

Isozyme Studies of Bark Beetle Population Genetics and Systematics¹

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Electrophoretic analysis was used extensively in the 1970's to estimate genetic relationships among populations, species, and closely related genera of many different plants and animals, including many insect groups. Some of the first electrophoretic work on bark beetles was done by Anderson and others (1979), Anderson and others (1983), Florence and Kulhavy (1981), Florence and others (1982), and Namkoong and others (1979). These studies demonstrated that bark beetles are highly polymorphic and amenable to population studies using this technique. The first isozyme study of the mountain pine beetle (MPB) *Dendroctonus ponderosae* (Stock and Guenther 1979) showed differentiation among geographically separated populations. A subsequent study of MPB from four locations (Stock and Amman 1980) suggested that there might also be genetic differences at individual gene loci between MPB from lodgepole and ponderosa pine, and greater overall heterozygosity in beetles from ponderosa pine. Some preliminary data also showed differences between beetles emerging from trees with thin and thick phloem and between early- and late-emerging beetles from the same tree.

Inferences that could be made from these observations of differences related to host species were, however, confounded by geographic distance among sites. Sturgeon (1980) and Sturgeon and Mitton (1986) studied sympatric groups of MPB attacking different hosts in mixed pine stands and found significant differences related to host tree species. Further evidence of differentiation of MPB by host was found in a comparison of isozyme frequencies of MPB collected from lodgepole and ponderosa pine at a single site in Utah (Stock and Amman 1985). Deviations in genotype frequencies from Hardy-Weinberg (random mating) expectations occurred when isozyme data from all beetles were pooled (Wahlund effect); frequencies were much closer to random mating expectations when separate analyses were conducted for beetles from each of the host species. Once again, greater heterozygosity was observed in beetles from ponderosa pine. Overall, beetles from thin-phloem lodgepole were more heterozygous than those from thick phloem, though these differences were not detected in beetles from ponderosa pine.

Heterozygosity and Environmental Stress

Males were more differentiated than females among sites and numbers of emerging males more variable in thin-phloem trees (Stock and Amman 1980). In a later study (Stock and Amman 1985), males emerging from thin-phloem lodgepole pine were found to be more heterozygous and more variable from tree to tree, and fewer and more variable males (relative to females) emerged from thin phloem. We suggested that genetic diversity of MPB (as measured by average heterozygosity) might increase in response to increased severity of environmental conditions (i.e., stress), a response observed in numerous other organisms by other workers (e.g., Samollow and Soule 1983). Male MPB are generally thought to be more sensitive to microenvironmental conditions than females, and therefore would be expected to have greater genetic diversity if a generalized population-level response to increased selective pressures was an increased level of heterozygosity. Further evidence to support this hypothesis was provided by a study of isozyme variation observed over six consecutive generations of MPB laboratory-reared in thick and thin phloem; heterozygosity increased in both groups over the generations, but more rapidly in the beetles in thin phloem (Amman and Stock unpublished).

In *Ips pini* (Gast and Stock unpublished), genetic diversity was significantly higher in overwintered than in non-overwintered beetles. However, Langor and Spence (1991) observed changes in gene frequencies but no consistent increase in heterozygosity in MPB with overwintering. These authors attribute the genetic differentiation they observed to differential survival in the hosts, rather than to differential host preference of beetle genotypes. Thus, one might see, as did Gast and Stock (unpublished), greater differences between beetles that have lived for a longer time in different hosts or in the same host species under more severe environmental conditions. Other evidence supports this hypothesis. For example, Gast (1987) reported that overwintered *I. pini* responding to pheromone were significantly more heterozygous than overwintered beetles responding to host volatiles. Heterozygosity levels in host-responding, pheromone-responding, and nonresponding beetles that had not overwintered were very similar across response categories.

Time of collection can therefore have an important influence on the type of genetic variation observed and interpretations that can be made from it. Langor and Spence (1991) and the work of Gast (1987) suggest that laboratory rearing of beetles collected from logs cut before winter reduces the differential selective influence of host tree species. This possibility could help explain the fact that Sturgeon (1980) and Sturgeon and Mitton (1986) found genetic differences between MPB

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from lodgepole and limber pine, whereas Langor and Spence (1991) did not. Sturgeon's (1980) beetles were collected in summer, just before emergence from their host trees; they represented survivors of within-tree mortality estimated to average 85 to 99 percent of the brood which had been established the previous fall. Langor and Spence (1991) used beetles reared in the laboratory and collected in the fall and suggested that data collected from laboratory-reared beetles better reflect possible effects of host selection behavior and the genetic makeup of colonizing beetles. We compared heterozygosity of early- and late-emerging MPB reared from fall-collected lodgepole logs from six sites (Stock and Amman unpublished). In beetles from four of the six sites, heterozygosity went up over the emergence period. It is possible that such trends would be even more apparent in samples of beetles collected as they emerged in the field the following year.

Heterozygosity and Species Distribution

Langor and Spence (1991) attributed the low levels of heterozygosity (10.0-11.2 percent) they observed in MPB from Alberta and British Columbia (compared to heterozygosity in MPB from U.S. studies) to the location of their populations near range margins. In contrast, our studies suggest that populations near the margins of species distribution (presumably living under suboptimal conditions) could be expected to be more heterozygous. The highest level of polymorphism that we observed in a MPB population (more than 17 percent) occurred in a population from California, another area that might be considered near the range margin for this species (Higby and Stock 1982). Heterozygosity levels of Langor and Spence's (1991) Canadian populations were lower than in this California population, but approximately equal to heterozygosities in almost all other populations we studied—including a population from the Black Hills (South Dakota), a third area that could be considered marginal for the species and which had an average heterozygosity of 11.1 percent (Stock and others 1984).

