This is to certify that the

thesis entitled

GENETIC VARIATION WITHIN AND
BETWEEN SYMPATRIC POPULATIONS OF
PISSODES STROBI ON TWO HOST SPECIES
EASTERN WHITE PINE AND JACK PINE

presented by

Charley Adrian Chilcote

has been accepted towards fulfillment
of the requirements for

MASTER OF SCIENCE degree in ENTOMOLOGY

Gary A. Simmons
Major professor

Date 10-10-85

GENETIC VARIATION WITHIN AND BETWEEN SYMPATRIC POPULATIONS OF PISSODES STROBI ON TWO HOST SPECIES: EASTERN WHITE PINE AND JACK PINE

BY

Charley Adrian Chilcote

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Entomology

1985

ABSTRACT

GENETIC VARIATION WITHIN AND
BETWEEN SYMPATRIC POPULATIONS OF
PISSODES STROBI ON TWO HOST SPECIES
EASTERN WHITE PINE AND JACK PINE

BY

Charley Adrian Chilcote

Genetic variability within and between 10 sympatric populations of Pissodes strobi Peck on two host species were studied in Michigan's lower peninsula. Coefficients of genetic distance were very low and ranged from 0.000 to 0.007 and genetic distances averaged across hosts were extremely low, 0.001. Fixation indices (F_{IS} and F_{IT}) indicated that some alleles deviated from Hardy-Weinberg equilibria, but differentiation (F_{ST}) between populations was low, 0.018, and ranged from 0.009 to 0.027. Differentiation between host populations was even lower, 0.003. Hierarchial analysis provided evidence that most of the variation in these populations is related to local populations and not correlated with host or locality. No evidence of host races was found in this electrophoretic analysis. These populations seem to represent subpopulations of a polyphagus panmictic population.

To my parents, S.V. and Florence

and my wife, Lois

ACKNOWLEDGEMENTS

I would like to deeply express my appreciation to Gary Simmons for acting as my major advisor. His guidance, encouragement, and criticism have helped shape my philosophy of science and my abilities as a researcher. I also wish to express my gratitude to Guy Bush for the use of his facilities and his aid in analysis and editing. To the other members of my guidance committee, Roland Fischer and William Mattson, my thanks for their advice on many matters.

For technical assistance and early insight on developing a proper research plan, I am indebted to Daniel J. Howard. His training in electrophoretic procedures provided a resource uncommonly beneficial to my project.

I would like to thank those who provided discussion on many matters that aided in my program. In particular, I thank Todd Bierbaum, Ray Drapek, Norman Elliott, and Frank Sapio.

I would like to make a special acknowledgement of Gary Rathbun, who in high school encouraged and helped me develop an interest in biology and entomology.

TABLE OF CONTENTS

LIST OF TABLES

Table Page

LIST OF FIGURES

INTRODUCTION

Life history and impact of the white pine weevil

The white pine weevil, Pissodes strobi Peck, is an important pest of many species of pines and spruces in North America. The most frequently colonized trees include: eastern white pine, Pinus strobus L., western white pine, P. monticola Dougl., jack pine, P. banksiana Lamb., Scots pine, P. sylvestris L., Norway spruce, Picea abies (L.) Karst, Sitka spruce, P. sitchensis (Bong.) Carr, and Engelmann spruce, P. engelmanni Parry. In the East, eastern white pine is considered the preferred host. Sitka spruce is the preferred host in the West.

Pissodes strobi is one of the most important factors limiting reforestation of certain host species in North America (Belyea and Sullivan 1956). The weevil can do extensive damage to young plantations as well as young natural stands. Injury to a large percentage of the trees in natural and planted stands results from repeated attacks during the early life of the trees (3-20 years). Some plantations have shown 50 percent or more of the most vigorously growing trees to be damaged in one year (Cline and MacAloney 1931; Prentice and Hildahl 1957). In New York and New Hampshire, it has been estimated that 70 to 90 percent of all eastern white pine have been damaged before the age of fifteen years (Graham 1926; Plummer and Pillsbury 1929). Trees, thus, have little value as sawlogs or are scarcely worth cutting at all (Dirks 1964). In the New England states alone, it is

estimated that poor quality of weevilled trees results in a loss in excess of 7 million dollars annually (Garrett 1972).

The adult weevils overwinter in the duff beneath the trees. When environmental conditions are optimal, adults emerge and begin to feed on the terminal growth below the current year's bud. Adults congregate on the leader to feed and mate. After mating, females lay their eggs in small cavities beneath the bark. Larvae hatch and feed on the cambium beneath the bark and as their members increase, aggregate together to form a feeding ring that encircles the terminal below the bud. Larvae in the ring orient themselves downward and feed, girdling the terminal, causing the leader to wilt and turn brown. This dead or dying leader is the characteristic "shepard's crook" commonly seen in field examinations of weevilled stands.

As larvae mature, they fall behind the advancing feeding ring and produce an excelsior-covered chamber in which pupation and transformation into teneral adults occurs. These adults remain in the chamber for one to two weeks then emerge and feed on succulent new growth until environmental conditions force them into overwintering sites. A more detailed description of the biology of this weevil is presented in Appendix 2.

In many areas of the northeastern U.S., eastern white pine can be regenerated naturally with high success. Weevil damage, however, has been so severe and widespread that it has been difficult to promote the growth of this species. The weevil has caused serious damage to both eastern white pine and jack pine in Michigan and Wisconsin. A 1980 survey found 27 percent of the jack pine plantations surveyed

severely damaged (Michigan Pest Report 1980), and in sample plots in the AuSable State Forest, weevilling rose from 35 percent in 1980 to 65 percent in 1981 (Michigan Pest Report 1981-1982). Weevil damage was in fact reported in every county in Michigan's Lower Peninsula in 1979 (Michigan Pest Report 1979). Jack pine plantation damage ranged from 3 percent to 75 percent and many young stands (ten-year-old) in the Pere Marquette Forest were unmerchantable due to reduced growth and poor form (Michigan Pest Report 1981-1982).

The damage from the feeding activities and oviposition of the weevil has been catagorized into either a reduction in the recovered volume in a stand or planting or the reduction of lumber quality in recovered volume. The weevil, which normally causes the main terminal to die, seldom kills trees unless they are young. Laterals below this dead region then compete for dominance producing a tree with a crooked stem. If more than one lateral share dominance, the tree may become forked. Repeated weevilling, year after year, results in a "cabbage" or a "shrub" tree (Cline and MacAloney 1931). Although a tree may recover from a crook or fork, lumber cut from these trees may have serious defects (Belyea and Sullivan 1956) including cross grains, large knots and compression wood, all of which can be brittle and check (Spurr and Friend 1941). Reduction in growth, forking and crooks, and defects in wood thus reduce the quality and quantity of lumber produced from weevilled stands by as much as 30 percent (Plummer and Pillsbury 1929). Even if the trees are only used for pulp, large knots and cross grains create additional expense in processing pulpwood.

Control of the white pine weevil and host preference

Many techniques have been developed to control the white pine weevil. Some of these show merit and are considered feasible, while others are too costly or impractical. Pruning and reclamation was one of the first techniques used in the Northeast. This procedure involves making acceptable products from trees already damaged and is not practical on a large scale. Later, chemical control was extensively used in the East and Canada. Compounds such as DDT and Lindane as well as many others have been used with mixed results. Different spray techniques were tested such as backpack spraying and helicopter spraying. Currently, chemical control is not employed except on high value stands such as Christmas trees and high valued lumber trees such as eastern white pine.

Another control technique currently in use is forest manipulation. In this category, the stand is modified from the standard planting to a planting that allows some measure of control. Dense planting of trees has proven to effectively negate the damage caused by the weevil in eastern white pine. These plantings force weevilled trees to recover quickly or lose their competitive place in the stand. A second approach involving understory plantings of the crop tree in an existing thinned stand of mature trees has also produced excellent results. Normally mature hardwoods provide shading for the crop tree during the early spring when weevil activity is high which reduces oviposition activity (Sullivan 1959, 1960, 1961). Overstory trees are later removed when crop trees have outgrown weevil damage or damage is no longer a problem. Understory planting is probably one of the most practical of

the silvicultural controls available, especially on better sites and with shade tolerant trees such as eastern white pine (Graham 1926).

In Michigan, jack pine is an important crop tree because it is adaptable to sandy soils, benefits wildlife, and has excellent pulpwood qualities. However, it is not shade tolerant and dense plantings do not provide damage control (Morse 1958). Thus, some other methods for controlling the weevil on this host are needed. Two methods that have received only limited study are biological control and host plant resistance. Because the genetic variability of the white pine weevil may alter the effectiveness of these methods, a thorough understanding of the weevil's genetic variability must be gained. Resistance to the weevil may well be a factor of host preference or selection and biological control agents (i.e., parasites) that utilize some aspect of the host plant to find the host may not have the same efficiency on a new host or on different hosts.

Many studies have been conducted to determine the host preference of the weevil, but most have not included jack pine (e.g., Anderson and Fisher 1956), because it was confirmed only recently as a host of the white pine weevil (Smith and Sugden 1969). In one of the few experiments using jack pine in a preference test with other species of of pines, no significant differences were found (Alfaro and Borden 1982). Unfortunately, this study used a forced-feeding technique and a feeding-choice procedure, which did not reveal much about preference mechanisms in natural or plantation forest.

Models and hypotheses of sympatric speciation

For many years, it has been an accepted hypothesis that speciation can occur when populations are separated geographically by some kind of barrier (i.e., allopatric speciation). The primary reason for the widespread acceptance of this theory is the ease with which it can be intuitively accepted. It is quite easy to imagine conditions such that genetic variation caused by different environments and no apparent gene flow lead to reproductive isolation. However, this theory has been accepted almost entirely on intuitive logic and very little hard evidence has been generated to support it (Bush and Howard 1985). It has not been as easy to accept the notion of speciation occurring within the cruising range of conspecific populations (i.e., sympatric speciation). In fact, many of the early models of sympatric speciation were refuted by Mayr (1963) because of "unsupported and unrealistic assumptions". Two of the models of sympatric speciation refuted by Mayr (1963) include: Fisher (1930)- speciation by disruptive selection; Laven (1959)- speciation by cytoplasmic sterility. Recently, many new models and evidence have been generated that support sympatric speciation. Models have been developed that support disruptive selection (Maynard Smith 1970; Bush 1975; Rice 1984), assortative mating based on host utilization (Bush 1969; Rice 1984), and reproductive character displacement (Maynard Smith 1966). The amount of evidence supporting sympatric speciation is much greater than the evidence supporting allopatric speciation (Bush and Howard 1985).

Recent sympatric speciation models have incorporated many of the features of early models and added new aspects and more evidence.

Early principles such as conditioning have been rediscovered. Walsh (1864) was probably the first to formally present the idea that species might form in sympatry if the populations adapted (conditioned) to different hosts. Later, this idea was developed as the Hopkins host selection principle (Craigehead 1921) which basically stated that an adult would tend to oviposit on the same host that was used by the immature stage as food. This principle lost credibility for being too simplistic and being applied too frequently on the basis of host association only. Today, it is still used frequently and more rigorous tests have provided evidence for its usefulness. Other models have gained new acceptance and additional evidence is making sympatric speciation seem plausible. Maynard Smith (1970) pumped new life into the principle of sympatric speciation by disruptive selection. The important aspect of Smith's model is the assumption that a population first develops a stable polymorphism in response to a heterogeneous environment. Many studies have stressed the importance of this assumption (Maynard Smith 1970; Bush 1975; Tauber and Tauber 1977; Tavormina 1982). Mayr (1963) had argued that no mechanism (of sympatric speciation) consistent with the known facts of genetics could be suggested for sympatric speciation. Smith's model seems to solve one of the biggest problems with previous models of sympatric speciation and presents a method by which current genetics can be applied to sympatric speciation. These models have also been supported by experimental evidence (Pimentel et al. 1967; Thoday 1972; Soans et al. 1974; Bush 1974) and theoretical studies (Dickinson and Antonovics 1973).

Current models stress the need for stable polymorphisms developing.

They further incorporate the assumption that host selection occurs in conjunction with mate selection (Diehl and Bush 1984). In this way the host is not chosen completely on the basis of its own merits (i.e., chemical cues, shape, etc.). This type of mate selection can easily lead to assortative mating. Mutations in genes that control host selection may lead to host shifts. Other mutations in genes controlling the surviveability of larvae on a host and timing of development can also lead to reduced gene flow between populations (Diehl and Bush 1984). Coupled with assortative mating, new host races may form that eventually lead to new species.

The new push for information about sympatric speciation has lead to new and detailed information about many populations that were once believed to be one wide ranging species. The white pine weevil is one of these species. It ranges from Nova Scotia to British Columbia and its host range is wide. In the East, it was given the species name of its preferred host, Pissodes strobi for Pinus strobus, eastern white pine. In the West, it received the names of the two hosts it most frequented, P. sitchensis for Picea sitchensis, Sitka spruce, and P. engelmanni for Picea engelmanni, Engelmann spruce. Recent evidence has made it clear that these species actually represent one species (Smith and Sugden 1969; Phillips 1985). This insect choses its mate in conjunction with its host which makes it a good candidate for host shifts. Detailed study of the possible correlation of genetic distance and geographic distance has been carried out by Phillips (1985). It was the intention of this study to gain information about the genetic variability within the cruising range of the insect and to establish the relative variability associated with host utilization.

