrupt water transport. The role of symbiotic fungi in the biology of bark beetles has been studied extensively, especially in the *Ophistoma* spp./*Dendroctonus* spp. system (reviewed exhaustively in Schowalter and Filip 1992; see for example, Owen and others 1987, Parmeter and others 1989, 1992). In *Ips* spp./fungi associations, *Ophistoma* spp. have been shown to inhibit oviposition but not development (Yearian and others 1972) and to interrupt water transport (Parmeter unpublished data, Homvelt and others 1983). Although no specialized morphological structures are apparent in *Ips* spp. for carrying these fungi [such as the pronotal mycangium of *Dendroctonus brevicornis* and *D. frontalis* and maxillary mycangia of *D. jeffreyi* and *D. ponderosae* (Whitney 1982)], there is a consistent and ubiquitous association between bluestain fungal invasion of the sapwood and phloem and the gallery systems of these bark beetles. Therefore, studies of relatedness and divergence in *I. mexicanus* and *I. plastographus* would be enhanced by similar analyses of these phoretic symbionts. Evidence of divergence with either or both taxa would be of great value in understanding the evolution of these species.

Studies of relatedness in *Ips* spp. have focused on *I. pini* (eastern/western populations) and on the *grandicollis* group sibling species (*paraconfusus*, *confusus*, and *hoppingi*). In *I. pini*, no post-mating barrier has evolved between eastern populations (Ontario, Canada) crossed with western populations (California and Arizona) (Lanier 1972). However, a premating barrier has evolved between these two populations. Their pheromone component, ipsdienol, is produced by both populations. However, in the California population (as well as in most western North American populations—see Seybold and others 1992d), ipsdienol is produced as a mixture of 98 percent (-) and 2 percent (+)-ipsdienol. The (+)- isomer is an interruptant, whereas the (-)- isomer is a powerful aggregation attractant (Birch and others 1980; Seybold and others 1992d). In the eastern population a mixture of about 40 percent (-) and 60 percent (+)-ipsdienol is produced. Here both isomers function as an attractant. We have found only a few beetles responding to the racemate in California (Seybold and others 1992d). Eastern populations respond at low levels to 98 percent (-)-ipsdienol (Teale 1990). In addition to ipsdienol, lanierone (2-hydroxy-4,4,6-trimethyl-2,5-cyclohexadien-1-ol) has recently

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been isolated and shown to be an important component of the pheromone of the eastern population (Teale and others 1991). Although this compound caused an increased response to ipsdienol in California, it has not been found in California populations (Seybold and others 1992c). As with ipsenol in the montane population of *I. mexicanus*, the presence or absence of lanierone and the differences in enantiomeric composition of ipsdienol offer opportunities to investigate the genetics of these two populations of *I. pini*.

In the work with the *grandicollis* group, Lanier (1970a and b) and Merrill (1991) established the presence of postmating barriers among *I. paraconfusus*, *I. hoppingi*, and *I. confusus*. Premating barriers are weakly developed or not developed at all (Cane and others 1990c, Fox and others 1991 a). Although there is pheromonal cross-attraction among these species (Lanier and Wood 1975; Cane and others 1990a, 1990c), we have found nonreciprocal divergence in these pheromone-elicited behaviors, i.e., *I. paraconfusus* females do not discriminate between conspecific and *I. confusus* males, whereas *I. confusus* females prefer conspecific males in the host pine species (Cane and others 1990c). Furthermore, females join heterospecific males in host and nonhost pines (Fox and others 1991 a). Morphometric, electrophoretic (Cane and others 1990b), and cuticular hydrocarbon (Page and others 1992) analyses support the species status of these sibling 5-spined *Ips* described by Lanier (1970b).

Thus we see the evolution of postmating and weak-to-nonexistent premating barriers in the sibling *grandicollis* group and the reciprocal scenario in *I. pini*. The studies we propose will add to the knowledge base developed over the past 20 years on the biosystematics of these two *Ips* spp. complexes. At the same time, expanding our investigation to include the symbiotic fungi should give us greater insights into the evolution of this group of organisms.

Also of importance to the proposed study is the recent discovery of the pitch canker fungus, *Fusarium subglutinans*, in Monterey pine (*P. radiata*) plantings in the vicinity of Santa Cruz, Calif. Our studies have shown that *I. paraconfusus* and *I. mexicanus* adults carry propagules of this fungus (Fox and others 1990, 1991b). Furthermore we have demonstrated that attraction of *I. mexicanus* to Monterey pines results in pitch

canker infections associated with attacking adults. This fungus, which is native to pines of the southern U.S. (Correll and others 1992), causes extensive tip dieback followed by numerous resinous bole-cankers. We believe that this fungus further weakens trees so that they are more easily killed by these *Ips* species.

Our studies suggest that progress of this disease from urban plantings of Monterey pine in the Santa Cruz area into our native forests will likely be a consequence of propagule transmission via *I. plastographus maritimus* and *I. mexicanus*. We predict transmission from *I. mexicanus* to *I. plastographus* in the northernmost native stands of Monterey pine (Ano Nuevo State Park) to urban plantings of Monterey and bishop pine (*P. muricata*), to native stands of bishop and shore pine (*P. contorta contorta*) on the coast and ultimately inland to the Cascade and Sierra Nevada Mountains, perhaps via *P. ponderosa*. *Ips mexicanus* and *I. plastographus* cohabit these coastal pine species as well as *P. contorta murrayana* (*I.p. plastographus*) in the above mountain ranges. Thus our studies on the coastal and montane populations of these *Ips* spp. will be important in predicting the ultimate distribution of this newly introduced fungal pathogen. At the same time we are in a unique position to investigate the coevolved system of these bark beetle species with their symbiotic fungi. Such studies will provide baseline information which can be used to understand the new symbiotic relationship that we expect will develop when *F. subglutinans* becomes a part of this community.

Research Objectives

1. Determine the extent of postmating barriers to gene flow between coastal and montane populations of *I. mexicanus*.
2. Determine the extent of premating barriers to gene flow between the above populations.
3. Determine the presence or absence of ipsenol in the above crosses of *I. mexicanus* and in the backcrosses.
4. Compare the genetic constitution of the above populations using mitochondrial DNA and cuticularhydrocarbon analyses.
5. Compare the genetic constitution of the fungal symbionts commonly associated with the above populations using mitochondrial DNA analyses.