Genetic Indicator of Population Phase

Some very early electrophoretic work with the Douglas-fir tussock moth (Stock and Robertson 1977) revealed an esterase allozyme that appeared only in low-density populations. Populations sampled two or more times during 1976 and 1977 showed changing frequencies of this allozyme (relative to the other two allozymes at that locus) that seemed to parallel changes in population density. At that time, we hypothesized that relative frequencies of these three allozymes might be used as a genetic indicator of future population trends in the Douglas-fir tussock moth. Hayes and Robertson (these proceedings) describe allozyme variation at an esterase locus showing similar correlation with population status in the MPB.

Average heterozygosity also appears to be related to population phase, reaching highest levels as epidemic populations reach peak numbers and begin to decline (Stock and others

unpublished). Thus, there may be a correlation between factors of increased environmental stress that act to end an outbreak and the higher levels of heterozygosity observed at this time. One might infer a relationship between heterozygosity and population size, but we need to keep in mind that the processes underlying observed genetic variation are very complex and that factors that influence genetic variation under one set of conditions (e.g., during outbreaks or epidemics) can be very different under another set of conditions (e.g., during the low-density or endemic population phase). For example, inbreeding in low-density populations might act to reduce heterozygosity and counterbalance any tendencies toward increased heterozygosity related to environmental stressors that might be keeping the population at a low level. In the field, heterozygosity appears to be lower in endemic than in epidemic populations (Stock and others unpublished), and laboratory studies (Amman and Stock unpublished) showed that small numbers of parent beetles per log resulted in an F_1 population with a much lower level of heterozygosity than the parental population.

On the basis of these ideas, Stock and others (unpublished) hypothesized that heterozygosity increasing over time might indicate that a population was approaching the end of an epidemic; conversely, a stable or decreasing heterozygosity in a high-density population could indicate that the population was unstressed and that population numbers would increase or stay high. Predictions of population trend based on these hypotheses for six MPB populations were good in four of six cases.

Systematics of *Dendroctonus* Species

Using electrophoretic techniques, Higby and Stock (1982) identified diagnostic isozymes for sympatric populations of the sibling species *D. ponderosae* and *D. jeffreyi* in California. An earlier study (Stock and others 1979) showed very similar levels of differentiation between *D. pseudotsugae* populations from Idaho and Oregon, but the wide geographic separation between these two populations precluded any definitive statements about species status.

Wood (1963) used anatomical and biological characters to rank *Dendroctonus* species in order of increasing evolutionary specialization relative to other Scolytidae. Lanier (1981) also suggested an order of specialization based on cytogenetic characteristics. Although Wood and Lanier placed the species into nearly identical species groups, their postulated order of specialization of these groups were nearly opposite. Bentz and Stock (1986) used electrophoretic data from 22 populations, representing 10 *Dendroctonus* species, in an attempt to unravel the discrepancies in phylogenetic interpretations. Although the species groups identified using electrophoretic data were similar to those previously identified by both Wood (1963) and Lanier (1981), the implied phylogeny was independent of the species groupings. Some members of a species group were more specialized than other members in the same group. On the basis of these data, it appears that no single species group can be said to be more advanced or more primitive than another.

The phylogenetic tree produced using electrophoretic data suggests that *D. rufipennis*, *D. adjunctus*, and *D. approximatus* were the most primitive species, followed by *D. ponderosae* and *D. brevicomis*; *D. valens*, *D. terebrans*, *D. simplex*, *D. pseudotsugae*, and *D. frontalis* are among the more evolutionarily advanced species in the genus. There were no discernible trends in level of heterozygosity (by species) based on evolutionary position in the hypothesized phylogenetic tree. These interpretations of the phylogenetic relationships among *Dendroctonus* species could be useful in analysis of behavioral strategies used in scolytid genera, such as production and response to semiochemicals, mating strategies, and host tree associations.

Stock and others (1987) compared the great European spruce bark beetle, *D. micans*, to the 10 North American *Dendroctonus* species studied by Bentz and Stock (1986). Average heterozygosity was 5.3 percent in *D. micans*; North American species ranged from 11.4 to 22.6 percent. Cluster analysis suggests that *D. micans* is most closely related to *D. terebrans* and *D. valens*, species with which it shares the characteristic of gregarious larval feeding in the phloem of living hosts. More recently, Gast and Furniss (unpublished) compared isozyme characteristics of *D. micans* with its morphologically similar, spruce-infesting, North American counterpart, *D. punctatus*.

Research Needs

(1) Test the heterozygosity model of Stock, Schmitz, and Amman for predicting phase (endemic, building, epidemic, postepidemic) of MPB populations.

(2) Investigate further the change from endemic to building phase: What are the influencing factors associated with it (population size, mixing of demes, synchrony of emergence and attack, host characteristics, etc.)?

(3) Continue to investigate differences in MPB response to verbenone (and possibly other pheromones): Are they genetically based and, if so, what factors are causing selection?

(4) Dispersal: Are there physiological and/or genetic differences among individuals with different flight behavior (long vs. short fliers) and do these relate to population phase?

(5) The effect of temperature on MPB is very important in Rocky Mountain populations. Is cold tolerance genetically based, possibly showing up as geographic differences associated with elevation and latitude?

(6) Continue study of bark beetle systematics using electrophoresis and other, newer, molecular genetic techniques.