STUDY AREAS

Field collection of specimens was conducted in northern Lower Michigan. Two counties were selected as study areas, Wexford and Manistee. A preliminary examination of eastern white pine and jack pine stands provided 10 that were deemed adequate for study. Figure 1 is a map of these counties and the numerical labels are the sites described in Table 1. Plantations were chosen on the basis of stand uniformity (i.e., white pine only or jack pine only) and their relative distances from different stands of the same host. The choices of stands are represented in the hierarchy presented below;

White Pine
- Adjacent
  - Site B W Pine
  - Site C W Pine
- Close
  - Site D W Pine
- Outside
  - Site A W Pine
  - Site E W Pine

Jack Pine
- Adjacent
  - Site B J Pine
  - Site C J Pine
- Close
  - Site A J Pine
- Outside
  - Site D J Pine
  - Site E J Pine

Two stands of each host were chosen as adjacent stands (i.e., stands separated by only a few hundred feet at the most). One stand was chosen

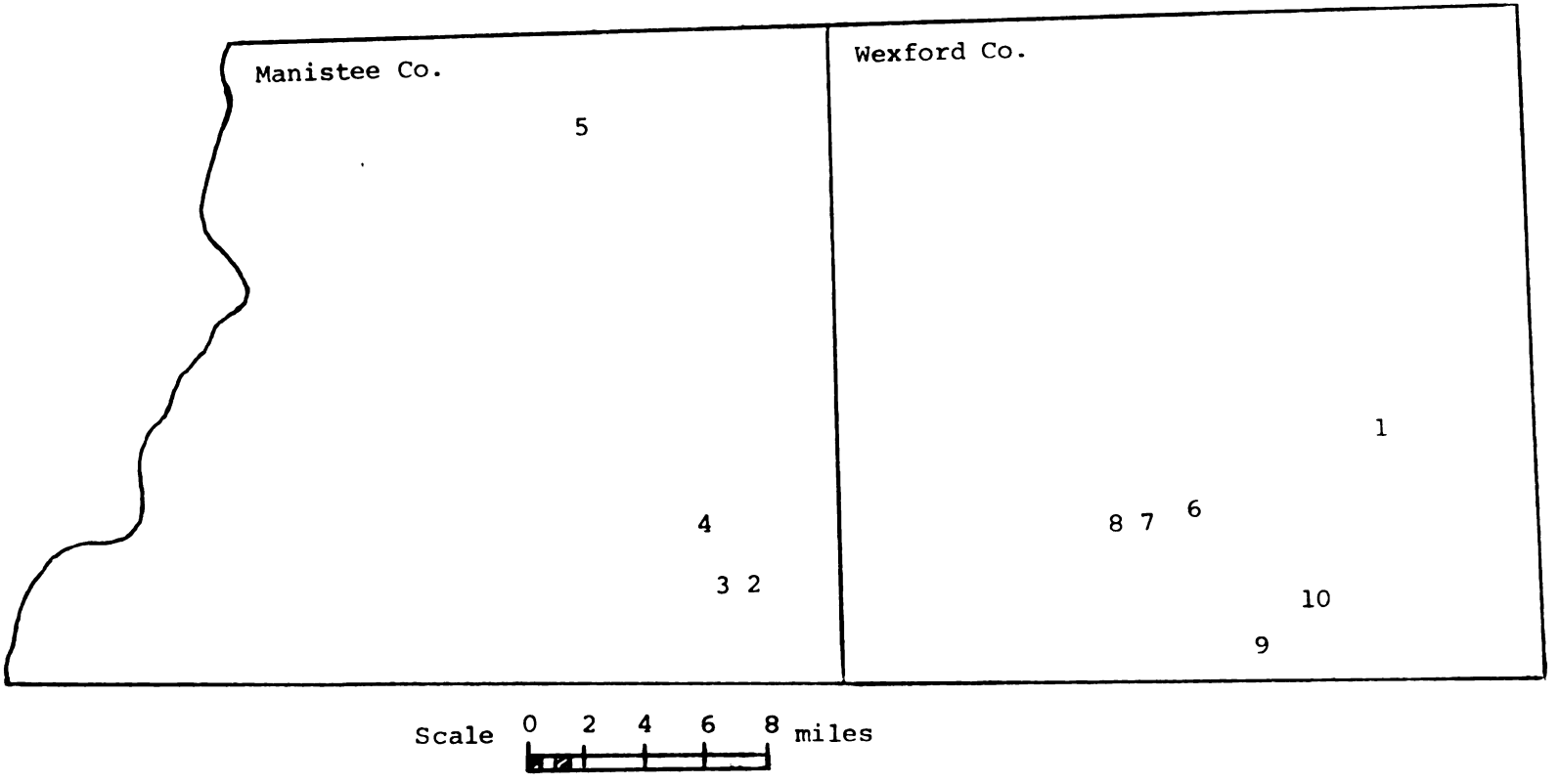

Figure 1. Map Of Study Sites For Pissodes strobi. Numbers coincide with those in Table (1).

Table 1. Study Site Locations And Descriptions For Examination Of *P. strobi*.

	Site	Abbrev.	Location	Description
1	Site A White Pine	A WP	T22N ;R9W; Sec. 17 Wexford Co., MI	Approx. 20 acres; Height 10-20 feet; planted stand surrounded by open fields on the north, east, and west sides and by red pines (30 feet tall) on south side
2	Site B White Pine	B WP	T21N; R13W; Sec. 2 Manistee Co., MI	Approx. 60 acres; Height 15-30 feet; planted stand, very dense; surrounded by hardwoods on the east and north sides, by newly planted red pine on south side and by Site C White Pine on west side
3	Site C White Pine	C WP	T21N; R13W; Sec. 3 Manistee Co., MI	Approx. 100 acres; Height 15-30 feet; planted stand, very dense; surrounded by hardwoods on the west, south, and north sides and by Site B White Pine on east side
4	Site D White Pine	D WP	T22N; R13W; Sec. 26 Manistee Co., MI	Approx. 40 acres; Height 10-30 feet; natural regeneration, sparce; surrounded on all sides by hardwoods and sparce pines
5	Site E White Pine	E WP	T24N; R14W; Sec. 22 Manistee Co., MI	Approx. 60 acres; Height 15-25 feet; planted stand, very dense; surrounded by hardwoods on east, west, and south sides and by red pine (25 feet tall on north side

Table 1. Continued.

	Site	Abbrev.	Location	Description
6	Site A Jack Pine	A JP	T22N; R11W; Sec. 35 Wexford Co., MI	Approx. 40 acres; Height 8-18 feet; natural regeneration after burning; surrounded on south and west sides by hardwoods and on the east and north sides by open fields
7	Site B Jack Pine	B JP	T21N; R11W; Sec. 2 Wexford Co., MI	Approx. 20 acres; Height 10-20 feet; natural regeneration after burning; surrounded on east and west sides by red pine and on south side by hardwoods and open fields on north side
8	Site C Jack Pine	C JP	T21N; R11W; Sec. 2 Wexford Co., MI	Approx. 80 acres; Height 8-20 feet; natural regeneration after burning; surrounded on east. west, and north sides by red pine and on south side by hardwoods
9	Site D Jack Pine	D JP	T21N; R10W; Sec. 33 Wexford Co., MI	Approx. 40 acres; Height 8-18 feet; natural regeneration after burning; surrounded by mature jack pine on all sides
10	Site E Jack Pine	E JP	T21N; R10W; Sec. 27 Wexford Co., MI	Approx. 20 acres; Height 10-25 feet; surrounded on east and west sides by mature jack pine and on north and south sides by hardwoods

for its close proximity to the adjacent stands of the same host (i.e., not more than two miles from the adjacent stands). Two additional stands of each host were selected to be not more than 20 miles away from the adjacent stands of the same host.

Stands were chosen to allow examination of genetic variation within a small geographic area (i.e., less than 300 square miles). Selection of stands at various distances was made to test for subtle differences in genetic variation which could result from host race formation at various levels and geographic distances.

Within these stands, weevilled trees were selected at random. To accomplish this, a starting point was chosen along the perimeter of the stand. A random number was chosen from a random number table and converted to a vector based on its relation to a compass reading (i.e., 45 became 45 degrees when standing on the west side of the stand and 135 degrees when standing on the north side of the stand). Weevilled trees along this vector were the trees to be sampled and their leaders were clipped and numbered for later reference. If a vector did not produce sufficient leaders upon going though the stand, a new vector was chosen at the point of exit from the stand and the procedure repeated until sufficient leaders were sampled. Leaders were surveyed until the larvae within them were starting to pupate. When this occurred, approximately 30 leaders from each stand were collected which allowed for the collection of mature larvae. The monitoring and collection of larvae was accomplished by cutting the bark away and removing the larvae from beneath it. About 25 larvae were removed from each leader. Those to be used in electrophoretic

analysis were placed in a cold vial and submerged in liquid nitrogen until the samples could be transferred to an ultra-cold freezer for storage at -80 degrees C. Monitoring continued until more than 50% of the insects in the leaders had pupated. At that time, leaders were clipped and placed in rearing chambers. Since birds often strip leaders in the field and eat many of the pupae, this chamber allowed collection of adults from the stands. Two hundred forty adults from each site were collected from these chambers, 20 leaders/ stand; 12 individuals/ leader. Again adults were placed in liquid nitrogen until transfer to an ultra-cold freezer.

MATERIALS AND METHODS

The electophoretic procedure used in this study was similar to those used by Berlocker and Bush (1982), Coyne et al. (1979), and Howard (1982). An 11.75% horizontal starch gel, which consisted of 47 grams of Electostarch, lot #392, (Electrostarch Co., Madison, WI) added to 400 ml. of the appropriate buffer solution, was used. The solution was heated until about 7 seconds after a phase change had occurred. The starch was aspirated and poured into a mold about 1 cm. thick and allowed to set. After allowing the gels to set for 24 hours, slits were cut though the gels toward one end. Wicks of filter paper (2 X 9 mm.) containing a small amount of insect homogenate were placed into the slit. A D.C. electric current then was applied to the gels which caused the enzymes contained within the homogenate to migrate though the gels. The amount of current depended on the buffer system used. Appendix 3 gives a detailed description of the electrophoretic procedure as well as the buffers used, currents applied, and run times.

Individuals from each population were crushed in a grinding buffer, 0.05 M tris/HCl pH 7.00, and the homogenate was applied to rectangular filter paper dits (2 by 9 mm.). Dits were placed into slits in the gels as described by Coyne et al. (1979). On each gel, twenty-three individual white pine weevils were run consisting of four individuals from each of the five populations chosen for that

runs and three individuals from the reference population. The reference population was the population chosen to be used as a standard and the other populations were scored relative to this population. During the enzyme survey, the most common allele for each locus in this population was noted and designated as 1.00. During subsequent runs alleles were measured relative to this allele (i.e., and allele measuring 1.50 cm. relative to the reference of 3.00 cm. was designated 0.50). Three individuals from the reference population were placed on the gel in the first, the tenth, and the twenty-third positions from the left which reduced errors in scoring gels.

Initially an enzyme-system survey was performed in which forty enzymes were tested on ten buffer systems (Table 2). This procedure allowed for evaluation of the proper buffer systems and enzyme stains for this insect. The enzymes which gave good resolution and strong banding under the systems tested are listed in Table 3. Ten enzymes, representing fourteen loci, were found to produce consistantly good results and were used in the analysis of genetic variation. Two enzymes, PEP and SDH, representing three loci, were later found to provide adequate banding, but were not used in the analysis.

Scoring of the bands was accomplished by measurement of the distance of individual bands from the origin. Individual weevils were assessed at each locus for the alleles they expressed. Those enzymes that were dimers expressed three bands in heterozygotes and one band in homozygotes. Monomers exhibited two bands in heterzygotes and one in homozygotes. Presumed subunit components for the enzymes examined in this study are presented in Table 3. The reason for the banding

Table 2. Enzymes And Buffer Systems Tested For Examination Of Pissodes strobi.

Enzymes Giving No Results	Enzymes Giving Results But Not Scorable	Enzymes Giving Results And Scorable
CK - Creatine kinase	ACP - Acid phosphatase	AK - Adenylate kinase
GDA - Guanine deaminase	ALD - Aldolase	ADH - Alcohol dehydrogenase
FUM - Fumarase	TRE - Trehalase	PGI - Glucose phosphate isomerase
ALP - Alkaline phosphatase	PGM - Phosphoglucomutase	IDH - Isocitrate dehydrogenase
XDH - Xanthine dehydrogenase	MPI - Mannose phosphate isomerase	MDH - Malate dehydrogenase
PER - Peroxidase	GDH - Glucose dehydrogenase	ACON - Aconitase
GLUD - Glutamate dehydrogenase	ME - Malic enzyme	GOT - Glutamate-oxaloacetate transaminase
HBDH - Hydroxybutyrate dehydrogenase	HK - Hexokinase	EST - Esterase
ODH - Octanol dehydrogenase	GP - General protein	GAPDH - Glyceraldehyde 3-phosphate dehydrogenase
HEXDH - Hexanol dehydrogenase	α-GDH - α-Glycerophosphate dehydrogenase	LAP - Leucine Aminopeptidase
F1,6P - Fructose 1,6-diphosphatase	6-PGD - 6-Phosphogluconate dehydrogenase	PEP - Peptidase
CAT - Catalase	DIA - NADH diaphorase	SDH - Sorbitol dehydrogenase
AO - Aldehyde oxidase	TO - tetrazolium oxidase	
	G6PDH - Glucose 6-phosphate dehydrogenase	
	LDH - Lactate dehydrogenase	

Buffer Systems Tested [1]

2 electrode: 0.03 M lithium hydroxide, 0.19 M boric acid; gel: 1:9 mix electrode buffer to 0.05 M tris, 0.0076 M citric acid

3 electrode: 0.30 M borate; gel: 0.076 M tris, 0.005 M citric acid

4 electrode: 0.22 M tris, 0.15 M citric acid (adjusted to pH 6.3); gel: 0.008 M tris, 0.003 M citric acid (adjusted to pH 6.3)

5 electrode: 0.687 M tris, 0.157 M citric acid; gel: 0.023 M tris, 0.005 M citric acid

Table 2. Continued.

