# 3. Genetic Variation in *Dendroctonus frontalis*, Within and Between Populations

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Many species of *Dendroctonus*, particularly the so-called aggressive species, are notorious outbreak organisms with more or less predictable or characteristic, cyclic patterns of outbreak locally if not regionally. *D. frontalis*, for example, exhibits an approximately 7-10 year cycle with 2-3 year duration of outbreak. The last south-wide outbreak *of D. frontalis* began in 1992 and peaked in 1995, with an estimated timber resource loss of $350 million in that year alone (Price and others 1998). The previous peak occurred in 1986. The presumption is that at epidemic levels, beetle populations move toward a maximum rate of increase; environmental resistance eventually imposes limits, and the populations begin to wane. Although numerous efforts have been made to determine the principle factor(s) responsible for contributing to this phenomenon, none have yet been identified. Turchin and others (1991) found that the pattern of *D. frontalis* population fluctuations in east Texas over a 30-year span most resembled a delayed density-dependent function. They suggested that this pattern is consistent with the notion that natural enemies exert the most significant influence and regulate bark beetle populations. There are numerous pieces of evidence to support this hypothesis, such as the ability to forecast beetle populations for the present year using the ratio of bark beetles to natural enemies (in particular, a clerid predator *Thanasimus dubius*) captured in traps (developed by Dr. R. F. Billings, Texas Forest Service), and the recent body of work by Reeve (for example, 1997) which may lead to a possible year-in-advance predictive model based on clerid and beetle numbers.

Because of the regularity of these outbreaks and the apparent population phase shift from endemic to epidemic, it is tempting to speculate about the potential role of genetics in the epidemiology of bark beetles. Such a possibility is not inconsistent with the mathematical description of outbreak cycles and has long been speculated about by early researchers attempting to explain outbreaks in forest defoliators (for example, Chitty 1965, Wellington 1964) and bark beetles (Raffa and Berryman 1987). Whether cause or effect-–that is, as a consequence of expansion to epidemic levels or as a factor contributing to expansion-–the genetic structure of epidemic populations is presumably different from endemic populations (see discussion by Mitter and Schneider 1987). Indeed, the genetic changes in an explosive population over time may actually contribute to the decline (or "implosion") of the population, and thus contribute to the pattern of outbreak. Relatively high levels of genetic variation in *D. frontalis* (Anderson and other 1979, Florence and Kulhavey 1981, Namkoon and others 1979) and other bark beetles (reviewed in Mitton and Sturgeon 1982, and in Hayes and Robertson 1992) were initially detected with enzyme electrophoretic studies. Florence and others (1982) were among the first, using a polymorphic esterase in *D. frontalis*, to document the connection between genetic structure and population behavior, finding that genetic diversity is maintained with dispersal. Similarly, isozyme variation in bark beetles has been related to differences in host species and/or the margin of a particular host species range (reviewed by Stock and other 1992 and Lakatos this proceedings). Stauffer (1997b) has found enzyme differences between first-attacking and later-arriving beetles. However, no studies to date have provided direct genetic evidence of differences between endemic and epidemic populations.

Current molecular techniques and sophisticated programs have opened up new opportunities for genetic studies of bark beetle populations (Hayes and Robertson 1992). A technique as simple as RAPD-PCR (random amplification of polymorphic DNA through polymerase chain reaction--see Carter, this proceedings for a detailed discussion of this technique) may permit the fingerprinting of a population and snapshots through time as an infestation progresses. Ultimately, a look at *D. frontalis* populations across the southeastern United States may provide a new predictive tool, if changes in infestations or population structure are consistent in epidemic vs. endemic populations. Differences between disparate populations, such as those found in the United

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States compared to those in Mexico or Central America, may be equally important. The identification of unique biotypes may have significant bearing on the development of different resource management programs.

