

ELECTROPHORETIC INVESTIGATION OF GENETIC CHARACTERISTICS
IN THE PINE VOLE

by

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INTRODUCTION

The pine vole (Microtus pinetorum), a small fossorial rodent native to the eastern deciduous forest of the United States, has during the last century successfully colonized new habitat in apple orchards. There its root-gnawing habit has created a serious pest control problem. Girdled roots seriously impair a tree's capacity to absorb moisture and nutrients from the soil, and eventually cause the tree's death. Eleven million dollars of damage in terms of lost production was attributed to pine vole activity in Virginia during 1973-75 (McCue, 1977). Endrin, a chlorinated hydrocarbon insecticide, has been applied as a ground spray for orchard vole control since 1954. Endrin is extremely toxic, in white rats up to 32 times more so than DDT (Edwards, 1973). In 1967, however, Webb and Horsfall reported that voles from an orchard sprayed annually with endrin for 12 years were about 12 times more tolerant to the compound than were voles from an unsprayed orchard--the first reported incidence of mammalian pesticide resistance developing in a natural habitat. In order to better understand both the causes and effects of endrin-resistance in the pine vole, more intensive study of toxicology, population dynamics, and genetics, as well as of basic reproductive and nutritional physiology, is required. This study was conducted to examine genetic variation among pine

vole populations in relation to two of these areas of research--endrin resistance and nutrition.

The mechanism by which endrin resistance develops in the pine vole has not been unequivocally identified. Petrella et al. (1975) attributed different urinary and fecal endrin metabolites from resistant and susceptible pine voles to differing metabolic efficiencies. In 1973, Webb et al. had observed first-generation offspring from crosses of resistant (R) and susceptible (S) voles with LD-50 values intermediate to those of their R and S parents. This led Hayes et al. (1975) to postulate that endrin resistance in the pine vole might involve a heritable component. Endrin, as the selective agent, would eliminate those animals with no genetic capability for efficient absorption, transport, storage, metabolism, or excretion of endrin. The population gene pool then would shift toward more resistant genotypes.

In laboratory nutrition studies, Merson (1979) did not postulate a mechanism to account for the different responses to restricted feed intakes he observed in male pine voles. In his studies, 58% of voles on 70% ad libitum feed intake died, but none on 100% ad libitum died. Specifically, those that died had lost one-and-one-half times more body weight than had the survivors on restricted feed intake and had retained only 1/5 the body fat content. Although Merson observed that dying voles failed to lower their daily

metabolizable energy requirements and thus used up their reserves more quickly than those that survived, he was unable to explain why. He did make the conjecture that starvation might prove a significant mortality factor in the wild. It therefore became meaningful to examine genetic predisposition to survival on restricted feed intake.

The objectives of this study were to determine if genetic differences existed between endrin-resistant and -susceptible pine vole populations and between voles living and dying on restricted feed intake. To do so required the essential repetition of the earlier works of Webb et al. (1973) and Merson (1979), with the addition of analysis of electrophoretic variants. Genetic homogeneity among populations of voles displaying these particular characteristics was tested in two disparate experiments linked by the common technique of horizontal starch-gel electrophoresis. Practically, the study was designed to determine genetic differences among discontinuous orchard populations by examining specific gene loci believed to be involved in endrin metabolism. It was meaningful to test the proposed usefulness of this approach in a laboratory with a small electrophoresis facility and limited funds. Specifically, electrophoretic separation of polymorphic enzyme markers was employed in the detection of genetic variation among (1) pine voles from endrin-treated and non-

treated orchards; and (2) voles surviving and dying on 70% ad libitum feed intake.

LITERATURE REVIEW

Population Genetics in Small Mammals

The inspiration for this study began with a literature review of electrophoretic characterizations of genetic variation in wild populations of small mammals. Two basic approaches were common. In the first, analysis of two or three genetic markers was used to estimate the processes of natural selection by monitoring allele frequencies in a single population over time. The second approach employed correlations of average population heterozygosity with ecologically important variations, such as growth and reproductive rates.

The first method of analyzing population genetic variation was represented by Krebs and his coworkers studying cyclic population fluctuations in Microtus pennsylvanicus and M. ochrogaster (Krebs et al., 1973). These workers admittedly sought evidence for Chitty's behavioral-genetic hypothesis (Chitty, 1958; Chitty, 1960). This hypothesis states that with respect to cyclic density fluctuations, individuals with a competitive edge are genetically selected for during increase and peak phases of the cycle, but are somehow intrinsically less fit and are selected against during the decline--in effect, natural selection on a short time scale (Krebs, 1978). Recent investigations have included reproductive, survival,

dispersal, and other fitness characteristics in addition to Chitty's behavioral types, in order to demonstrate that gene frequencies do differ at opposite phases of the cycle (Semeonoff and Robertson, 1968; Myers and Krebs, 1971; Gaines et al., 1971; Gaines and Krebs, 1971; Tamarin and Krebs, 1973; Pickering et al., 1974; Smith et al., 1975). For example, Gaines and Krebs (1971) found genotypes of two loci significantly correlated with individual parameters of fitness in fluctuating microtine populations of M. pennsylvanicus and M. ochrogaster. They concluded that during the increase phase when survival was good, selection favored genotypes responsible for reproductive advantage (indicated by higher growth rate). During the decline, though, when mortality was greatest and recruitment poor, selection favored different genotypes responsible for enhanced viability (indicated by higher survival rate). Tamarin and Krebs (1969), in the same populations, had found that density was negatively correlated with frequency of the transferrin e allele: selection reduced its frequency during the increase phase but favored it during the decline.

The problem with correlating changes in gene frequency with changes in population density concerns interpretation. Chitty's position that gene frequency changes are the driving force behind demographic fluctuations cannot be separated from the possibility that changes in gene

frequency are merely side effects of demographics (Charlesworth and Giesel, 1972). This cause/effect controversy has precluded any finite judgement on the role of genetics in cyclic microtine populations.

The second approach in small mammal genetics, which correlates overall heterozygosity with individual parameters of fitness on either a temporal or spatial basis, was supported by Selander et al. (1971) and Smith et al. (1975). Chitty's hypothesis was extended to include the dependence of behavioral types on "heterotic effects across the entire genome" (Smith et al., 1975:88). Measures of heterozygosity are based on the concept of heterozygote advantage and function as indices of overall inherited genetic variability. Instead of comparing two or three loci within the same population over time, however, an average of the proportion of heterozygous loci in each individual is estimated using as many as twenty or more different markers. Smith et al. (1975), working with Peromyscus polionotus, found a positive linear relationship between mean reproductive rate (number of offspring/female/month) and mean genic heterozygosity over a geographic range from South Carolina to Florida. The authors proposed that higher heterozygosities, higher reproductive rates, and higher population densities should all occur in more variable environments. The more heterozygous individuals were

presumed to maintain a greater reservoir of adaptive potential in a changeable environment, and were selected for because this enhanced adaptability was inherited by their progeny.

Endrin Resistance in Pine Voles

Physiological Mechanisms

The existence of endrin-resistant pine vole strains could potentially yield a clearer comparison of population genetic differences with environmental stress than do cyclic fluctuations in density. Therefore, a discussion of endrin resistance in the pine vole is appropriate.

Endrin-caused mortality in the orchard is presumably due to dermal contact with the sprayed compound and ingestion of contaminated vegetation (Brown, 1978). Since toxicant resistance in mammals is primarily controlled by the rate of toxicant metabolism (Walker, 1975), most research in endrin metabolism has dealt with the hepatic microsomal mixed-function oxidase (MFO) system. The MFO system is the body's most important detoxification system, catalyzing various reactions in the degradation of toxicants, steroids, vitamins, and drugs. Liposoluble compounds like endrin must be enzymatically transformed to water soluble metabolites or conjugates for excretion in the bile or urine. The necessary enzymes occur mostly in the smooth endoplasmic reticulum of liver microsomes. Some

compounds, including chlorinated hydrocarbons and barbiturates, induce the liver microsomal enzymes which metabolize them, thus increasing their degradation rate. For example, pentobarbital induces higher levels of microsomal enzymes which in turn rapidly metabolize the drug. Greater doses in experimental animals thus produce shorter sleeping times as the drug is degraded, providing an index to microsomal enzyme activity.

In white rats, Kashole and Pawar (1977) found that oral injections of endrin had no effect on MFO characteristics after 12 hours, but liver microsomal protein and electron transport components per gram of liver tissue increased significantly after 108 hours in a dose-dependent way. Since intraperitoneal injections also reduced pentobarbital sleeping times, the authors concluded that endrin had a non-specific inducing effect on the hepatic MFO system. When Webb and Horsfall (1967) discovered endrin resistance in pine voles, research at their laboratory had focused on similar physiological mechanisms.

Benzpyrene (BP) hydroxylase activity, another assay of hepatic microsomal activity, was reported higher in endrin-resistant than in endrin-susceptible pine voles by Webb et al. (1972) and Hayes et al. (1975). Hartgrove et al. (1974), however, found that phenobarbital induction of microsomal enzyme activity and cytochrome P-450 content (the

terminal oxidase in the hepatic MFO system) increased equally in both endrin-resistant and -susceptible strains. Neither were significant differences in activities at toxicant binding sites of cytochrome P-450 found between resistant and susceptible strains (Hayes et al., 1975). Both Hartgrove et al. (1974) and Hartgrove et al. (1977) concluded that endrin did not induce hepatic microsomal MFO activity in pine voles, although it did significantly influence this activity in white mice. Rather, resistance was associated with increased excretion rates of different hydrophilic endrin metabolites (Petrella et al., 1975). Urinary excretion rates were similar in both resistant and susceptible voles, but fecal excretion rates were twice as rapid in resistant voles. Both strains produced the same fecal metabolites, but the endrin-resistant animals produced a urinary metabolite not found in the resistant strain. Resistance is attributed, as far as known, more to metabolic differences between strains than to differences in levels of microsomal enzyme induction.

Preadaptive Aspects of Endrin Resistance

Rudimentary genetic observations were conducted in conjunction with the physiological research described above, since the authors recognized that endrin resistance may have preadaptive aspects. By definition, a preadaptive mechanism operates by "selection of genes for resistance already

present and not by the production of mutations" (Walker, 1975:75). With respect to the endrin-resistant pine vole population discovered by Webb and Horsfall (1967), researchers needed to know if resistance was a function of inherent detoxification capabilities or controlled by "acquired mechanisms" like enzyme induction.

The assays that characterize the inducibility of the hepatic microsomal mixed-function oxidase system, such as BP hydroxylase activity, also served to prompt speculation about a possible genetic component operating in endrin resistance in the pine vole. Webb et al. (1972) believed that the increased microsomal enzyme activity observed in resistant voles was inherited by their progeny. In the first generation from crosses of resistant animals, BP activity was only slightly lower than in the parents but was much higher than in susceptible animals. These results led Hartgrove et al. (1974) to suggest that resistance may involve both genetic and inductive effects.

The most persuasive piece of genetic evidence from these toxicology studies was reported by Webb et al. (1973). The original oral LD-50 values reported by Webb and Horsfall (1967) were 36.12 mg/kg for resistant voles trapped in an orchard sprayed yearly from 1956-1966, and 2.97 mg/kg for susceptible voles trapped in an unsprayed orchard. (In 1975, Hayes et al. reported LD-50's in the same populations

to be 18.91 and 2.56 mg/kg, respectively). From strains comparable in LD-50 to those discovered in 1967, resistant x susceptible (R x S) crosses yielded first-generation progeny with an LD-50 of 21.08 mg/kg, intermediate to those resulting from (R x R) and (S x S) crosses (Webb et al., 1973). These results suggested a codominant inherited component. Furthermore, Hartgrove et al. (1977) noted that the pine voles in their laboratory retained resistance over continued periods of nonexposure. Since they found no endrin residues in body tissues either, Hartgrove et al. (1977) agreed that a preadaptive component must be involved in endrin resistance.

Body Fat Content as a Physiological Indicator

Seasonal Fluctuations of Food Availability

In the study of population cycles in microtine rodents, Cole and Batzli (1978:817) stated that something "intrinsic to the population" must be involved in the periodic density fluctuations they observed in M. ochrogaster in Illinois. A local population decline seemed uninfluenced by diet quality, disease, parasitism, adverse weather, or excessive dispersal. Cole and Batzli (1979) examined closely the different densities of voles found in habitats with differing food availability. Alfalfa was known to be more digestible, to provide more crude protein and minerals, and to be preferentially selected by voles even when other

native grasses were more plentiful. When released into enclosures planted in alfalfa, voles grew faster and reproduced earlier than did other voles in bluegrass or prairie enclosures. Seasonal variations in fat reserves were noted as well. Fat levels in all voles gradually increased to a peak during the winter nonbreeding season. Animals in the alfalfa fields, however, were in better condition than those in the bluegrass, especially during the winter. The authors concluded that "higher forage quality accounted for greater peak densities of vole populations, but did not prevent population declines" (Cole and Batzli, 1979:455).

Kolodziej-Banach (1976) investigated fat content as an indicator of population fluctuations in a European vole species, M. agrestis. She too observed that increased fatness levels acquired during fall and winter declined upon commencement of the breeding season in the spring. She believed that fat content varied in the same way density, reproduction, and mortality did over a two-year cycle.

In contrast, the pine vole is not generally considered a cyclic species, although no hard evidence either for or against this view exists. Hamilton (1938:166), referring to the native pine vole then classified as Pitymus pinetorum scalopsoides, declared "Pitymus is a cyclic species", but cited no data other than the damage assessments of

orchardists. Since the cyclic nature of the pine vole is yet undefined, seasonal differences in fat content have not been related to phases of a cycle in the manner of Kolodziej-Banach (1976), above. Cengel et al. (1978), however, found body fat levels of wild pine voles changing in response to quality of the available forage. In a maintained orchard where the undergrowth was continuously mowed and thus more succulent and energy-rich, fat levels increased throughout the fall to a peak in November. An abandoned orchard, in contrast, had heavier growths of forbs and tough, older grasses, but the voles were surprisingly fatter. In September, both sexes in the abandoned orchard had high body fat levels that declined sharply in November. Fat content then increased again in January, when females were at their fattest. Complete cessation of reproduction in the abandoned orchard was observed over the fall and winter, as well. The data implied that voles conserved the available energy in forage to put on fat for over-winter survival, rather than expend energy in reproduction. Another link between food availability and seasonal fluctuations in reproduction was thus documented.