Buffer Systems Tested[1]
6 electrode: 0.50 M tris, 0.02 M EDTA, 0.65 M boric acid; gel: 1:9 dilution electrode buffer to deionized water
7 electrode: 18.78 g/l monobasic potassium phosphate, 2.48 g/l NaOH; gel: 1:9 dilution electrode buffer to deionized water
8 electrode: 29.1 g/l dibasic potassium phosphate, 5.7 g/l monohydrate citric acid; gel: 1.06 g/l dibasic potassium phosphate, 0.254 g/l monohydrate citric acid
9 electrode: 0.10 M tris, 0.10 M maleic acid, 0.01 M EDTA (adjusted to pH 7.4); gel: 1:9 dilution electrode buffer to deionized water
10 electrode: 1:20 dilution of stock sol. to deionized water; gel: 1:60 dilution of stock sol. to deionized water (Stock sol. - 90.8 g/l tris, 52.5 g/l citric acid)

[1] Buffer systems are those of Howard (1982); all buffers are made up with double deionized water.

Table 3. Enzymes And Procedural Information Used In Examination Of *P. strobi*.

Enzyme Name	Locus Abbrev.	Subunit Composition	Buffer System	Run Time	Migration	Voltage/[1] Amperage
Aconitase	ACON-1	monomer	5	12 hrs	anodal	100V/50mA
	ACON-2	monomer	5	12 hrs	cathodal	
Glutamate-oxaloacetate-transaminase	GOT	dimer	3	4½ hrs	anodal	250V/50mA
Malate dehydrogenase	MDH-1	dimer	5	5 hrs	anodal	100V/50mA
	MDH-2	dimer	5	5 hrs	cathodal[2]	
Isocitric dehydrogenase	IDH-1	dimer	5	5 hrs	anodal	100V/50mA
	IDH-2	dimer	5	5 hrs	anodal	
Alcohol dehydrogenase	ADH-1	dimer	5	12 hrs	anodal	100V/50mA
	ADH-2	unknown	5	12 hrs	anodal	
Phosphoglucoisomerase	PGI	dimer	6	7 hrs	anodal	150V/50mA
Adenylate kinase	AK	monomer	5	5 hrs	anodal	100V/50mA
Leucine Aminopeptidase	LAP	monomer	5	12 hrs	anodal	100V/50mA
Glyceraldehyde 3-phosphate dehydrogenase	GAPDH	tetramer	7	8 hrs	anodal	60V/50mA
Esterase	EST-1	monomer	6	7 hrs	anodal	150V/50mA

[1] The voltage was adjusted to the proper level after running the gels for a short time at a lower voltage.

[2] This enzyme appears near the origin and bands appear on both sides of the origin in the heterozygotes.

pattern expressed by these subunits are discussed by Brewer (1970). Relative relationships of electromorphs discussed earlier were used to assign allelic designations to each individual. Later these designations were converted to an alphabetic system with the slowest allele designated as "A" and each subsequent allele was given the next alphabetic character. This was done to facilitate analysis by computer.

The genetic basis for most of the enzymes in this study have been worked out independently by Phillips (1985). However, the genetic basis for ACON and LAP were assessed on the basis of the family group segregation (i.e., each leader sampled represented a family of weevils, since, in most cases, only one female oviposits in a leader-- results from data on electrophoretic study of 25 individuals from each leader produced no individuals that appeared to be from different parents; by testing members from family groups, segregation of genotypes could be examined and if it fit with normal Mendelian segregation, a genetic relationship was assumed for electromorphs under study).

Analysis of genetic identity and distance was performed with the aid of BIOSYS1, a computer program provided by Swofford and Selander (1981). This program calculates allele frequencies, Nei's genetic identities, Nei's genetic distances, F-statistics, Hardy-Weinberg equilibria, Chi-square goodness of fit for Hardy-Weinberg, as well as other functions not used for final analysis. A detailed description of the formulas and analysis techniques can be found in the User's Guide (Swofford and Selander 1981). This program allowed for manipulation of population structure to give more detailed information about the genetic variability within and between the populations.

RESULTS

Allele frequencies and heterozygosities

Ten enzymes, representing 14 loci, could be routinely resolved and scored. Most of these enzymes produced strong bands easily assigned to an electromorph category. Extremely long gel runs (i.e., about 12 hours) were needed to pull some bands apart sufficiently to determine heterozygotes (e.g., for ACON and LAP). Clear heterozygotes were expressed by most loci (ACON-1, ACON-2, MDH-1, MDH-2, IDH-1, IDH-2, AK, ADH-1, LAP, PGI, GAPDH, EST-1, and GOT). Four enzymes expressed no heterozygotes in any individuals (i.e., tetrazolium oxidase, TO; Glucose 6-phosphate dehydrogenase, G6PDH; Lactate dehydrogenase, LDH; and NADH diaphorase, DIA) and, because bands were indistinct or smeared, were seen as unreliable and not used in the analysis. Only one locus, ADH-2, was considered to be monomorphic as it did not express any heterozygote individuals in contrast to ADH-1, which did show heterozygotes on the same gel. Two additinal enzymes, SDH (sorbitol dehydrogenase) and PEP (peptidase), were found late in the study and provided good banding and exhibited heterozygosity. These two enzymes, however, were discovered too late to be included in analysis. Another locus, EST-2, was found to provide good resolution and banding, but it was difficult to determine a reference point from which to score the other alleles and it was not included in final examination.

The enzymes examined were highly variable and represented by a

wide number of alleles (Table 4). ACON-1, PGI, and EST-1 were extremely variable, exhibiting an average of 10 alleles each. EST-1 had 13 distinct alleles that combined to form electromorphs (Figure 2). Some of the observed combinations of alleles for ACON-1 and PGI are presented in Figures 3 and 4. Most of the other loci expressed 3 or more alleles.

Allele frequencies observed in the 10 populations of P. strobi are presented in Table 4. ACON-2, MDH-2, IDH-1, ADH-2, and ADH-1 expressed very little frequency variation between populations. PGI, ACON-1, and EST-1 displayed a high level of variation between populations. The "D" allele in ACON-1 had a range of frequencies from 0.389 in population E WP to 0.641 in population A JP. PGI also varied considerably with the "E" allele having a frequency of 0.481 in population E JP and 0.649 in population C JP. EST-1 expressed the highest degree of allele frequency variation. An extreme example of variation from EST-1 is found in the "J" allele, which varied from 0.083 in D JP to 0.432 in D WP. Except for these few cases, the allele frequencies were very similar in all populations studied. Mean heterozygosities, calculated by direct count, were quite similar in all populations and ranged from 0.210 in D WP to 0.307 in A WP. Table 5 presents mean heterozygosities and expected values calculated from Hardy-Weinberg expectations (Nei 1978). None of the direct-count measures of mean heterozygosity varied to any great extent from those expected under Hardy-Weinberg.

Using Levene's (1949) correction for small sample, it was found that 12 of the 112 possible variable loci in the 10 populations did

Table 4. Electrophoretic Allele Frequencies Of 10 Populations Of P. strobi On White Pine And Jack Pine.

		Population[1]									
Locus[2]	Allele[3]	A WP	B WP	C WP	D WP	E WP	A JP	B JP	C JP	D JP	E JP
ACON-1	(N)	(36)	(28)	(30)	(26)	(27)	(32)	(23)	(31)	(22)	(26)
	A	0.000	0.054	0.000	0.000	0.037	0.016	0.000	0.000	0.000	0.000
	B	0.028	0.125	0.050	0.038	0.074	0.094	0.043	0.065	0.114	0.019
	C	0.125	0.018	0.017	0.058	0.037	0.031	0.022	0.048	0.023	0.019
	D	0.500	0.429	0.583	0.558	0.389	0.641	0.565	0.613	0.568	0.596
	E	0.056	0.000	0.033	0.000	0.037	0.016	0.000	0.016	0.045	0.058
	F	0.194	0.321	0.283	0.250	0.333	0.172	0.283	0.226	0.182	0.231
	G	0.083	0.054	0.017	0.096	0.056	0.031	0.087	0.032	0.045	0.058
	H	0.014	0.000	0.017	0.000	0.037	0.000	0.000	0.000	0.023	0.019
	HDYWBG	NS	NS	NS	NS	NS	NS	NS	*	*	NS
	H	0.686	0.691	0.575	0.612	0.724	0.549	0.591	0.566	0.626	0.584
	H(UNB)	0.695	0.704	0.585	0.624	0.737	0.558	0.604	0.575	0.641	0.595
	H(D.C.)	0.694	0.607	0.600	0.692	0.519	0.563	0.652	0.516	0.636	0.538
ACON-2	(N)	(36)	(27)	(29)	(25)	(27)	(32)	(23)	(33)	(22)	(27)
	A	0.014	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	B	0.028	0.037	0.034	0.020	0.037	0.016	0.043	0.000	0.023	0.037
	C	0.944	0.963	0.966	0.980	0.963	0.984	0.957	1.000	0.977	0.963
	D	0.014	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	HDYWBG	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	H	0.107	0.071	0.067	0.039	0.071	0.031	0.083	0.000	0.044	0.071
	H(UNB)	0.108	0.073	0.068	0.040	0.073	0.031	0.085	0.000	0.045	0.073
	H(D.C.)	0.111	0.074	0.069	0.040	0.074	0.031	0.087	0.000	0.045	0.074

Table 4. Continued.

Locus[2]	Allele[3]	Population[1] A WP	B WP	C WP	D WP	E WP	A JP	B JP	C JP	D JP	E JP
PGI	(N)	(37)	(31)	(30)	(27)	(27)	(34)	(25)	(37)	(22)	(26)
	A	0.000	0.016	0.000	0.000	0.019	0.000	0.000	0.000	0.000	0.000
	B	0.000	0.000	0.000	0.019	0.019	0.029	0.000	0.000	0.000	0.000
	C	0.000	0.000	0.033	0.019	0.019	0.000	0.040	0.000	0.023	0.019
	D	0.351	0.419	0.383	0.296	0.352	0.382	0.400	0.311	0.409	0.404
	E	0.595	0.532	0.500	0.630	0.537	0.559	0.540	0.649	0.545	0.481
	F	0.014	0.000	0.033	0.019	0.037	0.000	0.020	0.041	0.000	0.019
	G	0.000	0.000	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.019
	H	0.041	0.032	0.017	0.019	0.019	0.029	0.000	0.000	0.023	0.058
	I	0.000	0.000	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	HDYWBG	NS	NS	NS	NS	**	NS	NS	NS	NS	NS
	H	0.521	0.540	0.600	0.514	0.585	0.540	0.546	0.481	0.534	0.601
	H(UNB)	0.528	0.548	0.610	0.524	0.596	0.548	0.558	0.488	0.547	0.613
	H(D.C.)	0.568	0.581	0.667	0.370	0.593	0.588	0.640	0.459	0.500	0.654
GOT	(N)	(36)	(29)	(29)	(27)	(27)	(33)	(22)	(37)	(21)	(26)
	A	0.000	0.000	0.000	0.000	0.000	0.000	0.023	0.027	0.000	0.019
	B	0.347	0.328	0.397	0.333	0.333	0.364	0.273	0.257	0.381	0.250
	C	0.014	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	D	0.611	0.672	0.569	0.648	0.667	0.606	0.682	0.689	0.595	0.712
	E	0.014	0.000	0.017	0.000	0.000	0.015	0.000	0.014	0.024	0.000
	F	0.014	0.000	0.017	0.019	0.000	0.015	0.023	0.014	0.000	0.019
	HDYWBG	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	H	0.505	0.441	0.518	0.468	0.444	0.500	0.460	0.458	0.500	0.430
	H(UNB)	0.513	0.448	0.528	0.477	0.453	0.508	0.470	0.464	0.512	0.439
	H(D.C.)	0.472	0.517	0.552	0.370	0.296	0.515	0.364	0.405	0.286	0.538

Table 4. Continued.

		Population[1]									
Locus[2]	Allele[3]	A WP	B WP	C WP	D WP	E WP	A JP	B JP	C JP	D JP	E JP
EST-1	(N)	(30)	(19)	(26)	(22)	(22)	(26)	(20)	(30)	(18)	(26)
	A	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	B	0.017	0.026	0.000	0.000	0.045	0.038	0.025	0.083	0.000	0.038
	C	0.050	0.026	0.000	0.068	0.023	0.038	0.125	0.000	0.111	0.019
	D	0.000	0.000	0.038	0.000	0.000	0.038	0.000	0.017	0.000	0.058
	E	0.150	0.026	0.231	0.068	0.091	0.038	0.175	0.083	0.083	0.077
	F	0.000	0.026	0.019	0.023	0.000	0.000	0.000	0.000	0.056	0.000
	G	0.067	0.079	0.058	0.000	0.023	0.000	0.050	0.017	0.056	0.019
	H	0.383	0.368	0.173	0.295	0.409	0.423	0.250	0.350	0.389	0.173
	I	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.056	0.000
	J	0.267	0.237	0.365	0.432	0.205	0.250	0.275	0.350	0.083	0.462
	K	0.067	0.132	0.115	0.091	0.182	0.115	0.075	0.083	0.139	0.154
	L	0.000	0.079	0.000	0.023	0.023	0.058	0.025	0.017	0.028	0.000
	HDYWBG	*	NS	**	NS	NS	**	**	NS	**	**
	H	0.748	0.776	0.765	0.708	0.746	0.736	0.806	0.733	0.793	0.722
	H(UNB)	0.760	0.797	0.780	0.724	0.763	0.750	0.827	0.746	0.816	0.736
	H(D.C.)	0.667	0.737	0.654	0.682	0.773	0.692	0.650	0.500	0.667	0.423
LAP	(N)	(35)	(19)	(26)	(22)	(27)	(28)	(21)	(32)	(22)	(27)
	A	0.057	0.026	0.038	0.045	0.074	0.036	0.000	0.016	0.023	0.056
	B	0.143	0.158	0.077	0.136	0.074	0.089	0.143	0.031	0.091	0.037
	C	0.643	0.816	0.846	0.750	0.778	0.875	0.833	0.875	0.727	0.796
	D	0.157	0.000	0.038	0.068	0.074	0.000	0.024	0.063	0.159	0.111
	E	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.016	0.000	0.000
	HDYWBG	NS	NS	NS	*	NS	NS	NS	NS	NS	NS
	H	0.538	0.309	0.275	0.412	0.379	0.225	0.285	0.229	0.437	0.349
	H(UNB)	0.546	0.317	0.281	0.422	0.386	0.229	0.292	0.233	0.447	0.356
	H(D.C.)	0.600	0.263	0.231	0.364	0.296	0.250	0.238	0.250	0.364	0.333

Table 4. Continued.