## Within-Population Variation--Differences in Paternity of Multiple Broods

Before the population structural dynamic can be understood, the mating pattern of bark beetles such as *D. frontalis* or *D. brevicomis*, which can produce multiple broods per generation, must be examined. Work by Gagne and others (1980) and Wagner and others (1981) uncovered the potentially important contribution of second and even third broods to the population dynamics of *D. frontalis*, but no studies have addressed the genetic consequences of multiple broods. Most significantly, the role of the male and his contribution with each successive mating are unknown. In their studies (Gagne and others 1980, Wagner and others 1981), new males were provided to females in production of second and subsequent broods. It is not known whether the sperm of the first male is expended or shunted prior to the next mating, whether there is sperm competition, mixing, or precedence, or whether a new male and/or additional sperm are required.

Paternity in insects has been assessed a number of ways using molecular genetic techniques (for example, Benken and others 1998; Fjerdingstad and others, in press; Hooper and Siva-Jothy 1996; Moritz and others 1995). I proposed to identify and use RAPD markers to examine the parents and offspring of multiple broods of *D. frontalis*. There are two major hurdles in this effort (the work described here involves multiple collaborations that are identified in Future Research Direction sections below and Hayes and others 1998). The first is finding suitably unique populations, that is, populations with unique polymorphisms or consistent proportions of polymorphisms. The second hurdle is arriving at a protocol that will permit the retrieval of parent adults as well as brood from two successive matings. This is a particular problem with *Dendroctonus* species because continuous rearing is problematic. A satisfactory solution has developed to the second problem, but not the first. However, while attempting to resolve the first problem, we (Hayes and others 1998) made some intriguing observations about two geographically isolated populations of *D. frontalis*, which will be described below.

To obtain brood from two successive matings, we employed a modification of methods developed by Rhodes and others (1998). A protocol was developed using prepared sections of freshly cut loblolly pine (*Pinus taeda*, a preferred host of *D. frontalis*) bole. These bolts were approximately 15-20 cm in diameter and length, each was halved, and all exposed areas were coated with paraffin to reduce desiccation. A newly emerged female and male pair was introduced into half of a gelatin capsule inserted into a small hole that had been cut with a cork borer through the bark to the phloem. After 7 to 14 days, the bark was carefully peeled off of the bolt to expose the parent adults and developing brood. The female was then paired with a second newly emerged male and introduced into a fresh bolt in the manner described above. Again, in 7 to 14 days this bolt was stripped and the parent adults retrieved along with eggs or larvae from the second brood. A minimum of 20 offspring from each brood is needed for adequate sample size. Additionally, a minimum of 20 first-brood bolts must be established to achieve adequate replication because for unknown reasons, a proportion of attempted matings (about 25%) are not successful in both first and second pairings. The longer the bolts are held before peeling the bark, the further developed the offspring are. For practical reasons, larval stages are easier to work with than eggs; however, we have successfully isolated DNA from individual eggs.

### Future Research Direction

To identify distinguishing markers, we screened nearly 50 primers using the robotic technology of the Genetic Institute of the South (USDA-FS, SRS, Saucier, MS). Taken altogether we found considerable variation within and among *D. frontalis* obtained from different infestation sources in central Louisiana; however, few distinctive polymorphisms were revealed. In a second screening effort, using about 20 primers, we identified distinctive markers for *D. frontalis* from Florida (supplied by Dr. J. Meeker, Florida Division of Forestry). Unfortunately, we were unable to obtain additional infested bark from Florida to correspond with the availability of newly-emerged individuals from central Louisiana or elsewhere. This effort will be continued.