Survival and Body Fat Content

Merson's work (1979) probed further into the relationship between food availability and survival in pine voles. In a controlled laboratory experiment, no mortality

occurred in male pine voles on ad libitum feed rations, and the animals lost <1% body weight during a three-week experiment. In contrast, animals on 85% ad libitum lost 17% body weight, and 3 of 14 animals died; 7 of 14 animals died in the 70% ad libitum group, and they lost about 20% of their original body weight. Body fat levels (carcass fat, % dry weight basis) of dying voles were 5.9 ± 0.4 in the 85% group and 5.5 ± 0.3 in the 70% group. Survivors on these restrictions, however, had significantly higher body fat levels: 20.4 ± 3.6 and 24.8 ± 6.1 , respectively, compared to voles on 100% ad libitum which maintained $43.2 \pm 3.3\%$ carcass fat.

In the wild, body fat content as an indicator of physiological condition depends on an animal's food supply, activity, age, time of birth, and reproductive status (Merson, 1979). Merson speculated that the herbivorous pine vole has not yet evolved mechanisms with which to cope with short-term starvation since its wide food base is rarely restricted throughout the year. This may not always be true, however, especially in abandoned winter orchards (Noffsinger, 1976). Why both survival with retention of body fat and mortality with depleted reserves should develop in response to the same feed restriction is unclear. Such individual responses point to possible inherited predispositions; in other words, some animals may be

genetically susceptible to starvation mortality during short-term periods of feed restriction.

Electrophoresis in Population Genetics

Most of the literature reviewed for this study necessarily was chosen for its emphasis of population genetics. Describing the genetic structure of wild populations is difficult, especially with microtine species which tend to be externally phenotypically uniform. Electrophoresis is a relatively simple technique for describing at the molecular level the genetic composition of an individual or population. Its success depends on the presence of polymorphic protein or enzyme systems.

Genetic polymorphism (at a two-allele locus, for example) exists when the two alleles are selectively maintained at intermediate frequencies, neither becoming fixed at the expense of the other, so that three genotypes are always produced in the population (Wallace, 1970). Continuous, permanent diversity within the population results (Ford, 1975). Although a balanced polymorphism is generally described as the selective maintenance of multiple phenotypes, processes other than selection can account for it. Gene flow via migration or mutation may result in polymorphism, even in a small population subject to random genetic drift effects. Polymorphism is generally ensured, however, whenever heterozygotes have a slight advantage over

any homozygotes at a particular locus (Ford, 1975).

A polymorphism arises when mutation causes the loss, gain, or substitution of nucleotide(s) in the DNA sequence of an allele that codes for a protein or enzyme, so that the change in amino acid sequence proves beneficial (or at least not detrimental) to the individual's survival. The resulting isozymes are multiple molecular forms of the same enzyme occurring either in an individual or within the species. The term isozyme refers specifically to different enzyme forms produced by different gene loci; allozymes are produced by combinations of multiple alleles at the same locus. For example, in most mammalian species the production of glutamate oxaloacetate transaminase is controlled by two gene loci designated GOT-1 and GOT-2, which occur in different cellular components. Each of the GOT-1 and GOT-2 isozymes also produce different allozymic forms in an individual, depending upon which alleles are present (i.e., genotype at that locus). Some changes in amino acid sequence (i.e., allelic change) cause a net change in enzyme charge, so that different allelic forms migrate faster or slower in an electric field. In this way, electrophoresis separates genotypes by identifying the products of all alleles. "Hidden" variation at the enzyme level can thus be used to identify physiological adaptations (Lewontin, 1974; Ford, 1975; Harris and Hopkinson, 1976).

Since in mammals no specific enzyme system has yet been linked to an environmental response by the individual, researchers have depended on arbitrarily chosen polymorphic markers to monitor genetic processes in the entire genome. The use of particular polymorphisms rests on the assumption that marker loci are a random sample of the entire genome (Selander et al., 1971). Powell (1975) maintained that this assumption is probably valid, since researchers can use only those loci for which diagnostic histochemical stains are available and which occur in detectable concentrations. Accordingly, "only a fraction of the variation in a fraction of the genome" can be electrophoretically analyzed (Powell, 1975:80). Specifically, only those DNA changes in genes that cause amino acid substitutions and thus a change in net charge are useful. The failure, therefore, to detect electrophoretic variation in a sample of 20 enzymes, for example, cannot be interpreted as absence of genetic variation in the population (Powell, 1975). Some polymorphic loci, however, are currently assumed to be "linked in a supergene complex with some ecologically relevant loci" (Tamarin and Krebs, 1969:204). Ford (1975) proposed that genes controlling polymorphisms act together in closely linked groups which prevent crossing over (recombination) among alleles, which then segregate in blocks. In some cases, a certain phenotype may be due to

additive effects of many such alleles acting together. Since selection operates on the individual rather than on single loci, the idea of genes acting together is intuitively satisfying (Wilson and Bossert, 1971). In any event, "if many loci were not involved, the probability of picking a genetic marker which showed correlation with population processes would be very low" (Tamarin and Krebs, 1969:208). The multitude of papers reporting such correlations tends to support the linkage concept.

MATERIALS AND METHODS

Endrin Experiment

Trapping and Animal Maintenance

Pine voles were trapped in both endrin-treated and non-treated apple orchards near Roanoke, Va., both before and after the endrin application. Orchardists apply a ground spray of endrin during the dormant season (usually in late November or early December), so a representative sample of each treated orchard's pine vole population was trapped from September to early November. Then another sample of animals was trapped after spraying (February through early March), before the next generation of young was produced the following spring. The same animals which had been subjected to and survived the endrin sprayings thus should have been captured in the spring sampling period.

Table 1 briefly describes each orchard trapped. History of endrin treatment is reported as accurately as known, but most orchardists kept infrequent records, so rates and dates of application could only be estimated. Three pints endrin/100 gal. water/acre is recommended for pine vole control, but Mr. Corbett applied "a little less" than this amount, Mr. Sprinkle "a little more", and Mr. Kinzie up to twice as much. All three orchardists had considerable vole problems, and while all maintained that endrin "worked", they still realized that vole populations

Table 1. Apple orchards in southwestern Virginia from which pine voles were collected, Fall 1980 and Spring 1981.

Orchard	Location	Dates Trapped	Endrin History
Endrin-treated Orchards			
Corbett	Troutville	Late Nov.- early Dec. 1980 Mid Feb. 1981	Endrin-sprayed for past 15 years
Kinzie	Troutville	Late Oct.- early Nov. 1980	Endrin-sprayed for past 15 years
Sprinkle	Daleville	Late Nov.- early Dec. 1980 Mid Mar. 1981	Endrin-sprayed for past 15 years
Nontreated Orchards			
Gross	Bedford	Early Jan. 1981 Early Apr. 1981	Not sprayed for past 15 years
Watson	Bedford	Mid Dec. 1980 Late Mar. 1981	Not sprayed for past 15 years
Wertz	Salem	Late Dec. 1980	Never sprayed

Daleville, Salem, and Troutville are located near Roanoke, Va.; Bedford is approximately 60 miles to the east.

were not checked. Sprinkle and Kinzie amplified their control programs by putting down the anticoagulant Rozol (chlorphacinone) in pelleted baits after the endrin application, and both believed the combination of chemicals more effective in controlling voles than endrin alone. Indeed, the Kinzie population was so effectively eliminated that only 2 animals were captured in a full day's trapping during the Spring sampling period (compared to 50-80 voles per day prior to treatment). Therefore, Kinzie's orchard was not included in the Spring sample, nor was the Wertz orchard. Although never subjected to endrin treatment, the pine vole population in that orchard was naturally too low and scattered to obtain an adequate sample size in the limited time available for trapping.

The primary trapping objective was procuring animals. Secondly, a representative sample of an orchard's population was necessary. Since the animals were to be removed (a form of artificial selection), only one section of an orchard was trapped in the Fall sampling period, leaving an untrapped section for the Spring sampling. Sections were separated by a buffer zone of at least three rows of trees. Each sampling period, 100 animals were required from each orchard. About 50 were live-trapped for LD-50 determinations, and the remaining 50 were snap-trapped. For the Spring sample, a total of 50 animals from

each orchard was considered acceptable, since population densities were lower at this time. Although time constraints permitted only one week for trapping any one orchard, in most cases these quotas were filled.

Voies were captured in small (16 x 7 x 6 cm) Sherman live traps, ordinary household Victor snap traps, and Museum Special snap traps. Live and snap traps were set in separate areas of the section to be trapped. Both types were set at active tunnels or runways, baited with apple, and covered with pieces of tar paper. Usually 2 traps were placed under each tree, but 1-4 were set depending on the level of vole activity observed. Live traps were checked and reset every 2-3 hours; snap traps every 1/2-1 hour. 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.

Snap-trapped carcasses were stored in small plastic Whirl-Paks and immediately placed on dry ice in the field. The whole carcasses were then stored in the Department of Fisheries and Wildlife freezer at about -20C.

Each live-trapped animal originally was to be toe-clipped with a unique identification number in the field. Because the excessive mortality sustained by these voles once they were settled in laboratory cages might be compounded by this stress, the marking procedure was

abandoned as the study progressed. The animals were brought to the Department's Animal Room and allocated by pairs (same sex) to hanging wire cages. Each cage was furnished with burlap bedding, apple twigs, and ad libitum water and Purina rodent chow. Fresh apple was provided to the voles during their first week of captivity. Animals were observed three times daily, at which time any carcasses were collected, wrapped in freezer foil or plastic, and stored at -20C. Photoperiod was held at a 14L:10D sequence. Relative humidity and temperature were not rigidly controlled but remained at the same levels as in the building at large.

LD-50 Determinations

The procedure for determining the 7-day oral endrin LD-50 for each orchard's pine vole population was designed to resemble as closely as possible that of Webb et al. (1973). Only adult animals were used, after a 3-week laboratory adjustment period. Most of the mortality due to trapping and relocation stress occurred in this time period, and any pregnant females could be identified and excluded from the test. Two days before the test, animals were individually caged and adequately provisioned with feed, water, bedding, and twigs. Animals were not fasted overnight before the test.

Varying dose ranges were used in the LD-50 determinations, with a varying number of animals per

treatment group, depending on the number of healthy voles available (see Table 2). The range of doses was selected to encompass LD-50 values for both endrin-resistant and -susceptible strains reported by Webb et al. (1973). In general, 4-6 voles were randomly assigned to each treatment group. Each group was half male and half female, since an insufficient number of either one sex could be trapped and maintained in the lab for any one test. The sequential order in which individuals were injected was also randomized, as was the location of cages on the rack. All LD-50 tests were begun at noon, with mortality observed hourly that first day, every 3 hours the next day, and at 6-hour intervals thereafter. After 7 days survivors were sacrificed, wrapped in freezer foil or plastic, and stored at -20C. Carcasses of voles collected during the 7-day observation period were similarly stored.

Commercial-grade endrin (manufactured by the Shell Chemical Co., New York, N.Y., and a gift of Mr. R.W. Young of the V.P.I.&S.U. Pesticide Analysis Laboratory) was used for LD-50 determination. Appropriate stock solutions were mixed in corn oil on an active-ingredient basis such that an average-weight vole (25.0 g) received a 0.5 ml-volume dose. Control animals received corn oil only. Endrin doses were calculated on a mg endrin/kg body weight basis, and injections were made by gavage. Any animal injured during

Table 2. Experimental design for LD-50 determinations of orchard pine vole populations trapped Fall 1980 and Spring 1981 (n=number of voles per treatment).

Orchard	Date of LD-50 Test	Treatment Levels (mg endrin/kg b.w.)	n
Fall			
Corbett	16 Dec. 1980	0, 1, 2, 4, 8, 16, 32	6
Kinzie	21 Nov. 1980	0, 1, 6, 36	4
Sprinkle	20 Jan. 1981	0, 1, 3, 9, 27	6
Gross	9 Feb. 1981	0, 1, 3, 9	6
Watson	31 Jan. 1981	0, 1, 3, 9, 27	6
Spring			
Corbett	14 Mar. 1981	0, 1, 3, 9	6
Sprinkle	25 Apr. 1981	0, 1, 6, 36	4
Gross	2 May 1981	0, 1, 3, 9	6
Watson	26 Apr. 1981	0, 1, 6, 36	4

the procedure was replaced.

Nutrition Experiment

Trapping, Laboratory Maintenance, and Feed Rations

Pine voles were live-trapped from a 1-1/2 acre section of L.W. Corbett's orchard in early April 1981, by the same trapping procedure described for the endrin experiment above. The animals were placed in hanging wire cages and allowed about 5 weeks for laboratory adjustment. Animals received the same care as that described for voles in the endrin experiment. During the second week of May, 48 animals (25 males, 23 females) were randomly assigned in the following way to either of two treatment groups: 12 voles (6 males, 6 females) to 100% ad libitum feed rations, and 36 voles (19 males, 17 females) to 70% ad libitum. Purina rodent chow ground in a large (2 mm mesh) Wiley mill was fed to both groups, with water always available. For four days prior to the actual experimental period, all voles received ad libitum ground chow to determine mean ad libitum consumption. From this amount the 30% restriction was computed on the first day of the experiment. Each day afterward, the feed consumed by the control (100% ad libitum) group was measured and the restriction computed from the mean ad libitum measured the previous day. Each vole was weighed on the first day and again either at death or upon sacrifice at the end of the 24-day experimental

period. Both animals and feed were weighed on a triple-beam balance.