		Population[1]									
Locus[2]	Allele[3]	A WP	B WP	C WP	D WP	E WP	A JP	B JP	C JP	D JP	E JP
IDH-1	(N)	(37)	(30)	(30)	(27)	(27)	(35)	(25)	(37)	(22)	(27)
	A	0.946	0.967	0.983	0.981	0.926	0.914	0.940	0.946	0.977	0.926
	B	0.054	0.033	0.017	0.019	0.037	0.057	0.060	0.054	0.023	0.074
	C	0.000	0.000	0.000	0.000	0.037	0.029	0.000	0.000	0.000	0.000
	HDYWBG	NS	NS	NS	NS	NS	NS	NS	NS	NS	*
	H	0.102	0.064	0.033	0.036	0.140	0.160	0.113	0.102	0.044	0.137
	H(UNB)	0.104	0.066	0.033	0.037	0.143	0.162	0.115	0.104	0.045	0.140
	H(D.C.)	0.108	0.067	0.033	0.037	0.140	0.171	0.120	0.108	0.045	0.074
IDH-2	(N)	(37)	(30)	(30)	(27)	(27)	(35)	(25)	(37)	(22)	(27)
	A	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.019
	B	0.000	0.000	0.000	0.000	0.019	0.000	0.000	0.000	0.000	0.000
	C	1.000	0.967	0.983	1.000	0.981	0.986	0.980	0.986	1.000	0.981
	D	0.000	0.033	0.017	0.000	0.000	0.014	0.020	0.014	0.000	0.000
	HDYWBG	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	H	0.000	0.064	0.033	0.000	0.036	0.028	0.039	0.027	0.000	0.036
	H(UNB)	0.000	0.066	0.033	0.000	0.037	0.029	0.040	0.027	0.000	0.037
	H(D.C.)	0.000	0.067	0.033	0.000	0.037	0.029	0.040	0.027	0.000	0.037
GAPDH	(N)	(30)	(28)	(26)	(20)	(23)	(21)	(21)	(31)	(22)	(17)
	A	0.817	0.768	0.962	0.950	0.913	0.929	0.905	0.871	0.841	0.824
	B	0.813	0.232	0.038	0.050	0.087	0.071	0.095	0.129	0.159	0.176
	HDYWBG	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	H	0.299	0.357	0.074	0.095	0.159	0.133	0.172	0.225	0.268	0.291
	H(UNB)	0.305	0.363	0.075	0.097	0.162	0.136	0.177	0.228	0.274	0.299
	H(D.C.)	0.367	0.250	0.077	0.100	0.174	0.143	0.190	0.194	0.227	0.353

Table 4. Continued.

		Population[1]									
Locus[2]	Allele[3]	A WP	B WP	C WP	D WP	E WP	A JP	B JP	C JP	D JP	E JP
MDH-1	(N)	(38)	(28)	(30)	(27)	(27)	(34)	(25)	(37)	(22)	(27)
	A	0.000	0.000	0.000	0.000	0.019	0.000	0.000	0.000	0.000	0.000
	B	0.013	0.018	0.017	0.019	0.037	0.000	0.000	0.000	0.000	0.019
	C	0.000	0.000	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	D	0.908	0.964	0.917	0.963	0.944	1.000	1.000	0.986	1.000	0.981
	E	0.066	0.018	0.050	0.019	0.000	0.000	0.000	0.014	0.000	0.000
	F	0.013	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	HDYWBG	NS	NS	*	NS	NS	NS	NS	NS	NS	NS
	H	0.171	0.070	0.157	0.072	0.106	0.000	0.000	0.027	0.000	0.036
	H(UNB)	0.173	0.071	0.159	0.073	0.108	0.000	0.000	0.027	0.000	0.037
	H(D.C.)	0.184	0.071	0.133	0.074	0.111	0.000	0.000	0.027	0.000	0.037
MDH-2	(N)	(38)	(28)	(29)	(26)	(27)	(33)	(24)	(37)	(22)	(26)
	A	0.974	1.000	0.983	1.000	1.000	0.985	0.979	1.000	1.000	1.000
	B	0.027	0.000	0.017	0.000	0.000	0.015	0.021	0.000	0.000	0.000
	HDYWBG	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	H	0.051	0.000	0.034	0.000	0.000	0.030	0.041	0.000	0.000	0.000
	H(UNB)	0.052	0.000	0.034	0.000	0.000	0.030	0.042	0.000	0.000	0.000
	H(D.C.)	0.053	0.000	0.034	0.000	0.000	0.030	0.042	0.000	0.000	0.000
ADH-2	(N)	(37)	(26)	(29)	(27)	(27)	(33)	(23)	(33)	(20)	(23)
	A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	HDYWBG	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	H	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	H(UNB)	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	H(D.C.)	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Table 4. Continued.

		Population[1]									
Locus[2]	Allele[3]	A WP	B WP	C WP	D WP	E WP	A JP	B JP	C JP	D JP	E JP
ADH-1	(N)	(37)	(26)	(30)	(27)	(27)	(33)	(23)	(32)	(22)	(27)
	A	0.014	0.000	0.033	0.000	0.000	0.015	0.000	0.031	0.023	0.000
	B	0.973	1.000	0.950	1.000	1.000	0.985	0.978	0.969	0.932	0.926
	C	0.014	0.000	0.017	0.000	0.000	0.000	0.000	0.000	0.023	0.037
	D	0.000	0.000	0.000	0.000	0.000	0.000	0.022	0.000	0.023	0.019
	E	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.019
	HDYWBG	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	H	0.053	0.000	0.096	0.000	0.000	0.030	0.043	0.061	0.130	0.141
	H(UNB)	0.054	0.000	0.098	0.000	0.000	0.030	0.043	0.062	0.133	0.143
	H(D.C.)	0.054	0.000	0.100	0.000	0.000	0.030	0.043	0.063	0.136	0.148
AK	(N)	(37)	(31)	(30)	(27)	(27)	(33)	(25)	(37)	(22)	(27)
	A	0.014	0.000	0.000	0.000	0.019	0.061	0.000	0.041	0.000	0.000
	B	0.014	0.000	0.000	0.000	0.000	0.000	0.060	0.041	0.000	0.000
	C	0.946	0.952	0.950	1.000	0.926	0.924	0.900	0.892	0.955	1.000
	D	0.000	0.016	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	E	0.027	0.032	0.050	0.000	0.056	0.015	0.040	0.027	0.045	0.000
	HDYWBG	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	H	0.104	0.093	0.095	0.000	0.139	0.142	0.185	0.201	0.087	0.000
	H(UNB)	0.106	0.095	0.097	0.000	0.142	0.144	0.189	0.203	0.089	0.000
	H(D.C.)	0.108	0.097	0.100	0.000	0.148	0.152	0.200	0.216	0.091	0.000

[1] Host trees: WP = eastern white pine, *Pinus strobus* L.; JP = jack pine, *P. banksiana* Lamb.

[2] Designations for locus codes given in text.

[3] (N) refers to number of individuals examined. HDYWBG refers to Levene's test (1949) for conformance to Hardy-Weinberg equilibrium; NS = not significant at $P<0.01$; * = significant at $P<0.01$; ** = significant at $P<0.001$. H = Hardy-Weinberg expected heterozygosity; H(UNB) = Unbiased estimated heterozygosity (Nei 1978); H(D.C.) = "direct" count heterozygosity.

Table 5. Genetic Variability At 14 Loci In All Populations Examined For *P. strobi*.[1]

	Population	Mean Sample Size Per Locus	Mean No. Of Alleles Per Locus	Percentage Of Loci Polymorphic*	Mean Heterozygosity	
					Direct-Count	HDYWBG Expected**
1	Site A WP	35.7 (0.7)	3.8 (0.5)	76.9	0.307 (0.072)	0.303 (0.075)
2	Site B WP	27.2 (0.6)	3.1 (0.6)	46.2	0.256 (0.073)	0.273 (0.077)
3	Site C WP	28.8 (0.5)	3.7 (0.6)	61.5	0.253 (0.072)	0.260 (0.074)
4	Site D WP	25.4 (0.7)	2.5 (0.6)	46.2	0.210 (0.071)	0.232 (0.076)
5	Site E WP	26.3 (0.5)	3.5 (0.7)	69.2	0.244 (0.068)	0.277 (0.077)
6	Site A JP	31.5 (1.1)	3.3 (0.6)	61.5	0.246 (0.070)	0.243 (0.071)
7	Site B JP	23.2 (0.5)	3.1 (0.5)	61.5	0.251 (0.068)	0.265 (0.074)
8	Site C JP	34.5 (0.8)	3.3 (0.6)	61.5	0.213 (0.055)	0.243 (0.069)
9	Site D JP	21.6 (0.3)	3.2 (0.7)	53.8	0.231 (0.067)	0.273 (0.079)
10	Site E JP	25.8 (0.7)	3.5 (0.6)	61.5	0.247 (0.065)	0.267 (0.072)

[1] Values in parentheses are standard errors.
* A locus is considered polymorphic if the frequency of the most common allele does not exceed 0.95.
** Unbiased estimate (See Nei, 1978).

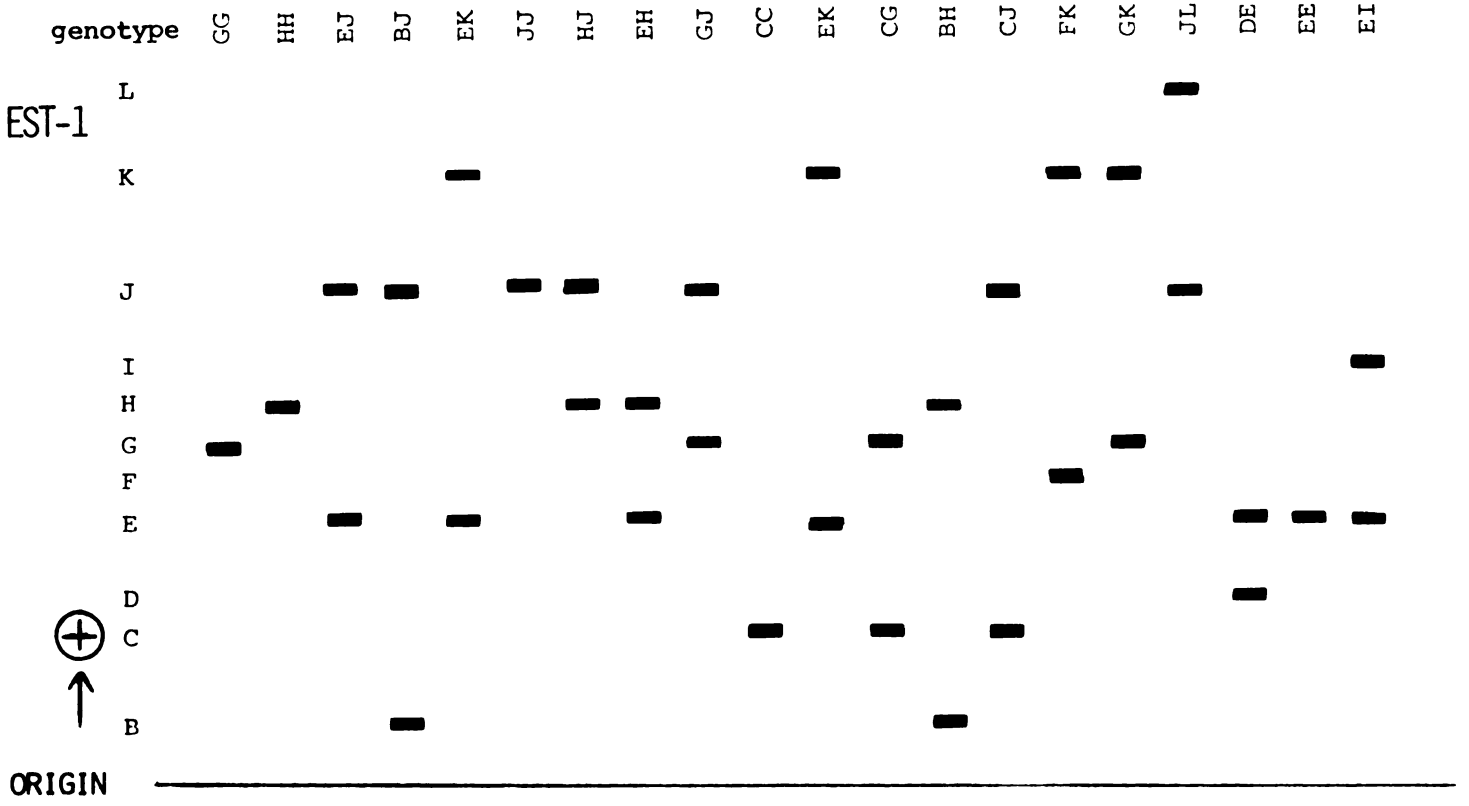

Figure 2. Allozyme banding patterns observed in P. strobi. Not all observed patterns are represented.

