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Introduction

The objective of this study was to test for genetic homogeneity among several discontinuous orchard pine vole populations, particularly between those that had been treated with endrin and those that had not. Endrin-resistant pine vole strains have been documented (Webb and Horsfall 1967; Webb et al. 1973), but have not been characterized genetically. Practically, this study was designed to determine if genetic differences existed among endrin-treated and nontreated orchard populations over two seasons of the year, by examining a few specific gene loci believed to be involved in endrin metabolism in small mammals.

Materials and Methods

A representative sample of pine voles from each of 3 endrin-treated and 3 nontreated apple orchards in southwestern Virginia was trapped during Fall 1980 and again in 2 orchards of each type during early Spring 1981. The same animals which had been subjected to and had survived the endrin treatments applied between sampling periods thus should have been captured in the Spring. All treated orchards had been sprayed annually with endrin for the past 15 years, and the nontreated orchards had not received treatment for this same amount of time. Since the animals were to be removed (a form of artificial selection), only one section of an orchard was trapped at either sampling period, leaving a buffer zone of at least three rows of trees between the Fall- and Spring-trapped sections. Traps were relocated on the second or third day of trapping so that eventually every tree with vole sign in the designated section was trapped. Carcasses were placed on dry ice in the field and then stored in the laboratory at about -20C.

Seven enzyme systems were surveyed in each orchard population for possible use as polymorphic genetic markers using horizontal starch-gel electrophoresis. Changes in activity levels of five of these systems had been reported to occur after endrin injection in small mammals (Kacew and Singhal 1973; Ludwicki 1974; Hendrickson and Bowden 1976; Meenä et al. 1978): AcP (acid phosphatase), β - GUS (β -glucuronidase), FDP (fructose-1, 6-diphosphatase), GOT (glutamate oxaloacetate transaminase), and LDH (lactate dehydrogenase). In addition, two other arbitrarily chosen systems were surveyed: IDH (isocitrate dehydrogenase) and MDH (malate dehydrogenase). It was necessarily assumed that none of the loci observed influenced the probability an animal was captured.

Kidney tissue was used in electrophoresis of the above enzyme systems. The tissues were homogeneized, centrifuged, and applied to filter-paper wicks that were inserted into a 12.5%-starch gel. At all stages of preparation, carcasses, excised kidneys, homogenates, sample wicks, and prepared gels were kept frozen, refrigerated, or on ice to prevent enzyme degradation. Recipes for electrophoretic buffers and histochemical stains were modified Harris and Hopkinson (1976) formulations reported by Guse (1980 and pers. comm.). After electrophoresis, gels were stained for the desired enzymes and banding patterns were immediately scored by genotype.

Results and Discussion

AcP, β -Gus, FDP, GOT-1, and MDH-1 were found to be monomorphic in all the pine vole populations studied. GOT-2, IDH-1, LDH-1, and MDH-2 were found to be polymorphic and were used to electrophoretically characterize each individual sampled (McBride 1981). For the four polymorphic loci in each orchard population, χ^2 independence tests of genotype frequency and sex were performed. IDH-1 and MDH-2 genotypes were found to be sex-dependent (α=0.05). These sex dependencies occurred in different sprayed orchards for the two loci at both seasons, and for IDH-1 also in a nonsprayed orchard in the Fall. Greater numbers of heterozygous females than those expected (and a concomitant lesser number of heterozygous males) occurred in the sprayed orchards for IDH-1 and MDH-2, whereas the opposite occurred for IDH-1 in the Fall nonsprayed orchard.

Three-way independence tests of orchard, season, and genotype frequency conducted for each polymorphic locus by the G log-likelihood ratio test demonstrated that genetic structure at the four loci differed among orchards, as expected, since these discontinuous populations have virtually no contact with each other. Orchard, season and genotype frequency were jointly dependent variables with significant interactions (α=0.05) at all four polymorphic loci. Differences in genotype frequencies were sigificant due to orchard type (endrin-treated or nontreated) only at the IDH-1 and LDH-1 loci, with heterozygotes comprising greater proportions of the populations in nontreated orchards for IDH-1 and in treated orchards for LDH-1.

Mean individual heterozygosity (mean number of heterozygous loci per individual) decreased slightly from Fall to Spring in 3 of the 4 orchards that were sampled both seasons, but increased slightly in one orchard that had been treated with chlorophacinone (Rozol) as well as endrin. Greater heterozygosities did not occur consistently in either endrin-sprayed or nonsprayed orchards at either season, nor were sex differences consistent with respect to orchard type or sampling time. Heterozygosity averaged over the 4 orchards, however, was slightly higher in nontreated orchards both seasons. The average also decreased from Fall to Spring in both sexes and in both orchard types.

Heterozygosity as a measure of inherited variability is commonly used as an index to the adaptive potential of a population, since the more heterozygous individuals are believed to have greater capacities to survive and change with their environment, thus successfully reproducing their kind (Selander et al. 1971; Manlove et al. 1975; Smith et al. 1975). The reductions in heterozygosity that occurred in 3 orchards may indicate the intermittent random drift effects that can result from severe local or periodic reductions in population density (Wilson and Bossert 1971), such as pesticide use and adverse winter weather conditions. The unique increase in heterozygosity that occurred in the one orchard treated with chlorophacinone as well as endrin, however, may suggest the greater adaptability and selective advantage of the more heterozygous animals, since presumably voles that survived both mortality factors would be more heterozygous than those facing just one. Alternatively, this one increase in heterozygosity may have been a random occurrence.

Summary and Conclusions

The presence of known, differing sources of mortality in the endrin-treated and nontreated orchards of this study provided a preliminary baseline for meaningful comparisons of population genetic indices. Since population genetic composition at the loci observed did not vary appreciably or consistently with respect to endrin treatment, no conclusions can be stated as to the genetic consequences of endrin-induced mortality in wild pine vole populations. Population genetic structure did seem to vary somewhat from Fall to Spring among orchards, regardless of pesticide use. Therefore, the orchard environment, with its predictable source of chemical-induced mortality, provides an excellent natural situation for further genetic observations.

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