During the first 2 weeks of the experiment, little mortality occurred. Two control animals appeared to be eating obsessively, up to 9 and 10 g per day, thereby inflating the mean ad libitum from which the restriction was computed. Even though presumably random representatives of the entire group, these 2 animals were thought to be behaving abnormally and were removed from the experiment. Over the first 17 days, then, mean ad libitum consumption was 4.6 ± 0.3 g, and 70% of this was 3.2 ± 0.2 g. For the last 7 days of the experiment, mean ad libitum was 4.1 ± 0.3 g, and the restriction was increased to 60% of this, or 2.5 ± 0.2 g. The animals were fed at the same hour each day.

Checks for mortality occurred every 6 hours so that carcasses were collected before excessive tissue degradation began. Each carcass was weighed, wrapped in freezer foil or plastic, and immediately frozen at -20°C .

Electrophoretic Procedure

Genetic Markers

Changes in activity levels of certain enzymes have been reported in the literature as resulting from endrin injections in small mammals. Whether these enzymes are involved in endrin metabolism or changes in their activities are simply a result of it is unknown, but it was planned to

monitor endrin resistance in the pine vole using such enzymes as genetic markers. Of the enzyme systems already known to be polymorphic in the pine vole (Gourley, pers. comm.), lactate dehydrogenase (LDH) and glutamate oxaloacetate transaminase (GOT) were reported as endrin-responsive. Three other endrin-responsive enzymes of unknown polymorphic nature for which electrophoretic procedures were convenient in our laboratory were surveyed for polymorphisms suitable for use as genetic markers: acid phosphatase (ACP), beta-glucuronidase (beta-GUS), and fructose-1,6-diphosphatase (FDP).

Meena et al. (1978) reported significantly increased levels of GOT in the liver and kidneys of male white rats 12-48 hours after a single intraperitoneal dose of 10 mg endrin/kg, while both acid and alkaline phosphatases significantly decreased. Activities of ACP and beta-GUS were inhibited in isolated lysosomal fraction of rat liver (Ludwicki, 1974), and a single oral dose of 50 mg endrin/kg administered to male rats resulted in increased activity of FDP (Kacew and Singhal, 1973). Evidence for endrin-inhibited LDH activity in rabbit muscle was reported by Hendrickson and Bowden (1976).

Three other known polymorphic loci were selected arbitrarily for electrophoresis as comparisons for the endrin-responsive enzymes. The metabolic function of

isocitrate dehydrogenase (IDH) is not fully known beyond its rate-limiting activity in the tricarboxylic acid cycle (Johnson, 1974). Malate dehydrogenase (MDH) also functions in this cycle and is important in gluconeogenesis (Lehninger, 1971). Phosphoglucomutase (PGM) catalyzes the conversion of glucose-1-phosphate to glucose-6-phosphate and is important in both glycolysis and gluconeogenesis (Lehninger, 1971; Johnson, 1974).

Thus a total of 8 polymorphic enzyme systems was electrophoretically surveyed for each pine vole population. It was necessarily assumed that none of the polymorphisms observed influenced the probability an animal was trapped.

Tissue Preparation

Gourley (pers. comm.) recommended kidney as the best tissue for resolution of the pine vole polymorphisms studied. Storing whole carcasses at -20C preserved these tissues for several months. When tissues are removed from the whole carcass, however, the rate of enzyme degradation increases, and increases at a faster rate for homogenized tissues in solution, unless such tissues are refrozen at or below -90C. Kidneys were excised from animals trapped in the fall sampling period (either upon discovery of the carcass after laboratory mortality or upon removal of carcasses from dry-ice storage in the field), wrapped in freezer foil, and stored in the V.P.I.&S.U. Department of

Animal Science freezer at -90C. Kidneys were excised from the later samples (stored as whole carcasses in the Department of Fisheries and Wildlife freezer at -20C) before electrophoresis only in allotments that could be processed within the next 2-3 days, for which time refreezing at -20C was adequate. For electrophoresis, frozen paired kidneys were homogenized in 0.5 ml of a tissue grinding buffer using an electrically-powered teflon bit (see Appendix for recipes of all solutions). Two subsamples from each individual were aliquotted to marked plastic 400-microliter centrifuge tubes. Homogenates were then immediately prepared for electrophoresis or refrozen at -20C for use the next day. Just before electrophoresis, samples were centrifuged at 1500 x g, 0-4C, for 30 minutes. The supernatant was then withdrawn by 20-microliter pipette and applied to 9 x 5 mm filter wicks, which were then inserted into the prepared gel. At all stages of the above procedures, carcasses, excised kidneys, homogenized tissues, and prepared sample wicks were always in frozen storage, under refrigeration, or on ice while in actual preparation.

Electrophoresis

The enzyme markers observed are listed, with appropriate buffers and running times, in the Appendix, as are the recipes for histochemical stains. All recipes are modified Harris and Hopkinson (1976) formulas used by Guse

(1980 and pers. comm.). Starch-gels of 12.5% concentration were mixed with the appropriate buffer and Electrostarh Lot No. 392 (Electrostarh Co., Madison, Wisc.). Gel size was 14 x 14 x 1 cm. Gels were stored before use at 0-5C for up to 48 hours, but best resolution and ease of handling resulted if gels were used sooner, after 24 hours. Up to 18 sample wicks were inserted into a slice cut 2.5 cm from one end of the gel. The electrophoresis apparatus was installed in a 0-5C refrigerator and wired to an outside power source. After electrophoresis, each gel was cut into four horizontal slices, and both anodal and cathodal pieces of the inner two slices were placed in plastic boxes for application of the appropriate stain. Stained gels were incubated in the dark at 37C for 15-30 minutes or until bands appeared, then immediately scored by genotype (banding pattern). The first gels of each enzyme were diagrammed, but thereafter only patterns which were new or anomalous were drawn for later reference.

Data Analysis

Data were analyzed using the Statistical Analysis System (SAS) (Helwig and Council, eds., 1979). LD-50 values were predicted by the PROBIT procedure, which makes a probit transformation of the logistic response function. The PROBIT procedure also calculates 95% fiducial limits on the predicted LD-50. All LD-50 analyses were based on natural

logarithm of dose.

Body weight statistics for the nutrition experiment were computed by the MEANS and ANOVA procedures of SAS; ANOVA tests for differences between means by Duncan's Multiple Range test. For other data, correlations were performed by the CORR procedure; nonparametric statistics were calculated by the NPAR1WAY procedure.

Chi-squared tests of independence of genotype on sex, orchard, and level of feed intake were performed by the FREQ procedure of SAS. In each orchard population and in the group of animals on the nutrition experiment, deviations of observed allelic frequencies from those expected under Hardy-Weinberg equilibrium conditions were tested by chi-squared goodness-of-fit. The chi-squared analyses sometimes required the combination of classifications (i.e., rare genotypes) in order to avoid producing a contingency table too sparse (expected value of any cell <1.0) for a valid test. Degrees of freedom (df) then depended on the number of combined classifications.

Three-way independence tests of orchard, sampling time, and distribution of genotypes were conducted for each locus by the G log likelihood ratio test, which is approximated by the chi-squared distribution (Sokal and Rohlf, 1969a,b). This test, in its partitioning of the overall G value into separate subtests by individual degrees of freedom, produces

comparisons similar to those of analysis of variance.

Mean individual heterozygosity for each orchard pine vole population and for each feed intake group was calculated by dividing the observed number of heterozygous genotypes by the product of the number of loci and the total number of individuals surveyed.

All decisions were made at the $\alpha=0.05$ level of probability.

RESULTS

Electrophoresis of Genetic Markers

Three enzymes that respond by increased or decreased levels of activity to endrin injections in small mammals (ACP, beta-GUS, and FDP) were surveyed for use as possible polymorphic markers in accordance with the rule reported by Guse (1980:14), in which a sample of 18 individuals was sufficient to detect at the 99% level of probability "any polymorphic locus with a rare allele frequency of at least 0.12, a frequency which is still useful in the analysis of data". These enzymes were found to be monomorphic in the pine vole populations studied, and were dropped from further analysis.

The following enzymes were found to be polymorphic and were used for genetic analyses: GOT-2, IDH-1, LDH-1, MDH-2, and PGM-2. The genetic control of these polymorphisms is autosomal and similar in most mammalian species (Selander et al., 1971; Manlove et al., 1975; Harris and Hopkinson, 1976; Bowen and Yang, 1978). Polymorphic nomenclature varies throughout the literature; that of Manlove et al. (1975) is applied here. Isozymes are designated by numerals (1, 2, 3) in decreasing order of relative anodal mobility (e.g., isozyme 1 being most anodal). Allozymes (allelic forms at each locus) are designated by letters (a, b, c, d), also in decreasing order of anodal mobility (e.g., a bands being

most anodal, or in the case of cathodally-migrating forms, closest to the origin). A description of electrophoretic behavior observed in this study follows.

GOT (alleles a,b), Fig. 1(a). GOT-2 migrated cathodally; GOT-1, migrating anodally, was monomorphic in the populations observed. Banding pattern was dimeric (3-banded heterozygotes).

IDH (alleles a,b,c,d), Fig. 1(b). IDH-1 migrated anodally and exhibited dimeric form. Several variant banding patterns appeared for heterozygotes (all 3-banded), probably resulting from interaction with the rare alleles a and d at this locus. Examples of all possible heterozygous banding patterns did not appear in the populations studied and are therefore not depicted in Fig. 1(b).

LDH (alleles a,b,c,d), Fig. 1(c). The production of LDH is controlled by 3 loci (see Selander et al., 1971, and other literature cited above), 2 of which were manifested in the kidney tissue analyzed. The alleles of LDH-1 combined tetramERICALLY with those of LDH-2 to form 5-banded homozygote patterns and heterozygote patterns of up to 11 bands, all migrating anodally. In the pine vole populations observed, LDH-2 was not consistently scorable but apparently was monomorphic. As for IDH-1, only those heterozygous banding patterns observed are depicted in Fig. 1(c).

MDH (alleles a,b), Fig. 1(d). MDH-2 migrated

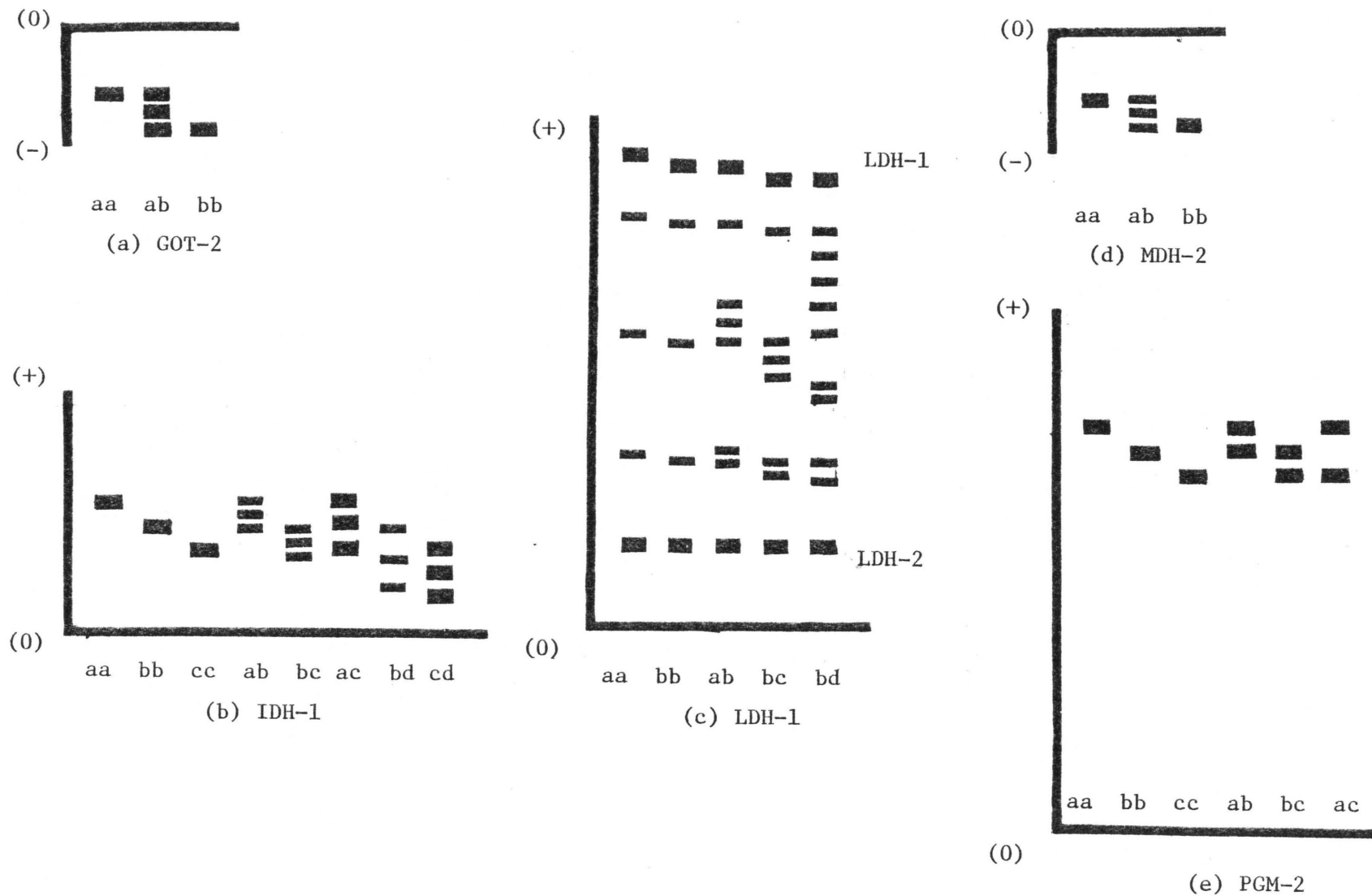


Fig. 1. Electrophoretic banding patterns of polymorphisms examined for population genetic variation in orchard-trapped pine voles, Fall 1980 and Spring 1981.

cathodally; MDH-1 migrated anodally and was monomorphic. Although dimeric (3-banded heterozygote patterns), MDH-2 also produced a secondary isozyme in the most anodal position of each pattern.