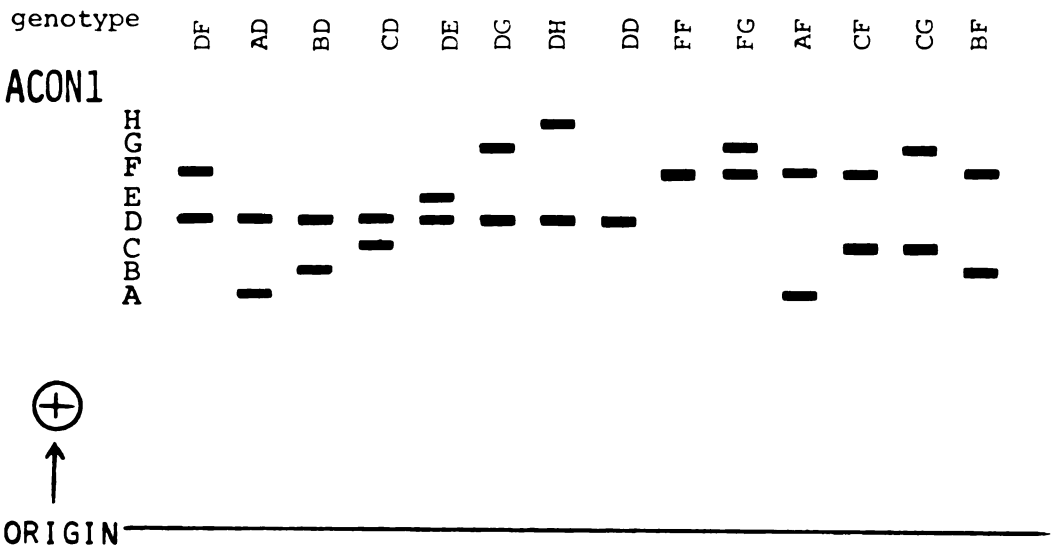

Figure 3. Allozyme banding patterns observed in P. strobi. Not all observed patterns are represented.

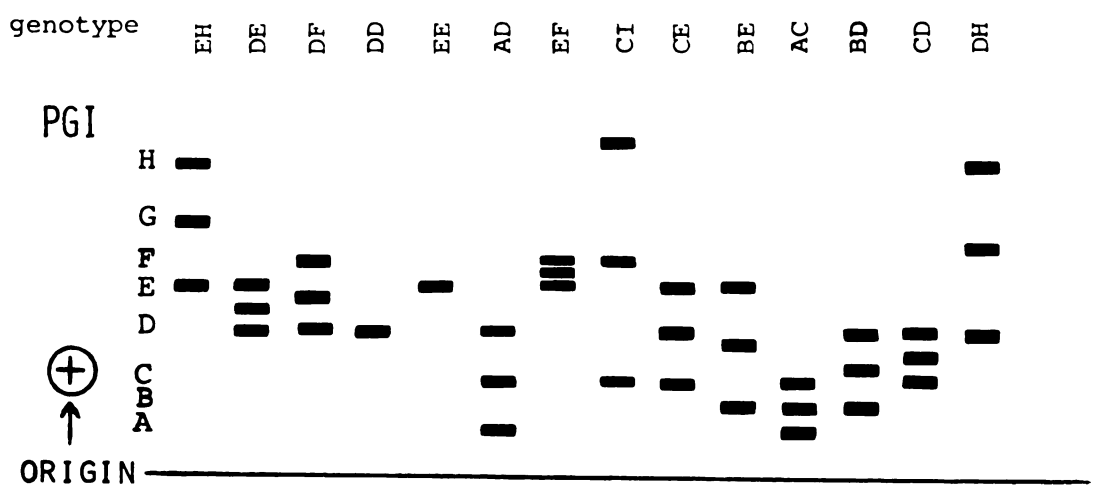

Figure 4. Allozyme banding patterns observed in P. strobi. Not all observed patterns are represented.

not conform to Hardy-Weinberg and were significant at P<0.01. EST-1 was significant at P<0.001 in 5 of the 10 populations. Only one other locus was significant at P<0.001 using Levene's test (i.e., population E WP for PGI). However, using exact probabilities (analogous to Fisher's (1930) exact test), only one population was significant at P<0.001 for the Chi-square test for Hardy-Weinberg conformance for any locus (i.e., population E JP for EST-1). Levene's test is suspect when expected frequencies of some classes are low (Swofford and Selander 1981) and exact probabilities may obscure real deviations from Hardy-Weinberg expectations because of the pooling involved in its calculation. To examine the significance of Hardy-Weinberg deviations, a test was conducted to determine if the populations conformed to the Whalund Principle (Crow and Kimura 1970). For this test, all populations were combined and treated as one large population. A Hardy-Weinberg equilibrium test was performed with some loci exhibiting an excess of homozygotes. No loci were found to differ significantly from expected values using exact probabilities (Table 6). The populations do not seem to exhibit a reduction in homozygosity expected for partially isolated isolates that were pooled into a single panmictic population.

Genetic diversity using genetic distance and similarity

Measurement of genetic diversity for conspecific populations was calculated by pairwise comparisons. Nei's (1978) estimates for unbiased genetic identity and distance were used to compare the mean number of allele differences at three levels of hierarchy described earlier. At the individual population level, demes (=subpopulations) were treated

Table 6. Significance Test For Whalund Effect.[1]

Locus	R1	R2	R3	P
ACON-1	89	128	64	0.186
ACON-2	264	17	0	1.000
MDH-1	275	19	1	0.308
MDH-2	285	5	0	1.000
IDH-1	268	28	1	0.537
IDH-2	289	8	0	1.000
ADH-1	268	16	0	1.000
PGI	92	148	56	0.906
AK	262	34	0	0.610
LAP	167	76	16	0.088
GOT	126	118	43	0.093
GAPDH	184	50	5	0.390

[1] Test using exact probabilities; (Swofford and Selander 1981)
R1 = Homozygotes for most common allele
R2 = Common/ rare heterozygotes
R3 = Rare homozygotes and other heterozygotes

as independent populations and genetic distances ranged between 0.000 and 0.007 with EST-1 included (Table 7). The same distances were between 0.000 and 0.004 when EST-1 was removed from the analysis (Table 8). The greatest distance occurred between populations within the same host. No pattern of divergence was evident from deme level genetic distances. These levels are very low and are well within those expressed for within population variation.

Genetic distances expressed for the hierarchial level of locality were also very low (Table 9) and ranged between 0.000 and 0.003. The greatest distance was found between the two adjacent populations on white pine and between the close population on jack pine and the outside populations on white pine. These results indicate that the variation between populations in this level are not significantly different. No direct relationship was found for any locality and genetic distance.

Estimates of genetic distance averaged by host exhibited no differences between the two hosts (Table 11). With each of five populations from each host being used in the average means, the within host variation was greater than between host distances. Host populations were essentially identical with the distance between the two hosts equal to 0.001, which was not significantly different from the within host distances of 0.001 for white pine and 0.000 for jack pine.

Single-loci genetic identities showed no significant patterns. Identities between populations for ACON-1 ranged from 0.931 to 1.000 with the least identity expressed between populations E WP and A JP (Table 13). LAP, GOT, GAPDH exhibited some differences in identity between populations with GOT differing only in populations collected

Table 7. Matrix Of Genetic Similarity And Distance Coefficients With EST-1 Included.[1]

	Population	1	2	3	4	5	6	7	8	9	10
1	Site A WP	*****	0.998	0.995	1.000	0.999	0.996	0.999	0.997	1.000	0.995
2	Site B WP	0.002	*****	0.993	0.997	1.000	0.998	1.000	0.997	1.000	0.996
3	Site C WP	0.005	0.007	*****	1.000	0.996	0.997	1.000	0.997	0.994	0.998
4	Site D WP	0.000	0.003	0.000	*****	0.999	0.999	1.000	1.000	0.996	0.999
5	Site E WP	0.001	0.000	0.004	0.001	*****	0.999	1.000	0.998	1.000	0.994
6	Site A JP	0.004	0.002	0.003	0.001	0.001	*****	1.000	1.000	1.000	0.995
7	Site B JP	0.001	0.000	0.000	0.000	0.000	0.000	*****	1.000	0.999	1.000
8	Site C JP	0.003	0.003	0.003	0.000	0.002	0.000	0.000	*****	0.996	0.999
9	Site D JP	0.000	0.000	0.006	0.004	0.000	0.000	0.001	0.004	*****	0.993
10	Site E JP	0.005	0.004	0.002	0.001	0.006	0.005	0.000	0.001	0.007	*****

[1] Below Diagonal: Nei's (1978) Unbiased Genetic Distance
Above Diagonal: Nei's (1978) Unbiased Genetic Identity

Table 8. Matrix Of Genetic Similarity And Distance Coefficients Without EST-1 Included.[1]

	Population	1	2	3	4	5	6	7	8	9	10
1	Site A WP	*****	0.998	0.997	1.000	0.999	0.996	0.999	0.997	1.000	0.999
2	Site B WP	0.002	*****	0.997	0.998	1.000	0.997	1.000	0.997	1.000	0.999
3	Site C WP	0.003	0.003	*****	1.000	1.000	1.000	1.000	0.999	1.000	0.999
4	Site D WP	0.000	0.002	0.000	*****	1.000	1.000	1.000	1.000	1.000	0.999
5	Site E WP	0.001	0.000	0.000	0.000	*****	0.998	1.000	0.999	1.000	0.999
6	Site A JP	0.004	0.003	0.000	0.000	0.002	*****	1.000	1.000	1.000	0.999
7	Site B JP	0.001	0.000	0.000	0.000	0.000	0.000	*****	1.000	1.000	1.000
8	Site C JP	0.003	0.003	0.001	0.000	0.001	0.000	0.000	*****	1.000	1.000
9	Site D JP	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	*****	1.000
10	Site E JP	0.001	0.001	0.001	0.001	0.001	0.001	0.000	0.000	0.000	*****

[1] Below Diagonal: Nei's (1978) Unbiased Genetic Distance
Above Diagonal: Nei's (1978) Unbiased Genetic Identity

Table 9. Matrix of Genetic Distance Coefficient Averaged By Locality.[1]

	Locality	No. Of Pops.	1	2	3	4	5	6
1	Adjacent White Pine	2	0.003 (0.003-0.003)					
2	Close White Pine	1	0.001 (0.000-0.002)	***** (*****-*****)				
3	Outside White Pine	2	0.001 (0.000-0.003)	0.000 (0.000-0.000)	0.001 (0.001-0.001)			
4	Adjacent Jack Pine	2	0.001 (0.000-0.003)	0.000 (0.000-0.000)	0.001 (0.000-0.003)	0.000 (0.000-0.000)		
5	Close Jack Pine	1	0.001 (0.000-0.003)	0.000 (0.000-0.000)	0.003 (0.002-0.003)	0.000 (0.000-0.000)	***** (*****-*****)	
6	Outside Jack Pine	2	0.000 (0.000-0.001)	0.000 (0.000-0.001)	0.000 (0.000-0.001)	0.000 (0.000-0.000)	0.000 (0.000-0.001)	0.000 (0.000-0.000)

***** Only one population included.

[1] Nei (1978) Unbiased Distance

(Values in parentheses represent the range of values expressed in the level examined)

Table 10. Matrix Of Genetic Similarity Coefficient Averaged By Locality.[1]

	Locality	No. Of Pops.	1	2	3	4	5	6
1	Adjacent White Pine	2	0.997 (0.997-0.997)					
2	Close White Pine	1	0.999 (0.998-1.000)	***** (*****-*****)				
3	Outside White Pine	2	0.999 (0.997-1.000)	1.000 (1.000-1.000)	0.999 (0.999-0.999)			
4	Adjacent Jack Pine	2	0.999 (0.997-1.000)	1.000 (1.000-1.000)	0.999 (0.997-1.000)	1.000 (1.000-1.000)		
5	Close Jack Pine	1	0.999 (0.997-1.000)	1.000 (1.000-1.000)	0.997 (0.996-0.998)	1.000 (1.000-1.000)	***** (*****-*****)	
6	Outside Jack Pine	2	1.000 (0.999-1.000)	1.000 (0.999-1.000)	1.000 (0.999-1.000)	1.000 (1.000-1.000)	1.000 (0.999-1.000)	1.000 (1.000-1.000)

***** Only one population included.

[1] Nei (1978) Unbiased Genetic Identity

(Values in parentheses represent the range of values expressed in the level examined)

Table 11. Matrix Of Genetic Distance Coefficient Averaged By Host.[1]

	Host	No. Of Pops.	1	2
1	White Pine	5	0.001 (0.000-0.003)	
2	Jack Pine	5	0.001 (0.000-0.004)	0.000 (0.000-0.001)

[1] Nei (1978) Unbiased Genetic Distance
(Values in parentheses represent the range of values expressed in the level examined)

Table 12. Matrix Of Genetic Similarity Coefficient Averaged By Host.[1]

	Host	No. Of Pops.	1	2
1	White Pine	5	0.999 (0.997-1.000)	
2	Jack Pine	5	0.999 (0.996-1.000)	1.000 (0.999-1.000)

[1] Nei (1978) Unbiased Genetic Identity
(Values in parentheses represent the range of values expressed in the level examined)

Table 13. Matrix Of Single-locus (ACON-1) Genetic Similarity Coefficient.[1]

	Population	1	2	3	4	5	6	7	8	9	10
1	Site A WP	*****									
2	Site B WP	0.995	*****								
3	Site C WP	0.994	0.994	*****							
4	Site D WP	1.000	0.991	1.000	*****						
5	Site E WP	0.965	1.000	0.994	0.989	*****					
6	Site A JP	0.990	0.952	1.000	1.000	0.931	*****				
7	Site B JP	1.000	1.000	1.000	1.000	1.000	0.999	*****			
8	Site C JP	1.000	0.975	1.000	1.000	0.965	1.000	1.000	*****		
9	Site D JP	1.000	0.978	1.000	1.000	0.963	1.000	1.000	1.000	*****	
10	Site E JP	1.000	0.969	1.000	1.000	0.972	1.000	1.000	1.000	1.000	*****

[1] Nei's (1978) Unbiased Genetic Identity

Table 14. Matrix Of Single-locus (LAP) Genetic Similarity Coefficient.[1]

	Population	1	2	3	4	5	6	7	8	9	10
1	Site A WP	*****									
2	Site B WP	0.985	*****								
3	Site C WP	0.986	1.000	*****							
4	Site D WP	1.000	1.000	1.000	*****						
5	Site E WP	0.997	1.000	1.000	1.000	*****					
6	Site A JP	0.976	1.000	1.000	1.000	1.000	*****				
7	Site B JP	0.987	1.000	1.000	1.000	1.000	1.000	*****			
8	Site C JP	0.979	0.994	1.000	0.999	1.000	1.000	0.997	*****		
9	Site D JP	1.000	0.990	0.998	1.000	1.000	0.989	0.995	0.997	*****	
10	Site E JP	0.995	0.990	1.000	1.000	1.000	0.996	0.994	1.000	1.000	*****

[1] Nei's (1978) Unbiased Genetic Identity

from jack pine (Table 15). All other loci displayed no differences in identity between populations. None of the loci were significantly different in respect to host or locality.

