Novel Bark Beetle Research Possible with New Genetic Techniques[1]

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Prospects

The new molecular techniques in genetics offer solutions to questions about bark beetle biology in two principal areas. The first of these is dominated by questions of phylogenetic interest. For example, the unity of *Dendroctonus valens* as a species (e.g., Pajares and Lanier 1990) could be explored with precision. Geographic substructuring of other scolytid taxa, including regional differences in pheromone production and response, partial mating barriers in sibling *Ips* and so on, share this phylogenetic approach. Much of this work is well underway.

The second area, which is wide open for new ideas, uses the ability of the new techniques to discriminate individuals and populations to address questions from population dynamics and behavior (Slatkin 1987). It is possible now with mammalian and bird DNA to characterize individuals and verify genetic contributions of parents to their offspring. Paternity analyses of this sort have recently provided some surprising insights in ornithological literature where extra-pair copulations have been found to be much more common than was previously recognized. Little is known about bark beetle behavior under the bark—e.g., whether supposedly monogamous *Dendroctonus* females sometimes mate with more than one male before ovipositing their first clutch of eggs, whether there is selection by females for males with particular characteristics, and so on. The ability to identify individuals and their descendants would allow paternity analyses of eggs from scolytid galleries. Male-male competition may be occurring in *D. valens* galleries where there are three adults present (Hobson 1992). Paternity analysis of egg clutches of even single pairs of beetles could provide insights into the frequency of pre-emergence mating.

The ability to identify beetles from subpopulations and track their descendants may allow us to answer questions such as whether patch kills of trees by *D. brevicomis* are caused by beetles which are the descendants of an earlier nearby infestation or instead are beetles which are drawn from the general population of all dispersing adults.

Similarly, typical bark beetle dispersal distances can be assessed by looking at variability along transects through beetle populations. The number of migrants per generation between populations can be estimated from F_{ST} statistics as estimated using the allele frequencies of several different genes. Studies employing electrophoresis have had difficulty in the past finding enough polymorphism to conduct very fine-grained analyses of this sort. Now several of the new molecular techniques permit rapid analysis of DNA with enough resulting information to carry out gene flow and dispersal analyses with dozens of markers resulting in more precise estimates.

Contrasts that exist between beetles in an endemic or early infestation population and an epidemic or late infestation population may involve genetic drift or perhaps repeating cycles of shifting gene frequencies.

Methods

Sequencing of both mitochondrial and genomic DNA has provided information previously unattainable to systematists. These data, in conjunction with other chemical phenotype data, such as cuticular hydrocarbon analysis (e.g., Page and others 1990b), will provide answers to many bark beetle phylogenetic questions.

However, discrimination of bark beetle populations requires molecular techniques that detect higher levels of polymorphism. DNA sequencing of highly polymorphic regions of DNA can be useful, but these regions must first be located. Sequencing is costly and labor-intensive, so its application in population genetics can only be limited. Restriction Fragment Length Polymorphism (RFLP) has also been used for the detection of DNA polymorphism. However, RFLP analysis requires that knowledge of a polymorphic region to target exists, and its sequence must be known so that a DNA probe can be constructed. RFLP analysis is much less labor-intensive than sequencing, but it is still quite costly. Radioactivity is also necessary for both sequencing and RFLP. Because of the drawbacks of these DNA techniques, protein allozyme analysis has been the most popular method for performing genetic polymorphism studies. Allozyme analysis is much less expensive, but it is often difficult to find polymorphic loci.

The polymerase chain reaction (PCR) has made it much simpler and more cost-effective to perform molecular analyses at the level of the DNA (Arnheim and others 1990). Because PCR dramatically increases the copy number of specific DNA sequences, analyses using PCR can be performed with very little starting DNA. DNA sequencing of PCR-amplified DNA eliminates the need for cloning, which dramatically reduces the cost and effort. RFLP analysis using PCR-amplified DNA eliminates the need for a probe, so less sequence information is required, and the need for radioactivity is eliminated. However, the level of polymorphism detected using these techniques may still limit their usefulness in answering some population genetics questions.

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[1]An abbreviated version of this paper was presented at the Bark Beetle Genetics Workshop, May 17-18, 1992, Berkeley, California.

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In the last two years, a PCR-based technique called Randomly Amplified Polymorphic DNA (RAPD) has proven very successful for performing genetic analyses in plants (Williams and others 1990). Its advantages are that it detects higher levels of polymorphism than either isozyme or RFLP analyses, and that no prior DNA sequence knowledge is necessary. A disadvantage to this technique is that the electrophoretic bands it produces are inherited as completely dominant traits as opposed to semi-dominant traits as in RFLP or isozyme analysis, which can make genetic analyses somewhat more complicated. It has also been reported that some RAPD bands are not inherited in a Mendelian fashion (Riedy and others 1992). Success with this technique has been reported with some insects (Edwards, unpublished results), including haplodiploid species where the dominance problem can be avoided by performing the analysis on males.

The characterization of individuals, as needed for paternity analysis, requires an analysis that targets highly variable regions of DNA. In humans, this is accomplished by analyzing Variable Number Tandem Repeats (VNTR's), which are highly mutable (10^{-3}-10^{-4}) regions of repetitive DNA (Jeffreys and others 1985). The major disadvantage of this technique is that few VNTR's have been found in insects (Bigot and others 1990, Blanchetot 1991, Moritz and others 1991), so it is likely that the application of this analysis to the study of bark beetles would first require significant time and effort to locate the repetitive DNA. Once this was accomplished a very high degree of polymorphism would be available for free grain dispersal studies and behavioral investigations requiring characterization of an individual and its progeny.

Given the diversity of techniques for assessing polymorphism and discriminating groups under study, one of our first goals might be to survey the level of polymorphism that is found in the groups of interest. Then the appropriate technique for a particular problem can be chosen.