PGM (alleles a,b,c), Fig. 1(e). The production of PGM is controlled by 3 loci, all migrating anodally. PGM isozymes were not consistently scorable, probably because intervals between death and the freezing of carcasses were too long. PGM-2 was the most reliably scored locus in these pine vole populations. PGM-1, the most anodal form, was polymorphic where it appeared, and PGM-3 was monomorphic. All three loci were monomeric (2-banded heterozygote patterns).

Not every marker was electrophoretically observed for every individual animal. Enzyme denaturation after death reduced mobility in the electric field, and excessive degradation totally checked it. Such "dead" enzymes accounted for the loss of individual marker counts and reduction of the total sample size. Autolysis had progressed to such a state in some of the carcasses collected after laboratory mortality that all markers were absent in the gel. In other gels, only one or two enzymes appeared. PGM seemed to be the most delicate in this respect. Rather than tailor the final sample size to only those individuals displaying PGM-2 patterns, that marker was

eliminated from all mean heterozygosity estimates and the much greater sample size of individuals displaying the other four loci was used. The reader should therefore note that all PGM-2 statistics in the endrin experiment are based on a smaller sample size than are those for the other markers.

Endrin Experiment

LD-50 Determinations

Predicted oral endrin LD-50 (mg/kg) values for both endrin-treated and nontreated orchard populations, both before endrin treatment in Fall 1980 and after treatment in Spring 1981, are presented in Table 3. The LD-50 was predicted by probit regression analysis. Missing values in the table for 95% fiducial limits on the predicted LD-50 were due to the inability of the model to compensate for inadequate sample size. The values nevertheless roughly indicate the LD-50 for the different orchard populations.

Despite the assertions of orchardists that vole populations have remained unchecked or have increased over the past five years of heavy endrin treatment, no evidence of an endrin-resistant pine vole strain existed from the data taken in this study. Before the endrin spraying in late 1980, there was no appreciable difference among the LD-50's of the different orchards, whether endrin-treated or not.

The Kinzie population, however, deserves closer study.

Table 3. Predicted oral endrin LD-50 of pine vole populations trapped in both endrin-treated and nontreated apple orchards, Fall 1980 and Spring 1981.

Orchard	LD-50 (mg/kg)	95% Fiducial Limits
Fall, 1980		
Endrin-treated		
Corbett	2.2	0.1-8.7
Kinzie	7.7	---
Sprinkle	1.3	0.0-3.4
Nontreated		
Gross	3.4	---
Watson	2.1	0.9-4.4
Spring, 1981		
Endrin-treated		
Corbett	64.5	4.4- --
Sprinkle	5.5	0.1-2.8x10 ⁴
Nontreated		
Gross	2.8	---
Watson	1.2	---

The predicted LD-50 is based on questionable data, since the LD-50 test was conducted before the animals had adjusted sufficiently to laboratory conditions. Heavy mortality was occurring in animals not subjected to the test, and one test control animal died as well, so unknown mortality factors other than the endrin were present. (All other LD-50 tests were conducted after that of the Kinzie population and after adequate laboratory adjustment of the animals had reduced other sources of mortality.) Still, no animals died at the 8 mg/kg dose, although some died at all of the lower doses. More importantly, even at each of the highest doses (16 and 32 mg/kg), one animal survived. The LD-50 was reported on the strength of these unique survivals at high doses, since the value was underestimated at worst. It is still unknown if those animals which survived the high doses were representative of a resistant population or were merely uncharacteristic individuals. Unfortunately, time and available cage space precluded another LD-50 trial at that time, and by the Spring sampling period the population was so decimated that animals could not be trapped at all. Thus the barest suggestion from the data of endrin resistance is anomalous at best.

In Spring 1981 (after endrin treatment), the pine vole populations observed did not differ appreciably in LD-50 from the values obtained before the spraying, with one

exception. The Corbett population's LD-50 was dramatically higher (2.2 mg/kg before spraying, 64.5 after). In Sprinkle's orchard, the LD-50 increased slightly from 1.3 to 5.5 mg/kg, and the Spring value was higher than Spring LD-50's in the unsprayed orchards. It is difficult to interpret this slight increase to 5.5 mg/kg in the light of its extremely wide fiducial limits, but the value is definitely out of the endrin-resistant range reported by Webb et al. (1973). Animals more tolerant to endrin were obviously present in Corbett's orchard, but whether the tolerance was due to genetic selection of animals inherently more tolerant or the result of enzyme induction is unknown.

Genetic Analyses

Sample sizes of pine vole populations used for electrophoresis are listed in Tables 4-7. Males comprised 50.2% of the combined populations in Fall 1980 and 56.5% in Spring 1981. Total sample size was 492 in the Fall and 294 in the Spring. Only 4.9% and 6.5% of total sample size were subadult or juvenile age classes in the Fall and Spring, respectively.

Genetic variability among the different orchard populations from both sampling periods was analyzed in four ways: (1) by testing for the independence of genotype on sex; (2) by testing for independence of genotype on location and sampling period; (3) by testing for agreement with

Table 4. Sample sizes of pine vole populations trapped in both endrin-treated and nontreated orchards used in electrophoretic analyses of GOT-2, IDH-1, LDH-1, and MDH-2 loci, Fall 1980.

Orchard	Total	Sex (M:F)	Age Distribution		
			Ad.	Sub.	Juv.
Endrin-treated					
Corbett	118	58:60	111	7	0
Kinzie	98	54:44	94	4	0
Sprinkle	106	47:59	99	5	2
Nontreated					
Gross	77	42:35	73	4	0
Watson	70	37:33	69	1	0
Wertz	23	9:14	22	1	0

Endrin-treated orchards trapped late Oct.-early Dec., 1980; non-treated orchards trapped mid Dec.-late Dec., 1980.

Table 5. Sample sizes of pine vole populations trapped in both endrin-treated and nontreated orchards used in electrophoretic analyses of the PGM-2 locus, Fall 1980.

Orchard	Total	Sex (M:F)	Age Distribution		
			Ad.	Sub.	Juv.
Endrin-treated					
Corbett	40	18:22	38	2	0
Kinzie	58	30:28	55	3	0
Sprinkle	59	24:35	57	2	0
Nontreated					
Gross	5	4:1	5	0	0
Watson	34	18:16	34	0	0
Wertz	--	---	--	--	--

Endrin-treated orchards trapped late Oct.-early Dec., 1980; non-treated orchards trapped mid. Dec-late Dec., 1980.

Table 6. Sample sizes of pine vole populations trapped in both endrin-treated and nontreated orchards used in electrophoretic analyses of GOT-2, IDH-1, LDH-1, and MDH-2 loci, Spring 1981.

Orchard	Total	Sex (M:F)	Age Distribution		
			Ad.	Sub.	Juv.
Endrin-treated					
Corbett	90	54:36	86	4	0
Sprinkle	82	47:35	77	5	0
Nontreated					
Gross	54	28:26	52	0	2
Watson	68	37:31	60	6	2

Endrin-treated orchards trapped mid Feb.-mid Mar., 1981; non-treated orchards trapped late Mar.-early Apr., 1981.

Table 7. Sample sizes of pine vole populations trapped in both endrin-treated and nontreated orchards used in electrophoretic analyses of the PGM-2 locus, Spring 1981.

Orchard	Total	Sex (M:F)	Age Distribution		
			Ad.	Sub.	Juv.
Endrin-treated					
Corbett	35	21:14	33	2	0
Sprinkle	30	16:14	29	1	0
Nontreated					
Gross	25	13:12	24	0	1
Watson	35	16:19	32	3	0

Endrin-treated orchards trapped mid Feb.-mid Mar., 1981; non-treated orchards trapped late Mar.-early Apr., 1981.

Hardy-Weinberg equilibrium expectations; and (4) by estimating mean individual heterozygosity at GOT-2, IDH-1, LDH-1, and MDH-2 loci combined, and at each locus separately. In the following sections, reference is made throughout to observed or expected genotypes. Properly speaking, isozyme phenotypes were observed via electrophoresis, but since the "enzyme is a direct product of the gene,...analysis of the gene action [is] relatively straightforward" (Shaw, 1965:940).

All loci were tested at the $\alpha=0.05$ probability level for dependency on sex by chi-squared contingency tables. In Fall 1980, IDH-1 was dependent on sex in Kinzie's sprayed orchard and in Watson's unsprayed orchard. Greater numbers of heterozygous females than those expected (and a concomittant lesser number of heterozygous males than expected) were present in Kinzie's, whereas the reverse was true in Watson's. In Spring 1981, IDH-1 was dependent on sex in Sprinkle's sprayed orchard, and a greater number of female heterozygotes was observed than expected. MDH-2 was dependent on sex in Sprinkle's orchard in the Fall only, displaying a greater number of female heterozygotes than expected. All other loci were independent of sex. Since genotypic frequencies are assumed to be equal at conception, deviations of observed frequencies from expected imply some form of sexual selection. Therefore, the above loci were

considered unsuitable for tests of agreement with Hardy-Weinberg equilibrium conditions.

Extremely small numbers of subadult and juvenile animals were caught in any one orchard; in some orchards, not any were caught. Therefore testing for dependency on age (and therefore for age-dependent selection) was impossible. All subsequent genetic analyses were conducted with adult individuals only.

Results of chi-squared tests of agreement of observed genotype frequencies with those expected under Hardy-Weinberg equilibrium conditions are presented in Appendix Tables II-1,2,3,4,5. Each locus was found in frequencies inconsistent with equilibrium expectations in one or more populations. Table 8 lists those loci in each population which were not in equilibrium. Of those loci not in equilibrium, greater numbers of observed heterozygous genotypes than those expected under equilibrium conditions were interpreted as heterozygote advantage, since these genotypes were favored over the homozygotes. Thus heterozygote advantage occurred consistently for the MDH-2 locus in all orchards not in Hardy-Weinberg equilibrium at that locus, in both sprayed and unsprayed orchards and at both sampling periods. Heterozygote advantage was also observed in the Fall for GOT-2 in two unsprayed orchards and for LDH-1 in two sprayed orchards, as well as for IDH-1 in

Table 8. Loci for which observed genotype frequencies did not agree with Hardy-Weinberg equilibrium expected values, for adult pine vole populations trapped in both endrin-treated and nontreated orchards, Fall 1980 and Spring 1981.

Orchard	Loci Not in Equilibrium				
	GOT-2	IDH-1	LDH-1	MDH-2	PGM-2
Fall 1980					
Endrin-treated					
Corbett	x	H		H	x
Kinzie	*	H		H	x
Sprinkle				*	x
Nontreated					
Gross		H	x	H	
Watson	H	*	x	H	x
Wertz	H		x		-
Spring 1981					
Endrin-treated					
Corbett	x			H	x
Sprinkle	*	x		H	x
Nontreated					
Gross	x		x	H	x
Watson				H	x

H = observed number of heterozygotes > Hardy-Weinberg expectations.

x = observed number of homozygotes > Hardy-Weinberg expectations.

* = Hardy-Weinberg analysis invalid due to dependence of genotypes on sex.

one unsprayed orchard. In the Spring, MDH-2 was the only locus not in Hardy-Weinberg equilibrium, as discussed above.

Appendix Tables II-6 and II-7 present the observed allele frequencies of all loci for each orchard population, although it must be stated that such data are questionable in populations not in Hardy-Weinberg equilibrium. Since the allele frequencies are calculated from observed genotypic frequencies, deviations of the latter from the Hardy-Weinberg model cause inaccuracies in the allele frequencies. The allele frequencies were reported, however, to provide the reader with relative comparisons of frequencies among orchards that would have occurred if loci were in equilibrium.

The homozygote designated bb was rare for both GOT-2 and MDH-2 loci. Rare alleles at the LDH locus were LDH-1 c and d. In the Fall, c was absent from one unsprayed orchard but appeared at very low frequency in the Spring. This same pattern occurred for the d allele in both one sprayed and one unsprayed orchard. As for rare IDH-1 alleles, a was nowhere abundant, and disappeared entirely from one sprayed and one unsprayed orchard by Spring. The d allele was absent from all orchards except Corbett's sprayed orchard in the Fall, but appeared in low frequencies in all but Sprinkle's orchard in the Spring.

Results of 3-way tests of independence of orchard,

sampling period, and genotype frequency are listed for each locus in Table 9 and summarized in Table 10. The orchard x season x genotype independence test was significant at the $\alpha=0.05$ level for all five loci; the orchard x season x genotype interaction (a test similar in effect to 3-way analysis of variance) was significant for all loci except PGM-2. Genotypes of GOT-2 and PGM-2 loci were independent of orchard, and genotypes of GOT-2 and LDH-1 were independent of sampling period; all other loci were dependent upon orchard and sampling period, respectively. Differences in distribution of genotype frequencies were significant due to orchard type (endrin-treated or nontreated) at the IDH-1 and LDH-1 loci. Heterozygotes formed greater proportions of the populations in nontreated orchards for IDH-1, and in treated orchards for LDH-1. IDH-1, MDH-2, and PGM-2 genotypes were dependent on sampling period, as the heterozygous proportion of the populations was greater in the Fall for IDH-1 and PGM-2, but greater in the Spring for MDH-2. Sample size was dependent on sampling period (orchard x season test of independence) only for PGM-2, indicating specifically that a smaller sample of active PGM-2 markers was available in unsprayed orchards, particularly in the Fall. The results of these G-tests of independence for each locus are described below.

Both orchard x season x genotype dependence and orchard

Table 9. Results of 3-way G-tests of independence for the five loci observed in adult pine vole populations trapped in both endrin-treated and nontreated orchards, Fall 1980 and Spring 1981.