Wright's fixation indices

Wright's (1965, 1978) fixation indices were used to assess the levels of fixation between the populations. These indices are defined in terms of expected and observed heterozygosities (Nei 1977). The formula

$$1 - F_{IT} = (1 - F_{IS})(1 - F_{ST}) \qquad (1)$$

provides the basis for this analysis. F_{IS} is the fixation index for individuals relative to the subpopulation. F_{IT} represents the index of fixation for individuals relative to the total population. F_{IS} and F_{IT} measure the deviations of genotype frequencies from Hardy-Weinberg proportions in the subpopulations and in the total population. F_{ST} provides a measure of the amount of differentiation among subpopulations relative to the limiting amount under complete fixation (Nei 1973).

Fixation indices for each locus were calculated separately. $F_{IS(IK)}$ values for all loci were used to examine fixation of individual alleles within each subpopulation (i.e., F_{IS} for the K-th allele in the I-th subpopulation). Using chi-square analysis (Li 1955), it was found that most of the $F_{IS(IK)}$ values were insignificant in all subpopulations for all alleles. The prime exception to this occurred in ACON-1 (Table 16), where two alleles were significantly different from $F_{IS} = 0$ at $P<0.001$. Allele "G" was significant in two subpopulations,

Table 15. Matrix Of Single-locus (GOT) Genetic Similarity Coefficient.[1]

	Population	1	2	3	4	5	6	7	8	9	10
1	Site A WP	*****									
2	Site B WP	1.000	*****								
3	Site C WP	1.000	1.000	*****							
4	Site D WP	1.000	1.000	1.000	*****						
5	Site E WP	1.000	1.000	1.000	1.000	*****					
6	Site A JP	1.000	1.000	1.000	1.000	1.000	*****				
7	Site B JP	1.000	1.000	0.993	1.000	1.000	1.000	*****			
8	Site C JP	0.999	1.000	0.983	1.000	1.000	0.996	1.000	*****		
9	Site D JP	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.994	*****	
10	Site E JP	0.998	1.000	0.979	1.000	1.000	0.994	1.000	1.000	0.992	*****

[1] Nei's (1978) Unbiased Genetic Identity

Table 16. $F_{IS(IK)}$ Values For ACON-1 In All 10 Populations Of *P. strobi*.[1]

	Subpopulation									
Allele	A WP	B WP	C WP	D WP	E WP	A JP	B JP	C JP	D JP	E JP
A	---	-0.057	---	---	-0.038	-0.016	---	---	---	---
B	-0.029	0.184	-0.053	-0.040	-0.080	-0.103	-0.045	-0.069	-0.128	-0.020
C	0.111	-0.018	-0.017	-0.061	-0.038	-0.032	-0.022	-0.051	-0.023	-0.020
D	0.000	0.125	-0.029	-0.169	0.229	0.118	-0.062	0.184	-0.019	0.121
E	-0.059	---	-0.034	---	-0.038	-0.016	---	-0.016	-0.048	-0.061
F	-0.064	0.018	-0.067	-0.128	0.667*	-0.208	-0.179	0.077	0.083	-0.083
G	-0.091	0.648*	-0.017	-0.106	-0.059	-0.032	-0.095	-0.033	-0.048	0.646*
H	-0.014	---	-0.017	---	-0.038	---	---	---	-0.023	-0.020
Mean	-0.013	0.122	-0.043	-0.130	0.283	-0.025	-0.104	0.087	-0.017	0.077

[1] Chi-square analysis based on the formula; $\chi^2 = F_{IS(IK)}{}^2 \cdot N$: * significant at $P<0.001$.

B WP and E JP, suggesting an excess of this allele in these subpopulations. The only other case of an allele that was significant at $P<0.001$ was the "F" allele of ACON-1 in subpopulation E WP (Table 16). Again the suggestion from chi-square test was that there was an excess of this allele in that population. Other alleles that were significant at $P<0.025$ included: alleles "A" and "B" for IDH-1 in E JP; allele "B" for LAP in C WP and E WP; allele "D" for LAP and GOT in D JP.

Weighted averages (across subpopulations) for F_{ISK}, F_{ITK}, and F_{STK} were calculated using the formulas of Wright (1965, 1978) and Nei (1977). Although these averages varied considerably for alleles in different subpopulations, all excesses or deficiencies were insignificant at $P<0.01$. Extreme values for F_{ISK} and F_{ITK} occurred in LAP, with both the "B" allele and "C" allele expressing a level of excess. With a F_{ISK} of 0.104 for both the "B" and "C" alleles, these values were significant only at $P<0.10$. However, F_{ITK} values for these alleles were both significant at $P<0.05$, indicating that these alleles occurred in excess to the expected Hardy-Weinberg proportions at that level. GOT also had an allele that was significant at $P<0.05$ for F_{ITK} (allele "D"). No value of F_{STK} was significant for any allele in any subpopulation, indicating very little differentiation between subpopulations.

F_{IS}, F_{IT}, and F_{ST} values (weighted averages of F_{ISK}, F_{ITK}, and F_{STK} across alleles) were found to be insignificant for all loci (Table 17). The greatest values for these were again found in LAP and GOT (i.e., 0.072 for F_{IS} and 0.099 for F_{IT} in LAP; 0.087 for F_{IS} and 0.095 for F_{IT} in GOT). Again, these values indicate that these alleles

Table 17. Summary Of F-statistics At All Loci.

Locus	F(IS)	F(IT)	F(ST)
ACON-1	0.030	0.049	0.020
ACON-2	-0.036	-0.029	0.007
MDH-1	0.001	0.027	0.027
MDH-2	-0.021	-0.008	0.013
IDH-1	0.022	0.033	0.012
IDH-2	-0.022	-0.011	0.011
ADH-1	-0.040	-0.019	0.020
PGI	-0.029	-0.019	0.009
AK	-0.063	-0.040	0.022
LAP	0.072	0.099	0.028
GOT	0.087	0.095	0.010
GAPDH	-0.001	0.033	0.034
Mean	0.022	0.040	0.018

Table 18. Summary Of F-statistics At All Loci For Combined Hosts.

Locus	F(IS)	F(IT)	F(ST)
ACON-1	0.043	0.049	0.006
ACON-2	-0.029	-0.028	0.002
MDH-1	0.008	0.024	0.016
MDH-2	-0.009	-0.009	0.000
IDH-1	0.021	0.024	0.002
IDH-2	-0.011	-0.011	0.000
ADH-1	-0.023	-0.019	0.003
PGI	-0.023	-0.022	0.000
AK	-0.044	-0.041	0.003
LAP	0.078	0.083	0.005
GOT	0.081	0.082	0.002
GAPDH	0.045	0.045	0.000
Mean	0.032	0.035	0.003

deviate from Hardy-Weinberg proportions by some amount. However, none of these values were significant even at $P<0.10$.

To test the significance of allele excesses and deficiencies across hosts, subpopulations within each host were combined and the F-statistics presented above were recalculated. Values for $F_{IS(IK)}$ varied from -0.084 to 0.170 in alleles for LAP in white pine. This was the greatest variance expressed for any alleles in either white pine or jack pine. Values for F_{ISK} and F_{ITK} differed considerably, with some alleles more variable than others. F_{STK} values were very low for most alleles, below 0.007, with the greatest value found for the "D" allele in MDH-1, 0.024. Means of F_{ISK} , F_{ITK} , and F_{STK} values were not significant using Li's (1955) chi-square test for significance. This result indicates that the variance within each host and between hosts was very low.

Hierarchial and heterogeneity analysis

A hierarchial analysis of population differentiation was computed using Wright's (1978) formulation. Variance components and F-statistics were calculated for each level of the hierarchy, used for sample collection, relative to other levels. Individual loci values for variance components were highly variable. Values for each allele were also very variable but no direct relationship could be made between any pairs of levels and individual loci or alleles. The greatest variance component across all loci was expressed for the demes relative to the locality (Table 19). Of the six pairs of levels, the three comparing demes to other levels had variance components at least 3 times greater than any

Table 19. Variance Components And F-statistics Combined Across Loci.

Comparison		Variance	F_{XY}
X	Y	Component	
Demes	- Locality	0.01294	0.005
Demes	- Host	0.01126	0.004
Demes	- Total	0.00942	0.004
Locality	- Host	-0.00168	-0.001
Locality	- Total	-0.00352	-0.001
Host	- Total	-0.00184	-0.001

Table 20. Contingency Chi-square Analysis At All Loci.

Locus	No. Of Alleles	Chi-square	D.F.	P
ACON-1	8	79.458	63	0.079
ACON-2	4	17.196	27	0.926
MDH-1	6	49.954	45	0.283
MDH-2	2	7.343	9	0.601
IDH-1	3	20.353	18	0.313
IDH-2	4	27.043	27	0.461
ADH-1	5	35.884	36	0.474
PGI	9	63.863	72	0.742
AK	5	52.503	36	0.037
LAP	5	48.204	36	0.084
GOT	6	30.031	45	0.958
GAPDH	2	16.652	9	0.054
(TOTALS)		448.483	423	0.189

other comparisons, indicating that the greatest variance is related to the demes. However, values of F_{XY} were extremely low and none of the pairwise comparisons were significant.

A chi-square test for a M x N contingency table with (M-1)(N-1) degrees of freedom, where M is the number of populations and N the number of alleles, was employed to test heterogeneity. This analysis revealed that none of the loci differed significantly from expected values and that heterogeneity among populations was very low (Table 20). This test was also employed for the second level of the hierarchy and results indicated that heterogeneity between adjacent populations (Tables 21 and 22) and between outside populations (Tables 23 and 24) are extremely similar for both hosts. Neither of the locality levels were significantly different from expected values using Hardy-Weinberg equilibrium.

Table 21. Hierarchy Contingency Chi-square Analysis At All Loci.[1]

Locus	No. Of Alleles	Chi-square	D.F.	P
ACON-1	8	10.554	7	0.159
ACON-2	2	0.005	1	0.942
MDH-1	4	1.873	3	0.599
MDH-2	2	0.974	1	0.324
IDH-1	2	0.342	1	0.559
IDH-2	2	0.342	1	0.559
ADH-1	3	2.672	2	0.263
PGI	8	7.692	7	0.366
AK	3	1.202	2	0.548
LAP	4	2.875	3	0.411
GOT	4	2.881	3	0.410
GAPDH	2	8.457	1	0.004
(TOTALS)		39.810	32	0.161

[1] Locality: Adjacent; Host: White Pine

Table 22. Hierarchy Contingency Chi-square Analysis At All Loci.[1]

Locus	No. Of Alleles	Chi-square	D.F.	P
ACON-1	6	3.323	5	0.650
ACON-2	2	2.922	1	0.087
MDH-1	2	0.681	1	0.409
MDH-2	2	1.554	1	0.212
IDH-1	2	0.020	1	0.888
IDH-2	2	0.079	1	0.778
ADH-1	3	2.831	2	0.243
PGI	4	4.617	3	0.202
AK	4	2.418	3	0.490
LAP	5	6.354	4	0.174
GOT	5	0.782	4	0.941
GAPDH	2	0.280	1	0.597
(TOTALS)		25.862	27	0.526

[1] Locality: Adjacent; Host: Jack Pine

Table 23. Hierarchy Contingency Chi-square Analysis At All Loci.[1]

Locus	No. Of Alleles	Chi-square	D.F.	P
ACON-1	7	3.870	6	0.694
ACON-2	2	0.167	1	0.682
MDH-1	2	0.823	1	0.364
IDH-1	2	1.320	1	0.250
IDH-2	2	0.823	1	0.364
ADH-1	5	2.226	4	0.694
PGI	6	2.603	5	0.761
AK	2	2.506	1	0.113
LAP	4	2.361	3	0.501
GOT	5	4.621	4	0.328
GAPDH	2	0.042	1	0.838
(TOTALS)		21.362	28	0.810

[1] Locality: Outside; Host: Jack Pine

Table 24. Hierarchy Contingency Chi-square Analysis At All Loci.[1]

Locus	No. Of Alleles	Chi-square	D.F.	P
ACON-1	8	11.226	7	0.129
ACON-2	4	1.594	3	0.661
MDH-1	5	6.496	4	0.165
MDH-2	2	1.443	1	0.230
IDH-1	3	2.947	2	0.229
IDH-2	2	1.381	1	0.240
ADH-1	3	1.483	2	0.476
PGI	7	5.514	6	0.480
AK	4	1.444	3	0.695
LAP	4	3.943	3	0.268
GOT	5	2.417	4	0.659
GAPDH	2	1.991	1	0.158
(TOTALS)		41.880	37	0.267

[1] Locality: Outside; Host: White Pine

Discussion

Genetic variability within and between the 10 populations appears to extremely low using Nei's (1978) unbiased estimates. In the context of this study, identity between all populations was notably high. Even when the 5 populations from each host were combined, genetic identity was essentially the same as when populations were examined separately. From this analysis, it appears that these populations represent only a fraction of a panmictic population. At no sample hierarchy level was there any significant differences between populations or levels, with locality and host levels expressing indistinguishable identities. The only analysis, using Nei's estimates, that indicated differences was the single-locus analysis with loci exhibiting varying degrees of identity between populations. This may well indicate that genetic drift may be influencing the results for this analysis. Random genetic drift occurring independently in subpopulations can lead to genetic differentiation between subpopulations (Falconer, 1981). However, it seems that this drift may not manifest itself when allele frequency differences are averaged, as in Nei's (1978) estimates. It must be noted that other factors may be influencing these differences (i.e., selection, inbreeding, and effective population size).