## Between-Population Variation – Differences Between Allopatric Populations

An opportunity to conduct mating trials with a *D. frontalis* population from southern Chiapas, Mexico, presented itself in April 1998. The presumption was that, compared to beetles from central Louisiana, these

beetles were likely to be genetically distinctive, as revealed through RAPD-PCR techniques, given the extreme geographic isolation of the two populations. Live specimens of these presumed *D. frontalis* were collected from a known host tree *P. oocarpa* (Tovar and others 1995) and were transported on ice to the Alexandria Forestry Laboratory (USDA-FS, Pineville, LA) for genetic analysis. Surviving individuals that were apparently healthy (missing no tarsi and able to respond to stimuli), were introduced into six loblolly bolts as described above with the hope that the females could then be mated to males from central Louisiana. One of the six bolts was used to cross a female from Mexico to a male from Louisiana; this combination produced approximately 10 cm of gallery but no brood. Of the five Mexico by Mexico crosses, one pair failed to initiate a nuptial chamber, but 50% of the others produced both gallery and a small number of apparently viable eggs. No second matings were possible. Although these results indicate that the *D. frontalis* from Mexico can use a novel host, the results are inconclusive relative to the mating ability of members of these two allopatric populations (Hayes and others 1998). Previous efforts to mate geographically distinct *D. frontalis* populations (Lanier and others 1988, Vite and others 1974) showed relatively high interfertility, suggesting the absence of post-mating isolating mechanisms.

The preliminary results of subsequent RAPD-PCR analyses confirmed the genetic differentiation between individuals from the Mexico and Louisiana populations. Although a relatively small number of primers were screened (about 12). Several showed distinctive population identifying markers, as well as markers that could be used to distinguish between the two observed morphs found among the individuals collected in Mexico (Hayes and others 1998).

We observed two external morphological features, overall size and corresponding mycangial conformation of females of both sizes, that differed consistently among individuals collected from the Chiapas site and from the same host tree. *D. frontalis* size is known to vary over the flight season within a region (for example, Hedden and Billings 1977) and among regions (Lanier and others 1988). Among the beetles collected in Mexico, we found they fell into two relatively distinct size classes; males and females of one morph were about 40% (n=20) larger in surface area on average than males and females of the other morph; there was no difference in size between the smaller morph from Mexico and beetles from central Louisiana (Hayes and others 1998). Additionally, the mycangia, a characteristic cuticular collar-like structure containing symbiotic fungi, of the large females from Mexico was less pronounced and appears to be narrower when compared to females from the smaller morph or central Louisiana.

In their review of the geographic distribution of *Dendroctonus* throughout Mexico, Zúñiga and others (in press) indicate that both *D. frontalis* and *D. mexicanus*, a larger member of the *frontalis*-complex, use *P. oocarpa*. Additionally, *D. mexicanus* and *D. frontalis* are known to occur in the same host tree (Zúñiga and others 1995). Genetalia of males from the two Mexican morphs and from specimen from central Louisiana (n=6-12) were dissected, and seminal rods were isolated and mounted (following Lanier and others 1988). Seminal rod is one of several characteristics used by Lanier and others (1988) to distinguish members of the *frontalis*-complex, which includes *D. approximatus*, *D. frontalis*, *D. mexicanus*, and the little known *D. vitei* (compare Tovar and others 1995). Despite the distinctive external morphology differences, the male seminal rod does not appear to differ among the two morphs from Mexico, nor the samples from central Louisiana (Hayes and others 1998). Chromosome numbers also differ between *D. frontalis* (diploid no. = 16) and *D. mexicanus* (diploid no. = 12) (Lanier 1981, Salinas-Moreno and others 1994). Chromosomal preparations of approximately 20 individuals made by Cisneros and Zúñiga (IPN, Mexico City, Mexico) also suggested that these two morphs were *D. frontalis*. Whether the observed morphological differences are genetically based remains to be demonstrated.

## Future Research Directions

Additional genetic work is planned in collaboration with G. Zúñiga, R. Cisneros, and J. Macías-Sámano (see Zúñiga and others this proceedings). In addition to these efforts, samples have been supplied to Dr. Kelley, for preliminary mitochondrial DNA analysis using techniques described in Kelley and Farrell (1998) and in these proceedings.