Hypothesis Tested	G	p	df
GOT-2 (N=627)			
Orchard x season x genotype independence	0.63	0.890	3
Orchard x genotype independence	9.16	0.165	6
Season x genotype independence	4.92	0.086	2
Orchard x season x genotype interaction	<u>29.40</u>	<u><0.001</u>	<u>6</u>
Orchard x season x genotype independence	44.13	<0.001	17
IDH-1 (N=627)			
Orchard x season independence	0.63	0.890	3
Orchard x genotype independence	89.46	<0.001	9
Within treated orchards	18.37	<0.001	3
Within nontreated orchards	23.21	<0.001	3
Between orchard types	47.89	<0.001	3
Season x genotype independence	43.36	<0.001	3
Orchard x season x genotype interaction	<u>41.16</u>	<u><0.001</u>	<u>9</u>
Orchard x season x genotype independence	179.62	<0.001	24

Table 9 (continued). Results of 3-way G-tests of independence.

Hypothesis Tested	G	p	df
LDH-1 (N=627)			
Orchard x season independence	0.63	0.890	3
Orchard x genotype independence	23.39	<0.001	6
Within treated orchards	12.70	0.002	2
Within nontreated orchards	2.36	0.307	2
Between orchard types	8.34	0.016	2
Season x genotype independence	0.06	0.970	2
Orchard x season x genotype interaction	<u>18.05</u>	<u>0.006</u>	<u>6</u>
Orchard x season x genotype independence	42.14	<0.001	17
MDH-2 (N=627)			
Orchard x season independence	0.63	0.890	3
Orchard x genotype independence	24.60	<0.001	6
Within treated orchards	6.49	0.039	2
Within nontreated orchards	16.11	<0.001	2
Between orchard types	2.01	0.366	2
Season x genotype independence	14.95	<0.001	2
Orchard x season x genotype interaction	<u>17.57</u>	<u>0.008</u>	<u>6</u>
Orchard x season x genotype independence	57.76	<0.001	17

Table 9 (continued). Results of 3-way G-tests of independence.

Hypothesis Tested	G	p	df
PGM-2 (N=262)			
Orchard x season independence	23.42	<0.001	3
Within treated orchards	2.67	0.102	1
Within nontreated orchards	10.08	0.002	1
Between orchard types	10.67	0.001	1
Orchard x genotype independence	19.14	0.208	15
Season x genotype independence	12.05	0.034	5
Orchard x season x genotype interaction	<u>8.61</u>	<u>0.971</u>	<u>15</u>
Orchard x season x genotype independence	63.23	0.006	38

Table 10. Summary of 3-way G-tests of independence significant at the $\alpha=0.05$ level for five loci observed in adult pine vole populations trapped in both endrin-treated and nontreated orchards, Fall 1980 and Spring 1981 (sample size in parentheses).

Hypothesis Tested	Significant at observed loci:				
	GOT-2 (627)	IDH-1 (627)	LDH-1 (627)	MDH-2 (627)	PGM-2 (262)
Orchard x season independence					X
Within treated orchards					
Within nontreated orchards					X
Between orchard types					X
Orchard x genotype independence	X	X	X	X	
Within treated orchards	X	X	X	X	
Within nontreated orchards	X	X	X	X	
Between orchard types	X	X	X	X	
Season x genotype independence	X	X	X	X	X
Orchard x season x genotype interaction	X	X	X	X	
Orchard x season x genotype independence	X	X	X	X	X

x season x genotype interaction existed at the GOT-2 locus. interaction existed at the GOT-2 locus. These relationships were also significant at the IDH-1 locus, but genotype was further dependent upon both orchard and sampling period. Genotype frequencies were dependent upon whether the orchard was endrin-treated or not (heterozygotes comprising 37% of the populations in treated orchards and 64% in nontreated orchards), but also dependent upon individual orchard within each orchard type. With respect to season, 57% of the populations was heterozygous in the Fall and 36% was heterozygous in the Spring. In addition, AB genotypes appeared in 10% of one nontreated population in the Fall, but did not appear in any population by Spring; BD genotypes, which were in low frequencies in all populations in the Spring, had been absent in the Fall samples.

Orchard x season x genotype dependence and interaction resulted for IDH-1, due to dependence of genotype frequencies on orchard (but not on season). Whether the orchard was endrin-treated or not was significant, but there were also significant differences in genotype distribution within the sprayed orchards. In the sprayed orchards, 40% of the populations was heterozygous, compared to 32% in the unsprayed orchards. As for MDH-2, both orchard x season x genotype dependence and interaction existed, but due to dependencies of genotype frequencies on both orchard and

sampling period. Genotype frequencies were dependent upon orchard within both endrin-treated and nontreated types, but did not seem to differ because of the treatment. With respect to season, 75% of the populations was heterozygous in the Fall and 87% was heterozygous in the Spring.

As previously discussed, loss of PGM-2 activity limited the numbers of individuals available for electrophoretic analysis at that locus. Some of the variation present in the orchard x season x genotype dependence was likely due to restricted Fall sample size in the unsprayed orchards, although no interaction among the three variables existed. Although genotype frequencies were independent of orchard, they were not independent of sampling period. A greater proportion of the populations was heterozygous in the Fall (49%) than in the Spring (38%), a relationship that could have been affected by variable sample size as well as seasonal fluctuations in genotype frequencies.

Table 11 lists mean individual heterozygosities (mean number of heterozygous loci per individual) estimated for each population by dividing the number of observed heterozygous genotypes by the product of the number of loci and total number of individuals surveyed (Selander et al., 1971; Smith et al., 1975; Browne, 1977). As can be seen from Fig. 2, total overall heterozygosity decreased from Fall to Spring in all orchards except Sprinkle's, where it

Table 11. Mean individual heterozygosity at the GOT-2, IDH-1, LDH-1, and MDH-2 combination of loci for adult pine vole populations trapped in both endrin-treated and nontreated orchards, Fall 1980 and Spring 1981 (\pm SE).

Orchard	Total	Males	Females
Fall 1980			
Endrin-treated			
Corbett	0.509 (0.27)	0.523 (0.28)	0.496 (0.28)
Kinzie	0.551 (0.26)	0.510 (0.18)	0.601 (0.30)
Sprinkle	0.409 (0.22)	0.355 (0.19)	0.451 (0.24)
Nontreated			
Gross	0.490 (0.25)	0.494 (0.25)	0.485 (0.25)
Watson	0.598 (0.33)	0.590 (0.34)	0.606 (0.33)
Wertz	0.557 (0.29)	0.625 (0.33)	0.518 (0.26)
Spring 1981			
Endrin-treated			
Corbett	0.454 (0.25)	0.439 (0.26)	0.570 (0.30)
Sprinkle	0.494 (0.28)	0.439 (0.26)	0.570 (0.30)
Nontreated			
Gross	0.447 (0.24)	0.472 (0.25)	0.420 (0.24)
Watson	0.542 (0.30)	0.530 (0.30)	0.556 (0.30)

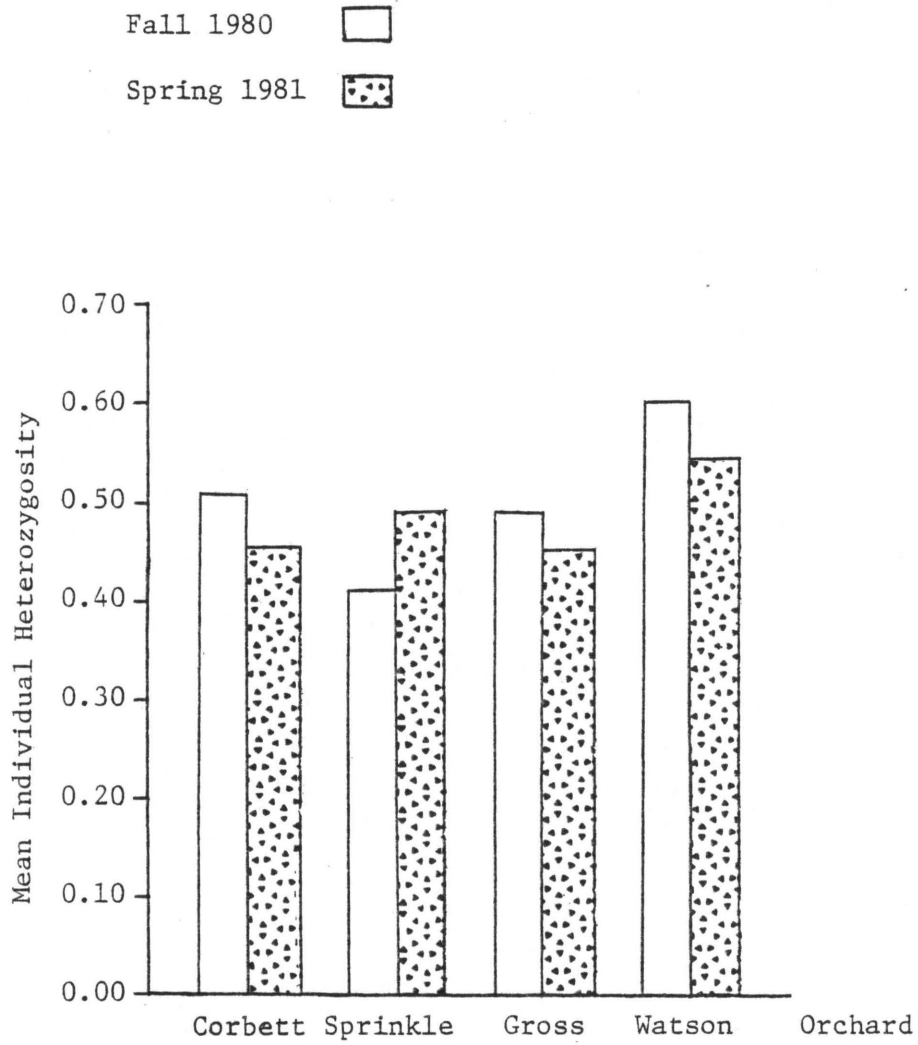


Fig. 2. Total heterozygosity at GOT-2, IDH-1, LDH-1, and MDH-2 loci combined for orchard-trapped adult pine vole populations, Fall 1980 and Spring 1981.

increased. Greater heterozygosities did not occur consistently in either sprayed or unsprayed orchards at either sampling period, nor were differences in heterozygosity between the sexes consistent with respect to orchard type or sampling time.

Table 12 lists total mean individual heterozygosity at all four loci combined and for each locus separately. According to Selander et al. (1971:77), any changes in heterozygosity for any one locus ≥ 0.100 identify loci which are responsible for "major contributions...to overall increase [or decrease] in heterozygosity". In this study, no particular locus contributed overwhelmingly to overall heterozygosity. In fact, all loci tended to be important in most orchards, but MDH-2 tended to contribute the most.

Averages of heterozygosities from both sprayed and unsprayed orchard populations were calculated by both methods commonly reported in the literature (Selander et al., 1971; Smith et al., 1975; Browne, 1977). Average heterozygosity of endrin-treated orchards, nontreated orchards, and all orchards combined were derived by (1) averaging mean individual heterozygosities in orchards, weighted by population sample size, and (2) averaging heterozygosities unweighted by sample size. Standard error of the mean thus produced for the former effectively describes the genetic variability among the populations

Table 12. Total mean individual heterozygosity at all four loci combined and at each locus separately for adult pine vole populations trapped in both endrin-treated and nontreated orchards, Fall 1980 and Spring 1981.

Orchard	GOT-2	IDH-1	LDH-1	MDH-2	Overall
Fall 1980					
Endrin-treated					
Corbett	0.307	0.360	0.496	0.874	0.509
Kinzie	0.436	0.394	0.606	0.766	0.551
Sprinkle	0.202	0.444	0.333	0.657	0.409
Nontreated					
Gross	0.288	0.658	0.411	0.603	0.490
Watson	0.507	0.884	0.145	0.855	0.598
Wertz	0.727	0.636	0.273	0.591	0.557
Spring 1981					
Endrin-treated					
Corbett	0.279	0.314	0.372	0.849	0.454
Sprinkle	0.442	0.588	0.351	0.922	0.494
Nontreated					
Gross	0.423	0.288	0.288	0.788	0.447
Watson	0.233	0.600	0.417	0.917	0.542

summed for the average; for the latter, it describes the variability among the heterozygosity estimates themselves. Both methods produced similar results, except that the SE was of course much lower for the average of the unweighted estimates. All averages are reported in Tables II-8 and II-9 in the Appendix.

Considering average heterozygosity revealed some slight trends in the data. When mean individual heterozygosity was averaged over all orchards at either sampling time, the estimate decreased from Fall to Spring (e.g., average heterozygosity decreased from 0.509 to 0.483). These decreases occurred in both sexes and in both sprayed and unsprayed orchards. Average heterozygosity was slightly higher in nontreated orchards both Fall and Spring.

Nutrition Experiment

Body Weight Changes

Weight data of pine voles on different feed rations appear in Table 13. Analysis of variance revealed no significant differences for initial body weights among voles on ad libitum feed intake, voles surviving on 70% ad libitum, and voles dying on 70% ad libitum ($p=0.607$), nor were there any significant differences due to sex in any group ($p=0.744$). (Mean squares of variables are reported in Table 14.) Final body weights differed only between ad libitum and restricted groups ($p=0.0001$); again, there was

Table 13. Body weight characteristics of adult pine voles on different feed intakes (\pm SE).

		Initial Body Weight (g)	Final Body Weight (g)	Weight Change ⁽¹⁾	Percent Weight Change ⁽²⁾
<u>Ad Libitum</u>					
Total	(N=10)	23.6 (1.2)	23.5 (0.7) *	-0.2 (0.8)	-0.4 (3.0) *
Males	(N=5)	24.0 (2.3)	23.6 (1.2) *	-0.4 (1.5)	0.2 (5.2) *
Females	(N=5)	23.3 (1.1)	23.3 (0.6) *	-0.0 (0.8)	0.0 (3.4) *
<u>Dying on 70% Ad Libitum</u>					
Total	(N=12)	25.2 (1.3)	18.8 (0.8) **	-6.4 (1.1)	-24.4 (0.0) **
Males	(N=9)	24.7 (1.6)	18.8 (1.1) **	-5.9 (1.1)	-23.1 (3.4) **
Females	(N=3)	26.6 (2.3)	18.7 (1.0) **	-7.9 (2.8)	-28.6 (8.1) **
<u>Surviving on 70% Ad Libitum</u>					
Total	(N=24)	24.6 (0.6)	19.2 (0.5) **	-5.4 (0.4)	-21.5 (0.0) **
Males	(N=10)	24.2 (1.1)	19.1 (0.8) **	-5.2 (0.8)	-20.9 (2.4) **
Females	(N=14)	24.8 (0.7)	19.3 (0.6) **	-5.5 (0.6)	-22.0 (2.0) **

Within a column, different starred symbols denote significant differences ($\alpha = 0.05$) resulting from Duncan's Multiple Range test.