Additional evidence for subpopulation differentiation is found when examining Wright's (1978) F-statistics. Levels of differentiation between subpopulations is extremely low. However, the levels

found indicate that subpopulations do differ from expected for a panmictic population (i.e., F_{ST} does not equal 0). More important than the between subpopulation variation is the within subpopulation variation. F_{IS} and F_{IT} values are significant in a few subpopulations. These values measure the deviation from expected Hardy-Weinberg. Deviations expressed indicate that within these subpopulations allele frequencies cannot be explained fully by expected Hardy-Weinberg equilibria. Something is causing either an excess or a deficiency of alleles within these subpopulations. Genetic drift, natural selection, inbreeding, or some other factor or any combination may be causing the subpopulations to express differences in allele frequencies.

The differences in F-statistics do not seem to be correlated to any level of the sampling hierarchy. This indicates that the genetic differences within subpopulations seem to be occurring randomly and are not related to host species association. The prime support for this comes from the hierarchy analysis of Wright (1978). Subpopulations (=Demes) seem to carry most of the variance with them. Variance components (when the deme level is included) are 3 times greater than any other combinations which lends support to the concept that populations once believed to be panmictic are really composed of small demes (Selander and Kaufman, 1973; Nei, 1975; Wright, 1978).

This substructuring of populations has become an important feature in population genetic theory (Phillips, 1985). Many recent studies have set out to examine the levels of substructuring in many different species (Bush et al., 1977- Rhagoletis; Nei and Imaizumi, 1965- Humans). This type of substructuring can lead to reduced gene flow between demes (Nei,

1975) which increases the possibility of genetic divergence (Diehl and Bush, 1984). It can also lead to inbreeding within demes, especially if the effective population size of demes was low (Falconer, 1981). If gene flow was greatly reduced and effective population size was low then substantial genetic drift could accumulate (i.e., founder effect). Substructuring that resulted in host race formation could also be very important (Bush, 1969) and demes that are host specific may become reproductively isolated from demes on other hosts. Accumulation of genetic diversity could be enhanced by genetic variation that effectively isolates subpopulations on the basis of hosts. Host selection based on chemical cues specific for the parent population may be altered by new genes that result from mutations. If gene flow is reduced and inbreeding is common, the new host selection genes may become common in the new demes (Bush, 1969). Substructuring of populations may be one of the first steps in the formation of races and species in sympatric populations.

If a population is composed of demes, it may be easy to imagine the fate of these demes if various factors influence genetic diversity between them. A reduction in gene flow could and probably would result in genetic drift between the demes. The same result would occur if the dispersal rate of the species was low (Phillips, 1985). This would in effect be similar to ecological isolation and might be regarded as allopatric isolation based on a lack of interbreeding. Within the demes, inbreeding could lead to reproductive isolation if gene flow between demes was greatly restricted. Thus, demic differentiation may result from any number of isolating mechanisms. Random drift within a deme,

inbreeding, assortative mating based on deme structure, low levels of dispersal may be the processes by which a deme may diverge from other demes.

In the current study, the demic differentiation was moderate. The hierarchy examined presented one was of trying to get a handle on the factors influencing differentiation. Demic differences could not be correlated with the hosts examined. However, it must be noted that the chi-square test utilized in this study has been the subject of some debate. Ward and Sing (1970) have stated that it may be necessary to examine large numbers of individuals ($>10^3$) in order to detect significant levels of differences from F-statistics. Small sample sizes and resulting sampling errors in this study may have influenced the results. Although some of the F-statistics were significant using this analysis, results do not provide adequate information about the causes of deviations from Hardy-Weinberg. Significant chi-square values obtained are such that it would be illogical to attribute the deviations from Hardy-Weinberg to inbreeding. Demic differentiation may be lower than would be predicted from the F-statistics. Despite high variation within demes and deviations from Hardy-Weinberg, F_{ST} values indicate that between deme differentiation is low. Values for all F-statistics were even lower when all host specific demes were combined, implying that genetic variation within and between demes is extremely low and not related to host specificity. It would seem that this group of populations is acting as one panmictic polyphagus species.

The importance of these results to managers of forests or private plantations may be limited. Controlling the weevil on one of the hosts

may not be as effective if the other host in in close proximity. Measures currently employed to control the weevil on eastern white pine may be subverted by high populations of weevils on nearby jack pine plantations. However, the greatest importance of this study may well be to managers trying to find resistant varieties. Trying to find varieties resistant to weevil infestation may be a very difficult endeavor. This insect would seem to be able to infest most species of pines with a degree of consistancy. Varieties of host species may prove ineffective in providing any measure of resistance to this type of insect. This is not to say that current programs utilizing host resistance are not worth continuing. However, the hope of finding varieties that provide control may be low. Pines such as red pine, Pinus resinosa Ait., do show resistance to weevil infestation and a thorough understanding of why they do may lead to varieties of other species that show resistance. Managers may want to be cautious about planting species of trees in close proximity to one another and to undertake programs for resistance with similar caution.

APPENDICES

APPENDIX 1

Record of Deposition of Voucher Specimens*

The specimens listed on the following sheet(s) have been deposited in the named museum(s) as samples of those species or other taxa which were used in this research. Voucher recognition labels bearing the Voucher No. have been attached or included in fluid-preserved specimens.

Voucher No.: 1985-2

Title of thesis or dissertation (or other research projects):

GENETIC VARIATION WITHIN AND
BETWEEN SYMPATRIC POPULATIONS OF
PISSODES STROBI ON TWO HOST SPECIES
EASTERN WHITE PINE AND JACK PINE

Museum(s) where deposited and abbreviations for table on following sheets:

Entomology Museum, Michigan State University (MSU)

Other Museums:

Investigator's Name (s) (typed)

Charley Adrian Chilcote

Date 11 Nov. 1985

*Reference: Yoshimoto, C. M. 1978. Voucher Specimens for Entomology in North America. Bull. Entomol. Soc. Amer. 24:141-42.

Deposit as follows:

Original: Include as Appendix 1 in ribbon copy of thesis or dissertation.
Copies: Included as Appendix 1 in copies of thesis or dissertation.
Museum(s) files.
Research project files.

This form is available from and the Voucher No. is assigned by the Curator, Michigan State University Entomology Museum.

APPENDIX 1.1

Voucher Specimen Data

Page 1 of 2 Pages

Species or other taxon	Label data for specimens collected or used and deposited	Number of: Eggs	Larvae	Nymphs	Pupae	Adults ♀	Adults ♂	Other	Museum where deposited
Pissodes strobi Peck	MI: Wexford Co.; T22N,R 9W,S.17 Site A White Pine		20		10	5	5		M.S.U.
Pissodes strobi Peck	MI: Manistee Co.; T21N,R13W,S.2 Site B White Pine		20		10	5	5		M.S.U.
Pissodes strobi Peck	MI: Manistee Co.; T21N,R13W,S.3 Site C White Pine		20		10	5	5		M.S.U.
Pissodes strobi Peck	MI: Manistee Co.; T22N,R13W,S.26 Site D White Pine		20		10	5	5		M.S.U.
Pissodes strobi Peck	MI: Manistee Co.; T24N,R14W,S.22 Site E White Pine		20		10	5	5		M.S.U.
Pissodes strobi Peck	MI: Wexford Co.; T22N,R11W,S.35 Site A Jack Pine		20		10	5	5		M.S.U.
Pissodes strobi Peck	MI: Wexford Co.; T21N,R11W,S.2 Site B Jack Pine		20		10	5	5		M.S.U.

(Use additional sheets if necessary)

Investigator's Name(s) (typed)

Charley Adrian Chilcote

Date 11 Nov. 1985

Voucher No. 1985-2

Received the above listed specimens for deposit in the Michigan State University Entomology Museum.

Curator

13 Nov-1985

Date

APPENDIX 1.1

Voucher Specimen Data

Page 2 of 2 Pages

Species or other taxon	Label data for specimens collected or used and deposited	Number of: Eggs	Larvae	Nymphs	Pupae	Adults ♀	Adults ♂	Other	Museum where deposited
Pissodes strobi Peck	MI: Wexford Co.; T21N,R11W,S.2 Site C Jack Pine		20		10	5	5		M.S.U.
Pissodes strobi Peck	MI: Wexford Co.; T21N,R10W,S.33 Site D Jack Pine		20		10	5	5		M.S.U.
Pissodes strobi Peck	MI: Wexford Co.; T21N,R10W,S.27 Site E Jack Pine		20		10	5	5		M.S.U.

(Use additional sheets if necessary)

Investigator's Name(s) (typed)

Charley Adrian Chilcote

Date 11 Nov. 1985

Voucher No. 1985-2

Received the above listed specimens for deposit in the Michigan State University Entomology Museum.

Curator

13 Nov. 1985

Date

Appendix 2

White Pine Weevil

The white pine weevil, Pissodes strobi Peck, belongs to the family Curculionidae (Coleoptera). This family reportedly contains more species than any other in the animal Kingdom. The family consists of some 60 subfamilies, which differ greatly in their biology and habits. Most plants are colonized by at least one species of this family and their damage to plants often cause extensive economic loss if not controlled (e.g., boll weevil). Damage can be inflicted to the fruit, nuts, stems, buds, and other tissue of the host plants as well as on forest, shade, ornamental trees and seedlings in natural stands, small ornamental plantings, and plantations. Adult weevil usually drill holes into the structure by feeding from the outside of the host and larvae usually live within the same host tissue.

The white pine weevil is an elongate, brownish insect about 4 to 6 mm. long. It is easily identified by irregular groups of brown and white scales that are found on the body and elytra. Their eggs are pearly white, usually about 1 mm. long and normally found in groups of two or three. Larvae are yellowish-white, legless scarabaeiform grubs, and are slightly longer than the adults. The pupae are creamy-white, exarate pupae and are about as long as the adults.

From March to May, adult weevils emerge from overwintering sites, in the duff beneath the host trees. Upon emergence, they make their way to the succulent new growth of their host. It is here that they feed until ready to copulate. Normally only a few days of feeding occur

before the male and female copulate. Feeding generally is confined to the area within 15 to 20 cm. of the dormant bud. During feeding and copulation, adults are gregarious and several may be found on the same leader.

Eggs are deposited in tiny cavities in the bark which are created by the female chewing out the inner bark with her curved snout. This produces a puncture in the outer bark with a larger chamber beneath. Upon completion of the cavity, one to three eggs are deposited within the chamber. This process of oviposition is normally confined to areas where the female has fed, but it is not uncommon for the female to oviposit on several trees.

Larvae eclose in about 7 to 10 days and begin to feed on the phloem and cambium in a random fashion. As more larvae emerge, they begin to orient themselves downward. Eventually the number of larvae is great enough that they arrange themselves in a compact feeding ring around the stem of the leader. This ring increases the chance of individuals surviving as feeding in a concentrated ring effectively stops resin flow that might drown individual larvae. However, the feeding ring leaves no nutrient behind, and individuals not at the forefront of the ring starve. As larvae mature, they fall behind the ring, bore into the wood, and use the wood chips produced to cover their chamber. The length of the larval period can vary greatly, depending on environmental factors, but usually lasts from 4 to 6 weeks.

Pupation occurs within the excelsior covered chamber with the pupal stage usually lasting approximately 2 to 3 weeks. Mature pupae change into pharate adults which remain within the pupal chamber for about 10

days. Mature adults emerge from the damaged leader in late July to mid-September, and sometimes as late as early October whereupon they feed on the succulent growth of the host. As winter approaches adults migrate down the trees into the duff where overwintering occurs in the pine litter below the crown dripline. Most overwintering sites are located at the interface level, an area that lies below the dry pine needles and above the moist organic debris zone.

Weevils go through only one generation per year as adults normally die shortly after mating and oviposition have occurred. Occasionally a weevil may live for several years, but this is not frequent (McMullen and Condrashoff, 1973).

Appendix 3

Gel Electrophoresis

Gel preparation

The preparation of starch gels follows those used by Howard (1982). An 11.75% horizontal starch gel was prepared using 47 grams of Electrostarch (The Electrostarch Co., Madison, Wisconsin), lot #392, in approximately 400 ml. of the appropriate gel buffer (Table 25). Buffer systems used were the ones found to give good results in the initial enzyme survey. In System 3, it was necessary to add an extra 10-15 ml. of buffer to the original amount to reduce the incidence of splitting at the origin which resulted from the gel contracting during the run. Extra buffer made the gel 2-3 mm. higher than the mold which allowed the ice container to press down directly onto the gel, keeping it together during the run.

Starch was placed into a 1000 ml. Ehrlenmyer flask and the contents swirlled until all large clumps of starch were dissolved. This prevented clumps from forming in the gel that could disrupt migration of the enzymes. The flask was then heated over a standard bunsen burner and vigorously swirlled until a noticeable phase change occurred. This change was recognized by the transformation of the milky white suspension of the starch to a clearer, more viscous, gelatinous fluid. For seven seconds after the phase change, the flask remained over the flame and swirlling continued. Because this mixture was boiling during the time after the phase change, many small air bubbles became trapped in the solution which could interfere with the migration of the enzymes

Table 25. Recipes For Buffer Systems Used In Electrophoretic Studies Of *P. strobi*.

System[1]	Electrode Buffer[2]	Gel Buffer[2]
3	0.30 M Borate, 18.55 g/l Boric Acid 2.40 g/l Sodium Hydroxide pH about 8.2 (not adjusted)	0.076 M Tris, 0.005 M Citric Acid, 9.21 g/l Tris 1.05 g/l Monohydrate Citric Acid pH about 8.7 (not adjusted)
5	0.687 M Tris, 0.157 M Citric Acid, 83.2 g/l Tris 30.0 g/l Monohydrate Citric Acid pH about 8.0 (not adjusted)	0.023 M Tris, 0.005 M Citric Acid, 2.77 g/l Tris 1.10 g/l Monohydrate Citric Acid pH about 8.0 (not adjusted)
6	0.50 M Tris, 0.02 M EDTA, 0.65 M Boric Acid, 60.6 g/l Tris 6.0 g/l Disodium salt of EDTA 40.0 g/l Boric Acid pH about 8.0 (not adjusted)	1:9 dilution of Electrode Buffer to Deionized Water
7	18.78 g/l Monobasic Potassium Phosphate 2.48 g/l NaOH pH about 6.7 (not adjusted)	1:9 dilution of Electrode Buffer to Deionized Water

[1] Systems used by Howard (1982).