(1) (Initial - Final) body weight.

(2) Weight change expressed as percent of initial body weight.

Table 14. Mean squares of body weight variables measured in adult pine voles on different feed intakes.

Variable	Sex	Group ⁽¹⁾	Error
df	1	2	42
Initial body wt.	1.4	6.6	13.1
Final body wt.	0.5	76.7 ⁽²⁾	5.8
Change in body wt. (as % of initial)	0.0	0.2 ⁽²⁾	0.0

⁽¹⁾ Voles on 100% ad libitum, voles surviving on 70%, and voles dying on 70%.

⁽²⁾ $p=0.0001$

no difference due to sex ($p=0.768$). Change in weight (expressed as percent of initial weight) was also significantly different between voles on ad libitum and those on restricted feed intake ($p=0.0001$), but was not significantly different between the sexes in either group ($p=0.886$). Duncan's Multiple Range test between group means revealed no significant differences ($\alpha=0.05$) between survivors and voles that died on 70% ad libitum for initial or final body weights or percent weight change. Mean day of death of animals on restriction was day 18.1 ± 1.5 (SE).

Because no differences in weight loss existed between animals that lived or died on restriction, analysis of body fat content was deemed unnecessary.

Genetic Analyses

None of the chi-squared tests for independence of marker genotypes on sex was significant (Appendix Table II-10), so no variation in genotype frequencies due to sex existed. These frequencies were also independent of "fate"--whether animals were on ad libitum feed rations, lived on 70% ad libitum, or died on 70% ad libitum (Appendix Table II-11). Of the five loci surveyed, only LDH-1 genotypic frequencies were found to agree with those expected under Hardy-Weinberg equilibrium conditions (Appendix Table II-12). Failure of observed frequencies to agree with Hardy-Weinberg expectations were probably the

effect of small sample size (N=46).

Chi-squared analysis for dependence of the number of heterozygous loci in each individual vole upon the fates described above was nonsignificant. The assumption that those individuals with the greater number of heterozygous genotypes were distributed equally among the three fates was not rejected ($P[X^2 \geq 9.41] = 0.309$, $df=8$). In other words, there were no differences among the fates with respect to the number of heterozygous genotypes an individual possessed for the five loci examined.

Mean individual heterozygosity estimates were calculated by summing all five loci (Table 15), and tended to be lowest in animals which died on 70% ad libitum, and highest in those that survived. Heterozygosity estimates for females were greater than those for males, both over all groups (0.809 and 0.625, respectively) and within each group. These differences were slight because all were within the variability ranges denoted by SE. Indeed, none of the differences in heterozygosity between groups or between the sexes was found significant at the $\alpha=0.05$ level of probability by the Kruskal-Wallis nonparametric test of the hypotheses that the groups or sexes were identical.

Laboratory Mortality Experiment

In a related matter, the reader should recall that

Table 15. Mean individual heterozygosity at the five loci examined for adult pine voles on different feed intakes (\pm SE).

	<u>Ad Libitum</u>	Dying on 70% <u>Ad Libitum</u>	Living on 70% <u>Ad Libitum</u>	Overall
Total (N=46)	0.620 (0.28)	0.576 (0.27)	0.742 (0.34)	0.670 (0.31)
Males (N=24)	0.560 (0.26)	0.511 (0.25)	0.760 (0.35)	0.625 (0.29)
Females (N=22)	0.680 (0.29)	0.733 (0.30)	0.871 (0.39)	0.809 (0.36)

animals housed in the Department of Fisheries and Wildlife Animal Room had very poor survival at first. Although all were caged and cared for as equally as possible, many died within a few days. An informal experiment conducted in the lab at this time was designed to test the effect of toe-clipping in the field on subsequent laboratory survival. Control animals died in numbers comparable to those that were toe-clipped, so the excessive laboratory mortality of animals trapped for LD-50 determinations probably had not been influenced by the marking procedure. The cause of this mortality, however, remained unidentified. For most of the animals trapped for LD-50 determinations but which died before the test, both capture date and date of death in the lab were recorded. Just as those animals that lived or died on restricted feed intake had been compared for possible genetic variability, so also were those that lived or died in the general laboratory environment. From correlations of number of days survived in the lab with individual heterozygosity (number of heterozygous genotypes at GOT-2, IDH-1, LDH-1, and MDH-2 loci divided by 4), no apparent relationship existed for either orchard population examined (Table 16). Analysis of variance revealed no significant differences in number of days survived between populations ($p=0.672$) or between sexes in an orchard ($p=0.833$), and no correlation existed between laboratory survival and

Table 16. Results of correlations of individual heterozygosity at the GOT-2, IDH-1, LDH-1, and MDH-2 combination of loci with number of days survived in the lab after capture for adult pine voles, Fall 1980.

Orchard		Mean Number of Days Survived (\pm SE)	Range of Days Survived	Mean Heterozygosity (\pm SE)	p	r ²
Corbett						
	Total (N=32)	6.1 (1.2)	1-31	0.484 (0.086)	0.07	0.33
	Males (N=15)	5.5 (1.2)	1-17	0.517 (0.067)	0.71	0.10
	Females (N=17)	6.6 (2.0)	2-31	0.456 (0.065)	0.06	0.46
Kinzie						
	Total (N=23)	6.8 (1.4)	1-24	0.576 (0.053)	0.64	0.10
	Males (N=14)	6.3 (1.6)	1-21	0.554 (0.060)	0.76	0.09
	Females (N=9)	7.7 (2.5)	1-24	0.611 (0.103)	0.80	0.10
Orchards Combined						
	Total (N=55)	6.4 (0.9)	1-31	0.523 (0.035)	0.75	0.24
	Males (N=29)	5.9 (0.9)	1-21	0.534 (0.044)	0.61	0.10
	Females (N=26)	7.0 (1.6)	1-31	0.510 (0.056)	0.09	0.34

individual heterozygosity when the two populations were combined.

DISCUSSION

Endrin Experiment

Endrin Resistance and LD-50

The objective of the endrin experiment was to determine if differences in genetic structure existed in pine vole populations from endrin-treated and nontreated orchards. The first step then was to demonstrate that voles in treated orchards were indeed resistant. Webb and Horsfall (1967) and Webb et al. (1973) discovered endrin-resistant strains in sprayed orchard populations trapped in the fall, just before endrin application. The LD-50's characteristic of the populations trapped in this study, however, did not appreciably differ from those estimated for voles trapped in nontreated orchards. The individuals composing endrin-treated populations in the Fall were those animals that had survived the endrin spraying of the year before as well as those that had been born since. Population responses to the endrin stress should have been most diverse in the Fall, compared to those of the Spring populations which were composed primarily of animals that had survived the endrin treatment applied that winter. Therefore the Spring LD-50 should have been higher, as it was in Corbett's. In Sprinkle's, however, the LD-50 increased only slightly. So the questions to be examined were (1) why did the LD-50 increase in one sprayed orchard and not in the other; and

(2) what caused the increased tolerance to endrin that was observed?

The most readily discernable difference between the two endrin-treated orchards was that Rozol (an anticoagulant rodenticide formulated with chlorphacinone) was applied after the endrin in Sprinkle's. Webb et al. (1973) reported that voles resistant to endrin were three times more susceptible to chlorphacinone. Perhaps the more tolerant individuals that did survive the endrin spraying were subsequently preferentially eliminated by Rozol in Sprinkle's orchard, thus depressing the LD-50. Alternatively, no endrin-tolerant animals may have ever existed in Sprinkle's population.

Additionally, the observed LD-50 increase in Spring 1981 in the Corbett population indicated a population more tolerant of endrin at that time, but not necessarily inherently resistant to it. Genetic selection of individuals with preadaptive capacities to better metabolize and excrete endrin may have occurred, but the greater LD-50 may just as well have been the result of microsomal enzyme inductive effects from the endrin application. Endrin induction of the enzymes responsible for degradation of the compound would effect a greater endrin tolerance in the population that would be reflected in the LD-50 just as selection for genetically superior individuals would be.

Nevertheless, a population with a significantly higher tolerance to endrin was present in one sprayed orchard in Spring 1981. It then remained to be seen if genetic composition in that population varied from that of the other populations or if it changed from Fall to Spring. In conjunction, it was imperative to follow seasonal genetic variation in the other orchards. Should such seasonal changes occur locally in all orchards, it would be impossible to infer a genetic predisposition to endrin tolerance in the one orchard with high LD-50. But if changes in mean individual heterozygosity significantly differed in the one tolerant population and not in the others, an argument for genetic predisposition might exist.

In spite of the increased LD-50 that occurred in Corbett's, one glaring fact remains. The resistant populations that Webb and Horsfall (1967) and Webb et al. (1973) studied were trapped in the fall, before endrin treatment, and the significantly higher LD-50's they observed were characteristic of the fall population. LD-50 results in the present study did not identify such an endrin-resistant population in Corbett's orchard in Fall 1980, before spraying. If selection had favored more endrin-tolerant individuals from the previous year's treatment, it was not apparent by Fall 1980. Given the short life span of the pine vole, few of those more tolerant

individuals from the 1979 spraying may have survived by the fall of 1980. That none of their progeny had inherited this supposed increased endrin tolerance by Fall 1980 is suggested by the low LD-50 discussed above. Thus the existence of an inherently endrin-resistant population is not supported by the reported data. Whether or not enzyme induction occurred is unknown, but genetic (and presumably selective) effects are discussed below.

Genetic Variation and the Hardy-Weinberg Equilibrium

The Hardy-Weinberg equilibrium is the fundamental model of population genetics. It is based on the binomial (Mendelian) distribution, and states that genotypes AA, AB, and BB occur in the proportions $p^2:2pq:q^2$ where p and q are the frequencies of the alleles A and B. When the assumptions upon which the model is based are fulfilled, these allele frequencies are constant in the next generations. In other words, the equilibrium is a distribution of genotypes that depends on the allele frequencies present at a particular time (Wallace, 1968). Examined in the same populations on a temporal or geographical scale, the model provides insight into "microevolutionary" changes (Wallace, 1968:66).

The utility of the Hardy-Weinberg model resides in its ability to distinguish unstable population genetic structures. The generality of the model, however, makes it

difficult to identify which population characteristics are responsible for deviations from equilibrium conditions. The model assumes that the population is a large, randomly-mating one in which migration, mutation, and selective forces are inactive. Mutation is generally discounted as its rate is too low (10^{-4} to 10^{-8} per generation) in natural populations to be effective. Migration or dispersal in orchard pine vole populations is also negligible; animals tend to be quite sedentary within the orchard, with individual movements confined to 2-3 trees within a row and rarely extended across rows (Tipton, pers. comm.). Effective population size is probably large enough (except in the Wertz orchard) in the vole-infested orchards studied, but there have been reports of "discrete, non-overlapping" families of voles concentrated around 2-3 trees in a single row within the orchard (FitzGerald and Madison, 1981:57). Although the possibility of interbreeding behavior could not be studied, it may have contributed to deviations from the equilibrium state.

The importance of Hardy-Weinberg chi-squared goodness-of-fit should not be overemphasized. Even when testing for the agreement of observed electrophoretic phenotypes with a standard genetic (binomial or multinomial) model, this test is unreliable for sample sizes of less than 200 individuals (Fairbairn and Roff, 1980). Another difficulty arises when

comparing changes in genetic composition over time. Strictly speaking, the Fall and Spring samples were not independent. Both were composed of the same generation of individuals, except that some new progeny were present in the Spring that were not yet born in the Fall. Genetic variation in populations with such overlapping generations is impossible to characterize concisely. Analysis of adult individuals only does not solve the problem since Spring adults could very well have been subadult, juvenile, or unborn the previous Fall. Adults could have been present at any one time that belonged to different generations. Even though reproductive activity decreases during the winter nonbreeding season, precise classification of adults into generation classes is impossible. Therefore, testing for independence of genotypes on sampling time is both unreliable and invalid unless individuals can be accurately aged. Indeed, the most significant violation of the Hardy-Weinberg model is encountered in samples with overlapping generations (Tamarin and Krebs, 1969; Birdsall, 1974). For this reason at least a two-year study of orchard vole populations is required to properly assess the local and seasonal differences that obviously occurred. None of the differences at particular loci among orchard populations was consistent at either sampling period, however, in occurring only in sprayed or only in unsprayed orchards. Neither did

any seasonal changes occur consistently in any one orchard or orchard type. Comparisons with a second year's data should greatly clarify the processes at work.

The genetic structure at the loci observed, however, was expected to differ among orchards, since these discontinuous "island" populations have virtually no contact with each other. In addition, orchard, sampling period, and genotype frequency were jointly dependent variables at all five loci examined, and a significant interaction for these three variables existed for all except PGM-2. The dependency of IDH-1 and LDH-1 genotype frequencies on whether the population was trapped in an endrin-sprayed or unsprayed orchard is deceptive. There could be other factors besides presence or absence of endrin treatment contributing to the dependencies. In other words, some other unique characteristics unrelated to endrin could have served to classify the orchards in the same sprayed vs. unsprayed groupings, with genotype frequencies dependent upon those unknown factors.

That gene frequencies change in cyclic microtine populations has been repeatedly demonstrated by Krebs' group of researchers (see Krebs et al., 1973, for review). From two sampling periods, it appeared that gene frequencies varied also in the pine vole, a species not known to be cyclic in orchards. Again, at least two years' data are

necessary to determine the magnitude and pattern of gene frequency changes over time. To derive any meaning from such data, however, one must define environmental or individual fitness parameters with which to reference or correlate genetic changes. In this study, the failure to find the postulated endrin-resistant pine vole strain in sprayed orchards aborted such comparisons. The conclusion of the experiment thus depended on the existence of genetic variation in the one orchard with greater LD-50 in the Spring.

Heterozygosity

Heterozygosity, a measure of inherited variability, is used as an index to the adaptive potential of a population, since the more heterozygous individuals generally have greater capacities to change with the environment. For instance, geographic variation of heterozygosity in P. polionotus was interpreted by Selander et al. (1971) as adaptive advantage. While it is true that heterozygosity is "essential" for the "genetical dissection" necessary to reveal forces influencing population genetic composition, still it is "a cloak for ignorance of gene action" (Berry, 1976:115). In the practical sense, however, no standard method of handling this estimate seems to exist, in spite of its popularity. No formal statistical test has been devised to define differences between populations, although

calculating the standard error of mean heterozygosity indicates the variability of the estimate. What magnitude of change in heterozygosity is considered significant is largely open to the interpretation of the researcher. Smith et al. (1973) interpreted decreases in mean individual heterozygosity occurring on a geographical continuum from 0.0814 to 0.0506 as noteworthy for P. polionotus. Selander et al. (1971:75) also claimed differences in populations ranging in heterozygosity from 0.086 to 0.054, but one population heterozygosity of 0.0857 was "slightly greater" than another of 0.0855. The question how slight is "slight" and how great is "great" naturally arises. Browne (1977) interpreted differences of 0.080 and 0.071 as significant when compared with their standard errors of 0.033. (this study). Lewontin (1974), however, asserted that such a SE range (representing up to 40% of the heterozygosity estimates) was too wide.

Nevertheless, heterozygosity did decrease slightly in three of the four orchard populations which were trapped both Fall and Spring. Heterozygosity did not differ appreciably, however, between sprayed and unsprayed orchards. It is difficult to resolve these results with the generally-held notion that heterozygosity indicates populations with greater survival advantages without taking into account the reduction in heterozygosity that

accompanies any severe local or periodic decrease in population density. That heterozygosity decreased in all orchards suggested that such intermittent random drift effects resulted from the lower numbers of voles that had survived pesticide treatments and winter weather conditions. Curiously, the one orchard which displayed unique changes in heterozygosity estimates from Fall to Spring was not Corbett's (with the higher LD-50) but Sprinkle's, where heterozygosity increased by Spring. Perhaps one would expect heterozygosity to be higher in Sprinkle's, since these animals needed to adapt to Rozol as well as to endrin. Presumably voles that survived both mortality factors would be more heterozygous than those facing just one. Unique genetic relationships at single IDH-1 and LDH-1 loci were suggested for the Corbett population. These two loci were found to be significantly dependent upon whether the orchard was endrin-treated or not, and both loci also exhibited dependency upon individual orchard within the treated type. The evidence in Corbett's orchard, however, was probably not strong enough to imply a genetic component in the greater endrin tolerance observed there that Spring.

Nutrition Experiment

Body Weight Characteristics of Voles on Restricted Feed Intake

Just as the LD-50 results failed to support reports in

the literature of endrin-resistance in the pine vole, so did the feed restriction experiment fail to repeat the results of Merson's (1979) work. In this experiment, no differences in final body weights were discovered between voles that lived or died on 70% ad libitum feed intake. Merson admitted that the voles which died on this restriction in his three-week experiment weighed less than the other voles to begin with. The male voles in this experiment weighed less both initially and at the end of the experiment than those in Merson's experiment. Expressed as percent of initial weight, the weight losses of voles dying in Merson's experiment represented about 20%, whereas 23% of initial body weight was lost by voles dying on restriction in this study. Due to the fact that mean ad libitum feed rations in this experiment were abnormally high, restricted rations were not comparable to Merson's until the final week of the experiment (see Methods), explaining why the average day of death for male voles in this experiment was later (18.1 compared to 12.4 in Merson's) and why fewer died (47% compared to 58%). Whether or not significant differences between voles living and dying on restricted feed intake in weight loss and consequently body fat content would have resulted if this experiment had been extended another week is debatable. The results remain, however, comparable to Merson's but for the fact that voles dying on 70% ad libitum

feed intake did not lose weight in amounts significantly different from those that survived, even though the percentage of initial weight lost was slightly greater than it was in Merson's study. Therefore, genetic variation in voles on different feed intakes could be referenced only to whether the individual lived or died on restriction, and not correlated with body fat content as originally intended.

Mean individual heterozygosity was generally greater in the voles that lived on restricted feed intake than in those that died, although the difference was not statistically significant. Females seemed to be somewhat less vulnerable, since only 3 out of the 12 animals that died were females, and female heterozygosities were greater than those of the males (again, not significant statistically). Nevertheless, heterozygosity in this case may have imparted some advantage with respect to survival for voles on short-term feed restriction.

Laboratory Mortality

Little need be said about the correlations of individual heterozygosity with number of days survived in the lab, except that they were nonsignificant. The relationship approached significance in one of the populations, however, and bears further study. Trapping and relocation to alien surroundings imposes a severe stress on the pine vole. The adaptive significance of heterozygosity

could be studied easily in this species by means of rigidly controlled laboratory experiments.

CONCLUSIONS

Testing an hypothesis involves more than verification--the hypothesis must be able to withstand repeated assaults or be discarded. Thus, "...it must be possible for an empirical scientific system to be refuted by experience" (Popper, 1968:41). It is just as important to document those cases which do not corroborate hypotheses as those that do.

In this study, the hypothesis that pine vole populations were developing a genetic resistance to endrin was tested and found wanting. Based on orchardists' reports and the work of Webb and Horsfall (1967), the existence of endrin-resistant strains was not corroborated in these populations trapped in southwestern Virginia. From the work cited above, a resistant population certainly existed, but the animals tested apparently were trapped in only one area (perhaps even from a single orchard) in Berryville, Va. Research documented the same high LD-50's in this population over five years, but geographically the phenomenon seemed to be restricted (Webb et al., 1973). LD-50 values in the populations trapped for the present study were not appreciably different among endrin-treated and nontreated orchards, except in one sprayed orchard after the treatment. Heterozygosity estimates at the particular loci examined did not imply a significant difference in genetic composition.

between pine vole populations from endrin-treated and nontreated orchards, although a dependency between orchard type and genotype existed at two loci.

Incompatibility of the results of this study with the original literature report (Merson, 1979) also occurred for the nutrition experiment. Although pine voles that died on 70% ad libitum feed rations lost the same percentage of their initial body weight as those that died in Merson's experiment, those that died had lost no more than those that lived. Since the study was initiated on the premise that survivors would retain body fat and lose less weight, failure to repeat Merson's 1979 results hampered genetic comparisons among the different feed intake groups. Nevertheless, heterozygosity was somewhat higher in the voles that survived the restriction.

A field study such as that designed to detect genetic variation among orchard-trapped pine vole populations would prove far more conclusive with two years' data, thus yielding repeatable comparisons of Fall and Spring characteristics. Increased tolerance to endrin did develop in one orchard, but heterozygosity decreased in all orchard populations (except in the one which suffered exposure to Rozol as well as to endrin) and estimates in sprayed orchards did not differ appreciably or consistently from those in unsprayed orchards. In addition, the greater

average heterozygosities and significant genotype/treatment dependencies at two loci in the unsprayed orchards did not necessarily suggest differences in genetic composition between endrin-treated and nontreated populations. All sprayed orchards were located around Troutville, but all unsprayed ones were about 60 miles away in Bedford. Therefore these apparent differences between orchard types probably reflect geographical variation, especially since endrin-resistant and -susceptible populations could not be identified. The conclusion that the increased endrin tolerance observed in Corbett's orchard does not include an inherited component is suggested by the LD-50 and heterozygosity data; another year's observations would be required to substantiate it.

As for the nutrition study, mean individual heterozygosity was somewhat greater in voles that survived on 70% ad libitum feed intake than in those that died, indicating a slight adaptive potential. More controlled laboratory experiments and accompanying field studies based on food availability may clarify further the role of heterozygosity in survival during times of food shortages.

Heterozygosity, as discussed previously, is an unsatisfactory indicator of population differences in that variability of the estimate cannot be quantified adequately. Lewontin (1974) maintained that heterozygosity estimates

from electrophoretic analyses of polymorphic markers are biased inasmuch as the markers are chosen for their known variability. For multivariate experimental designs and analyses that measure and demonstrate relationships among all possible population parameters, including heterozygosity, such a bias may be important. But for the purpose of comparing relative genetic variability among populations, heterozygosity is the most useful estimate of population genetic composition available. In a practical sense, however, heterozygosity must be studied in relation to the identification and control of environmental factors expected to affect the survival of the organism.

Although electrophoresis is touted as a fast, simple, and relatively inexpensive technique for screening population genetic variation, there is a baseline limit to the simplicity allowed. Heterozygosity estimates are greatly improved (reductions in standard error) if a greater number of loci is examined. Indeed, Lewontin (1974) insisted this requirement to be more important than the number of individuals sampled. But until specific enzymes are linked to observable characteristics that enhance individual survival, many random loci will have to be examined. In this study, GOT and LDH were thought to respond physiologically to endrin. Although neither locus overwhelmingly contributed to overall population

heterozygosity, even in the one population exhibiting greater endrin tolerance in the Spring, LDH-1 did exhibit a significant dependence on whether the orchard was endrin-treated or not. Whether changes in activity levels of these enzymes are merely artifacts of endrin metabolism, actually involved in resistance, or totally unimportant in endrin metabolism in the pine vole is unknown. Failure to detect genetic variability for GOT-2 proves only that, whatever its function, genetic changes at that locus did not parallel the development of endrin tolerance in this study. The LDH-1 relationship, however, demands further observation.

The promise of this study lies in the indication that greater genetic variability in animals subjected to environmental stress can be monitored. Controlled experiments designed in such a way that animals must adapt to an identified stress that produces quantifiable changes in the individuals are necessary. Such studies are important even if no genetic variation is discovered: an accumulation of such evidence is indicative that research effort would be more fruitfully spent in the examination of extrinsic factors.

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Appendices

Appendix I. Electrophoretic Procedures

Table I-1. List of enzymes used in electrophoresis.

Symbol	E.C. No.(1)	Enzyme Name
ACP	3.1.3.2	Acid phosphatase
Beta-GUS	--	Beta-glucuronidase
FDP	--	Fructose-1,6-diphosphatase
GOT	2.6.1.1	Glutamate oxaloacetate transaminase
IDH	1.1.1.42	Isocitrate dehydrogenase
LDH	1.1.1.27	Lactate dehydrogenase
MDH	1.1.1.37	Malate dehydrogenase
PGM	2.7.5.1	Phosphoglucomutase

(1)Commission on Biochemical Nomenclature, 1973.

Table I-2. Formulations for electrophoretic buffer systems.

System	Gel Buffer	Electrode Buffer
APM/Citrate pH 6.1	0.002M H3-Citrate 0.0035M APM ⁽¹⁾	0.16M H3-Citrate 0.073M APM ⁽¹⁾
EBT pH 8.6	0.9M Tris ⁽²⁾ 0.5M Boric acid 0.1M EDTA ⁽³⁾ At 1:25 dilution.	Same formulation as gel buffer but at 1:8 dilution
LiOH (discontinuous) pH 8.1/8.5	0.03M Tris ⁽²⁾ 0.005M H3-Citrate 0.0004M NaOH Add 20 ml electrode buffer to 1980 ml solution above pH 8.5	0.3M Boric Acid 0.06M LiOH monohydrate pH 8.1

⁽¹⁾Amino propyl morpholine

⁽²⁾Tris(hydroxymethyl)-aminomethane

⁽³⁾Ethylenediaminetetraacetic acid, disodium dihydride

Table I-3. Electrophoretic buffers, voltage potentials, and average running times appropriate to the enzymes studied.

Buffer	Enzymes	<u>Voltage Potential</u> (volts) (m.a.)		Hours
APM/Citrate	IDH, MDH	200	25-45 ⁽¹⁾	5
EBT	FDP	150	35-50 ⁽¹⁾	8
LiOH (discontinuous)	AcP, Beta-GUS, GOT, LDH, PGM	120	50	8

⁽¹⁾Raised to higher m.a. after 1/2 hour.

Table I-4. Enzyme concentrations used in histochemical stains.

Enzyme	Concentration
NAD (nicotinamide adenine dinucleotide)	10 mg/ml
NADP (nicotinamide adenine dinucleotide phosphate)	10 mg/ml
MTT (dimethylthiazolyl-diphenyltetrazolium bromide)	5 mg/ml
PMS (phenazine methosulfate)	2 mg/ml

Table I-5. Supplementary buffers used in tissue preparation and enzyme stains.

Tissue Grinding Buffer, pH 7.0

0.1M Tris
0.001M EDTA
0.00001M NADP
0.00001M NAD
1-2% mercaptoethanol
pH to 7.0 with HCl

Citrate Buffer, pH 4.5

0.05M H₃-Citrate
0.0825M NaOH

NaAcetate Buffer, pH 5.0

0.2M glacial acetic acid
0.128M NaOH

0.2M Tris/HCl Buffer, pH 8.0

0.2M Tris
0.1M HCl

Table I-6. Histochemical stains for enzyme markers. Use distilled, deionized water. Add starred (*) ingredients just before application to the gel. Incubate in dark at 37-44C unless otherwise noted. Fix MTT stains with 7% acetic acid; others with 1:5:5 acetic acid, methanol, water. All recipes for one gel.

ACP (Pre-incubate gel in citrate buffer at room temp. for 15 minutes.)
 50 ml 0.05M citrate buffer, pH 4.5
 50 mg alpha-naphthyl phosphate
 *50 mg Fast Garnet GBC salt

Beta-GUS (Pre-incubate gel in NaAcetate buffer at room temp. for 15 minutes.)
 50 ml 0.2M NaAcetate, pH 5.0
 10 mg AS-BI naphthyl glucuronide
 *10 mg Fast Garnet GBC salt

FDP 10.0 ml 0.2M Tris/HCl, pH 8.0
 20.0 mg fructose-1,6-diphosphate
 2.0 ml 0.1M MgCl₂
 0.5 ml NAD
 1.0 ml MTT
 *21.0 units glucose phosphate isomerase
 * 9.8 units glucose-6-phosphate dehydrogenase
 * 2.5 ml PMS
 Heat together 10 ml water and 0.3 g agar and add to stain just before use.

GOT 30.0 ml 0.2M Tris/HCl, pH 8.0
 10.0 ml water
 *150.0 mg aspartic acid
 *100.0 mg alpha-ketoglutaric acid
 * 5.0 mg pyridoxal 5-phosphate
 * 75.0 mg Fast Blue BB salt

Table I-6 (continued). Histochemical stains for enzymes.

IDH	(Do not incubate stained gel above 37C.) 10.00 ml 0.2M Tris/HCl, pH 8.0 50.00 mg isocitrate 0.25 ml 0.1M MnCl ₂ 0.25 ml 0.1M MgCl ₂ 1.00 ml NADP 0.50 ml MTT * 1.75 ml PMS Heat together 10 ml 0.2M Tris/HCl, pH 8.0 and 0.15 g agar, cool to lukewarm, and add to stain just before use.
LDH	20.0 ml 0.2M Tris/HCl, pH 8.0 20.0 ml water 0.9 ml 0.5 NaLactate, pH 7.0 2.0 ml NAD 0.8 ml MTT * 4.0 ml PMS
MDH	20.0 ml 0.2M Tris/HCl, pH 8.0 5.0 ml 2M malate, pH 8.0 1.0 ml NAD 4.0 ml MTT * 2.5 ml PMS
PGM	5.0 ml 0.2M Tris/HCl, pH 8.0 10.0 ml water 165.0 mg glucose-1-phosphate 2.5 ml NAD 4.0 ml MTT * 80.0 units glucose-6-phosphate dehydrogenase * 2.0 ml PMS Heat together 10 ml water and 0.2 g agar and add to stain just before use.

Appendix II. Supplementary Genetic Analyses

Table II-1. Results of chi-squared tests of agreement of observed GOT-2 genotypes with Hardy-Weinberg expectations, from adult pine vole populations trapped in both endrin-treated and nontreated orchards, Fall 1980 and Spring 1981.

Orchard	χ^2	p	df	N
Fall 1980				
Endrin-treated				
Corbett	0.15	0.698	1	111
Kinzie	1.66	0.198	1	94
Sprinkle	0.30	0.584	1	99
Nontreated				
Gross	3.23	0.072	1	73
Watson	5.83	0.016	1	69
Wertz	7.35	0.007	1	22
Spring 1981				
Endrin-treated				
Corbett	0.65	0.420	1	86
Sprinkle	2.66	0.103	1	77
Nontreated				
Gross	3.82	0.051	1	52
Watson	1.06	0.303(1)	1	60

(1) Contingency table too sparse (cells with expected values <1.0) even after combining rare genotypes; test invalid for $df \geq 1$.

Table II-2. Results of chi-squared tests of agreement of observed IDH-1 genotypes with Hardy-Weinberg expectations, from adult pine vole populations trapped in both endrin-treated and nontreated orchards, Fall 1980 and Spring 1981.

Orchard	χ^2	p	df	N
Fall 1980				
Endrin-treated				
Corbett	60.90	<0.001	3	111
Kinzie	20.71	<0.001	3	94
Sprinkle	2.50	0.114	1	99
Nontreated				
Gross	12.88	0.002	2	73
Watson	31.33	<0.001	2	69
Wertz	11.54	0.001 ⁽¹⁾	1	22
Spring 1981				
Endrin-treated				
Corbett	43.38	<0.001	1	86
Sprinkle	23.30	<0.001	1	77
Nontreated				
Gross	20.71	<0.001	1	52
Watson	0.71	0.701	2	60

⁽¹⁾Contingency table too sparse (cells with expected values <1.0) even after combining rare genotypes; test invalid for $df \geq 1$.

Table II-3. Results of chi-squared tests of agreement of observed LDH-1 genotypes with Hardy-Weinberg expectations, from adult pine vole populations trapped in both endrin-treated and nontreated orchards, Fall 1980 and Spring 1981.

Orchard	χ^2	p	df	N
Fall, 1980				
Endrin-treated				
Corbett	8.26	0.041	3	111
Kinzie	14.76	0.002	3	94
Sprinkle	2.30	0.129(1)	1	99
Nontreated				
Gross	33.20	<0.001(1)	1	73
Watson	--	-- (1)	--	--
Wertz	11.94	0.001	1	22
Spring 1981				
Endrin-treated				
Corbett	0.77	0.380	1	86
Sprinkle	10.08	0.006	2	77
Nontreated				
Gross	4.58	0.032	1	52
Watson	2.23	0.135	1	60

(1)Contingency table too sparse (cells with expected values <1.0) even after combining rare genotypes; test invalid for $df \geq 1$.

Table II-4. Results of chi-squared tests of agreement of observed MDH-2 genotypes with Hardy-Weinberg expectations, from adult pine vole populations trapped in both endrin-treated and nontreated orchards, Fall 1980 and Spring 1981.

Orchard	χ^2	p	df	N
Fall 1980				
Endrin-treated				
Corbett	62.10	<0.001	1	111
Kinzie	29.14	<0.001	1	94
Sprinkle	16.63	<0.001	1	99
Nontreated				
Gross	6.30	0.012	1	73
Watson	37.21	<0.001	1	69
Wertz	0.94	0.332	1	22
Spring 1981				
Endrin-treated				
Corbett	43.98	<0.001	1	86
Sprinkle	54.97	<0.001	1	77
Nontreated				
Gross	19.13	<0.001	1	52
Watson	42.10	<0.001	1	60

Table II-5. Results of chi-squared tests of agreement of observed PGM-2 genotypes with Hardy-Weinberg expectations, from adult pine vole populations trapped in both endrin-treated and nontreated orchards, Fall 1980 and Spring 1981.

Orchard	χ^2	p	df	N
Fall 1980				
Endrin-treated				
Corbett	28.70	<0.001	3	38
Kinzie	14.82	0.001	3	55
Sprinkle	33.14	<0.001	3	57
Nontreated				
Gross	--	-- (1)	--	--
Watson	12.78	0.002	3	34
Wertz	--	--	--	--
Spring 1981				
Endrin-treated				
Corbett	8.64	0.034	3	33
Sprinkle	10.26	0.017	3	29
Nontreated				
Gross	10.68	0.014	3	24
Watson	9.94	0.019	3	32

(1) Contingency table too sparse (cells with expected values <1.0) even after combining rare genotypes; test invalid for $df \geq 1$.

Table II-6. Observed allele frequencies of marker loci for adult pine vole populations trapped in both endrin-treated and nontreated orchards, Fall 1980.

Locus	Endrin-treated Orchards			Nontreated Orchards		
	Corbett	Kinzie	Sprinkle	Gross	Watson	Wertz
GOT-2					*	*
a	0.18	0.26	0.12	0.24	0.27	0.36
b	0.82	0.74	0.88	0.76	0.73	0.64
LDH-1	*	*		*	*	*
a	0.22	0.24	0.21	0.48	0.20	0.36
b	0.69	0.68	0.77	0.49	0.80	0.45
c	0.03	0.03	0.02	0.01	0.00	0.07
d	0.06	0.05	0.00	0.01	0.00	0.00
IDH-1	*			*		
a	0.10	**	0.03	0.07	**	0.05
b	0.29	**	0.27	0.31	**	0.41
c	0.60	**	0.70	0.62	**	0.54
d	0.01	**	0.00	0.00	**	0.00
MDH-2	*	*		*	*	
a	0.49	0.44	**	0.37	0.44	0.57
b	0.51	0.56	**	0.63	0.56	0.43
PGM-2	*	*	*	*	*	--
a	0.41	0.34	0.27	0.20	0.50	--
b	0.48	0.32	0.50	0.10	0.22	--
c	0.11	0.34	0.23	0.70	0.28	--

*Locus not in Hardy-Weinberg equilibrium.

**Locus dependent on sex, so calculation of Hardy-Weinberg expectations invalid.

Table II-7. Observed allele frequencies of marker loci for adult pine vole populations trapped in both endrin-treated and nontreated orchards, Spring 1981.

Locus	Endrin-treated Orchards Corbett	Orchards Sprinkle	Nontreated Orchards Gross	Orchards Watson
GOT-2				
a	0.15	0.25	0.21	0.12
b	0.85	0.75	0.79	0.88
LDH-1		*	*	
a	0.15	0.34	0.21	0.25
b	0.78	0.63	0.76	0.71
c	0.02	0.02	0.01	0.03
d	0.05	0.01	0.02	0.01
IDH-1	*		*	
a	0.00	**	0.00	0.01
b	0.30	**	0.36	0.33
c	0.63	**	0.60	0.55
d	0.07	**	0.05	0.11
MDH-2	*	*	*	*
a	0.45	0.50	0.43	0.47
b	0.55	0.50	0.57	0.53
PGM-2	*	*	*	*
a	0.38	0.43	0.25	0.34
b	0.22	0.22	0.21	0.30
c	0.40	0.35	0.54	0.36

*Locus not in Hardy-Weinberg equilibrium.

**Locus dependent on sex, so calculation of Hardy-Weinberg expectations invalid.

Table II-8. Average heterozygosity (weighted by sample size) at the GOT-2, IDH-1, LDH-1, and MDH-2 combination of loci for adult pine vole populations trapped in both endrin-treated and nontreated orchards, Fall 1980 and Spring 1981 (\pm SE).

Orchard	Fall 1980	Spring 1981
Endrin-treated		
Total	0.489 (0.28)	0.473 (0.33)
Males	0.470 (0.28)	0.444 (0.31)
Females	0.490 (0.28)	0.438 (0.32)
Nontreated		
Total	0.544 (0.34)	0.498 (0.36)
Males	0.548 (0.35)	0.504 (0.36)
Females	0.541 (0.33)	0.491 (0.35)
Combined		
Total	0.509 (0.22)	0.483 (0.24)
Males	0.498 (0.22)	0.504 (0.26)
Females	0.519 (0.22)	0.504 (0.26)

Table II-9. Average heterozygosity (unweighted by sample size) at the GOT-2, IDH-1, LDH-1, and MDH-2 combination of loci for adult pine vole populations trapped in both endrin-treated and nontreated orchards, Fall 1980 and Spring 1981 (\pm SE).

Orchard	Fall 1980	Spring 1981
Endrin-treated		
Total	0.490 (0.042)	0.474 (0.020)
Males	0.463 (0.054)	0.444 (0.004)
Females	0.499 (0.058)	0.441 (0.130)
Nontreated		
Total	0.548 (0.032)	0.494 (0.048)
Males	0.570 (0.039)	0.501 (0.029)
Females	0.536 (0.036)	0.488 (0.068)
Combined		
Total	0.519 (0.029)	0.484 (0.021)
Males	0.516 (0.038)	0.472 (0.020)
Females	0.526 (0.027)	0.502 (0.036)

Table II-10. Results of chi-squared tests of independence of observed genotypes on sex, for adult pine voles on different feed intakes (N=46).

Locus	χ^2	p	df
GOT-2	0.87	0.351	1
IDH-1	1.46	0.471	1
LDH-1	2.48	0.487	2
MDH-2	--	-- (1)	--
PGM-2	1.39	0.492	1

(1) Contingency table too sparse (cells with expected values <1.0) even after combining rare genotypes; test invalid for $df \geq 1$.

Table II-11. Results of chi-squared tests of observed genotype frequencies on outcome of 24-day feed restriction experiment (survival on 100% ad libitum feed intake, survival on 70%, and mortality on 70%; N=46).

Locus	χ^2	p	df
GOT-2	2.26	0.324	2
IDH-1	0.08	0.960	2
LDH-1	0.22	0.898	2
MDH-2	--	-- (1)	--
PGM-2	2.93	0.405	2

(1) Contingency table too sparse (cells with expected values <1.0) even after combining rare genotypes; test invalid for $df \geq 1$.

Table II-12. Results of chi-squared tests of agreement of observed genotype frequencies with Hardy-Weinberg expectations for adult pine voles on different feed intakes.

Locus	χ^2	p	df
GOT-2	7.14	0.008	1
IDH-1	150.44	<0.001	1
LDH-1	5.49	0.139	3
MDH-2	38.64	<0.001	1
PGM-2	11.31	0.001	1

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ELECTROPHORETIC INVESTIGATION
OF GENETIC CHARACTERISTICS IN THE PINE VOLE

by

Jeness Elizabeth McBride

(ABSTRACT)

Genetic homogeneity among endrin-resistant and -susceptible pine vole populations and between voles that survived or died on restricted feed intake was tested using starch-gel electrophoresis of 5 polymorphic enzyme markers.

Three apple orchards annually treated with endrin for the past 15 years and 3 nontreated orchards in southwestern Virginia were sampled in Fall 1980, and in 2 of each orchard type in Spring 1981 (N=786). LD-50's were uniformly low in all Fall orchards, increasing in only one endrin-treated orchard by Spring 1981 (from 2.2 to 64.5 mg/kg). Orchard, season, and genotype variables were jointly dependent at all 5 loci, but average heterozygosity was similar among the orchards (decreasing from 0.490 to 0.470 in sprayed orchards and from 0.548 to 0.494 in unsprayed orchards). The one incidence of increased endrin tolerance entailed no unique genetic component related unequivocally to endrin treatment.

Genetic heterogeneity was suggested among 36 pine voles subjected to 70% ad libitum feed intake for 24 days. Although approximately equally composed of males and females, only one-fourth of the 33.3% that died were

females. Weight losses did not differ significantly between the survivors and casualties on restriction (22% and 24% of initial weight, respectively). Mean individual heterozygosity was greater for females (0.809) than for males (0.625) over all groups, and greater in both sexes for survivors of the feed restriction (0.742) compared to those that died (0.576), but these differences were statistically nonsignificant. The data suggested a relationship between survival and heterozygosity that deserves closer study.