[2] All buffers are prepared with double deionized water.

and thus had to be removed. After the gel was removed from the flame, a vacuum was applied to the flask to remove the unwanted air bubbles. Vacuuming caused the gel to boil vigorously as most of the air trapped in the suspension escaped. This procedure was continued until large, regular bubbles rose from the suspension (i.e., about 1½-2½ min.; depending on the buffer used). The aspirated starch was then poured unformly and quickly into the mold in order to prevent premature setting. After all the gel had been poured, the mold was shaken gently side to side to smooth the surface and to insure all corners were filled. This also helped mend any cracks in the gel formed during pouring. Air bubbles and debris that might have been missed or introduced during aspiration or pouring were removed using a pasteur pipet. Only large bubbles and debris were removed, because care had to be taken not to produce holes in the gel that set up before they could be filled. After debris had been removed, the mold was once again shaken gently to fill in holes and smooth the gel surface then left to cool for about 20 minutes. After cooling, it was covered with Saran Wrap and placed in a cold room (about 4 degrees C.) until used, usually not less than 4 hours and not more than 48 hours after preparation.

Sample preparation

On the day of a gel run, individual adult and larval weevils had to be prepared. Vials containing samples of either larvae or adults were removed from the -80 degree C. storage and placed on crushed ice. One individual from each vial was transferred to spot plates which were placed on crushed ice to keep the enzymes from degrading.

Four individuals from each of the five populations under study were placed in individual spots on the plates. In addition, three individuals from the reference population (population A WP) were placed in the first, the tenth, and the twenty-third positions on the plates. These individuals were used to make sure that all gels were scored the same. After all individuals were transferred to spot plates, one drop of grinding buffer (0.05 M tris/HCl pH 7.0) was added to each spot with a pasteur pipet. Each weevil was then ground using a clean tissue grinding rod to insure that enzymes were not transferred between spots. Wicks of filter paper (2 by 9 mm.) were then added to each spot, the number of wicks varying, depending on the number of gels to be run. If two different systems of buffers were being used for different enzymes, two wicks were added to each spot. The wicks soaked up the homogenate containing the enzymes. In a few cases, it was necessary to retain some of the homogenate for future study. In which case, capillary tubes were used to collect a small amount of the fluid and the tubes held in a test tube submerged in a cold ice bath until all were collected. They were then transferred to an ultra-cold freezer for future use. When needed the tubes were removed, allowed to thaw, and the fluid emptied directly onto wicks placed in spot plates.

When all individuals were ground and wicks prepared, gels were cut near one edge. The cut was made using a scalple along a plastic guide, through the gel gel to the bottom of the mold. The cut (from here on termed the origin) was used to place wicks into the gel in preparation for electrophoresis. Wicks were removed from spots on the plates individually with care taken not to confuse individual wicks (i.e.,

each individual was assigned a particular position on the gel; individual #1 had to be placed into position #1 on each and every gel used). Each individual weevil provided sufficient homogenate to test for many enzymes. The fluid wetted enough wicks to be used on several gels. Each gel provided a test for up to four enzymes and each individual provided enough wicks for up to seven gels. When all wicks were placed into the gel, it was ready to be run.

Gel running

Running the gel was performed in a cold room at 4 degrees C.. Electrode trays were set up so that all electric leads led out of the cold room to power units. Electrode buffers, listed in Table 25, were placed in the appropriate trays. About 160 ml. of buffer were placed into each side of the tray. The amount of buffer was sometimes increased because fluid levels were lower than desired. Buffer levels were occasionally checked during long runs to insure that capillary action did not reduce the level to the point where electric contact was reduced. When ready to start a gel, protective mold feet were removed to allow good electric contact. Gel molds containing the gel with wicks in place were placed into the electrode trays. Electrodes were placed into trays with the red (+) electrode placed at the end of the gel away from the origin. Electrodes consisted of a small piece of platanum wire stretched on plastic legs and connected to a lead terminal. After the gels were in place and electrodes connected, they were ready to run. Run voltages and times depended on the buffers used and were those used by Howard (1982). Run times were increased in those cases

where bands were too close together to determine heterozygotes. An ice box was placed on each gel when it was determined that the gel was running properly (i.e., the power unit displayed a potential that was right for the system used). The ice box was filled with crushed ice and used to cool the gel during the run and in the cases described earlier, to press on the gel to keep the origin from separating. Periodic checks during the run were made to insure good electrical contact, proper electrode buffer level, and sufficient ice. All through the run, Saran Wrap was kept between the gel and ice box which reduced moisture loss from gels and prevented the gels from degrading. Thus, enzymes were not denatured or their movement though the gel impaired.

After the run was completed, gels were removed from electrode trays and sliced at the edge of each of the feet to remove them. A notch was cut at the origin on the left-hand side to provide a reference point when gel slices were examined later. Gels were then removed from the mold and placed on a slicing board. A slicer was then used to take four slices (about 2 mm. thick) from each gel. The front slice (wide slice) and back slice (narrow slice) were both cut and placed into individual stain boxes. In most cases only the front slice contained enzymes used in the study.

Staining

Stains used in this study were modified from those of Howard (1982). Table 26 gives the recipes and special instructions used to give good results. After slices were made, the proper stains for the systems used were added to stain boxes. Stain boxes were either placed into an

Table 26. Stain Recipes And Special Instructions For Electrophoretic Analysis Of P. strobi.[1]

Enzyme	Buffer[3]	Stain Recipe	Stain Time	Strength Of Staining	Resolution	Special Instructions
ACON	A	60 ml. Buffer 150 mg. cis Aconitic Acid 200 mg. $MgCl_2 \cdot 6H_2O$ 40 mg. Isocitric dehydrogenase 10 mg. NADP 10 mg. MTT 3 mg. PMS	1 hr.	Good	Very Good	Incubate at room temp. for 30 min. and then add PMS; place in an incubator and wait for bands to appear
MDH	B	60 ml. Buffer 10 ml. MDH substrate 50 mg. NAD 40 mg. NBT 2 mg. PMS	½ hr.	Very Good	Very Good	Substrate: 13.4 g. Malic Acid in H_2O adjusted to pH 7.0 w/ NaOH, volume to 100 ml.
IDH	A	60 ml. Buffer 150 mg. Isocitric Acid 100 mg. $MgCl_2 \cdot 6H_2O$ 20 mg. NADP 20 mg. MTT 4 mg. PMS	1 hr.	Very Good	Good	Solution is adjusted to pH 7.0 before PMS is added to the solution
ADH	C	60 ml. Buffer 10 ml. Isopropanol 50 mg. NAD 10 mg. MTT 5 mg. PMS	2 hrs.	Good	Good	Incubate at room temp. for 30 min. and then add PMS; place in an incubator and wait for bands to appear

Table 26. Continued.

Enzyme	Buffer[3]	Stain Recipe	Stain Time	Strength Of Staining	Resolution	Special Instructions
PGI	D	60 ml. Buffer 200 mg. $MgCl_2 \cdot 6H_2O$ 10 mg. Fructose 6-phosphate 10 mg. NADP 10 mg. MTT 80 units G6-PDH 4 mg. PMS	½ hr.	Very Good	Very Good	Stains very fast
AK	C	60 ml. Buffer 20 mg. $MgCl_2 \cdot 6H_2O$ 90 mg. Glucose 50 mg. ADP 160 units Hexokinase 80 units G6-PDH 30 mg. NADP 20 mg. MTT 5 mg. PMS	1 hr.	Very Good	Very Good	
GOT	A	60 ml. Buffer 400 mg. L-Aspartic Acid 200 mg. α-Ketoglutaric Acid 1 mg. Pyridoxal 5-phosphate 100 mg. Fast Blue BB	1-2 hrs.	Good	Good	Mix solution w/o Fast Blue BB; adjust pH to 7.0 w/ KOH and then add Fast Blue BB; pour quickly over gel

Table 26. Continued.

Enzyme	Buffer[3]	Stain Recipe	Stain Time	Strength Of Staining	Resolution	Special Instructions
EST	A	60 ml. Buffer 20 mg. Naphthyl proprionate 50 mg. Fast Blue RR salt	½ hr.	Very Good	Very Good	Dissolve Naphthyl proprionate in Acetone; titrate to buffer; add Fast Blue RR
GAPDH	A	60 ml. Buffer 150 mg. Fructose 1,6-diphosphate 100 units Aldolase 50 mg. NAD 40 mg. MTT 150 mg. Na_2HAsO_4 4 mg. PMS	1-2 hrs.	Good	Fair	Aldolase should be pre-incubated in 5 ml. buffer for 30 min.
LAP	A	60 ml. Buffer 20 mg. L-lencyl-β-naphthylamide: HCl 20 mg. Fast Garnet GBC	1-2 hrs.	Good	Very Good	Dissolve L-lencyl-β-naphthylamide: HCl in 2 ml. N,N-dimethyl formamide; soak gel slice 30 min. and then add Fast Garnet GBC
SDH[2]	A	60 ml. Buffer 25 mg. NAD 5 mg. MTT 500 mg. Sorbitol 5 mg. PMS	1-2 hrs.	Good	Fair	Incubate for 30 min. and then add PMS

Table 26. Continued.

Enzyme	Buffer	Stain Recipe	Stain Time	Strength Of Staining	Resolution	Special Instructions
PEP	A	60 ml. Buffer 90 mg. Glycyl-D-L-Leucine 40 mg. Peroxidase 20 mg. O-dianisidine di-HCl 20 mg. Snake Venom (Bothrops) 1 ml. 0.25 M $MnCl_2$	2-3 hrs.	Good	Fair	Stain box should be rotated during staining-- this prevents stain from settling and increases the band resolution

[1] Enzyme labels are those referred to in the text; NAD = β-Nicotinamide Adenine Dinucleotide, NADP = β-Nicotinamide Adenine Dinucleotide Phosphate, PMS = Phenzine Methosulfate, NBT = Nitro Blue Tetrazolium, G6-PDH = Glucose 6-phosphate dehydrogenase, MTT is abbreviation used by Sigma Chemical Co.

[2] Not used in final analysis

[3] Buffers used to prepare stains are: A = 0.2 M Tris/HCl pH 8.0; B = 0.1 M Tris/HCl pH 8.5; C = 0.05 M Tris/HCl pH 7.0; D = 0.05 M Tris/HCl pH 8.0.

incubator immediately or transferred to one after an appropriate time. Banding patterns were watched to judge when to stop the staining process. In some cases this was almost immediate and in others it took several hours (Table 26). After gels were stained for the appropriate enzymes, they were removed from the incubator and either scored immediately or fixed. The fixative that was used consisted of a 5:5:1 mixture of water to methanol to acetic acid. Some stained gels faded when placed into this solution and had to be scored immediately. Others were fixed and scored later. A mixture of methanol and water (1:1) was used to fix gels stained for peptidase. In most cases, pictures of stained gels were taken for future reference. Later, it was found that photocopies of gels could be taken on a standard copying machine. These provided for quick reference and maintained banding patterns for future examination.

LIST OF REFERENCES

LIST OF REFERENCES

Alfaro, R.I., and J.H. Borden. 1982. Host selection by the white pine weevil, Pissodes strobi Peck: feeding bioassays using host and nonhost plants. Can. J. For. Res. 12: 64-70.

Anderson, J.M., and K.C. Fisher. 1956. Repellency and host specificity in the white pine weevil (Pissodes strobi). Physiol. Zool. 29: 314-324.

Battenfield, S.L. (ed.) 1981-1982. Michigan Forest Pest Report. Michigan Cooperative Forest Pest Management Program. 39 pp.

Belyea, R.M., and C.R. Sullivan. 1956. The white pine weevil: A review of current knowledge. For. Chron. 32: 58-67.

Berlocher, S.H., and G.L. Bush. 1982. An electrophoretic analysis of Rhagoletis (Diptera: Anthomyiidae) phylogeny. Syst. Zool. 31(2): 136-155.

Brewer, G.J. 1970. An Introduction to Isozyme Techniques. Academic Press. New York. 186 pp.

Bush, G.L. 1969. Sympatric host race formation and speciation in frugivorous flies of the genus Rhagoletis (Diptera, Tephritidae). Evolution 23: 237-251.

Bush, G.L. 1974. The mechanism of sympatric host race formation in the true fruit flies (Tephritidae). p. 3-23. In M.J.D. White, ed. Genetic Analysis of Speciation Mechanisms. Aust. and New Zealand Book Co.

Bush, G.L. 1975. Modes of animal speciation. Ann. Rev. Ecol. Syst. 6: 339-364.

Bush, G.L., and D.J. Howard. (in press) Allopatric and nonallopatric speciation: Assumptions and evidence.

Bush, G.L., S.M. Case, A.C. Wilson, and J.L. Patton. 1977. Rapid speciation and chromosomal evolution in mammals. Proc. Nat. Acad. Sci. 74: 3942-3946.

Cline, A.C., and H.J. MacAloney. 1931. A method of reclaiming severely weevilled white pine plantations. Mass. For. Assoc. Bull. 152: 3-11.

Coyne, J.A., W.F. Eanes, J.A.M. Ramshaw, and R.K. Koehn. 1979. Electrophoretic heterogeneity of α-Glycerophosphate dehydrogenase among many species of Drosophila. Sys. Zool. 28: 164-175.

Craighead, F.C. 1921. Hopkins' host-selection principle as related to certain cerambycid beetles. J. Agr. Res. 22: 189-220.

Crow, J.F., and M. Kimura. 1970. An Introduction to Population Genetics Theory. Harper and Row, New York. 591 pp.

Dickinson, H., and J. Antonovics. 1973. Theoretical considerations of sympatric divergence. Am. Nat. 107: 256-274.

Diehl, S.R., and G.L. Bush. 1984. An evolutionary and applied perspective of insect biotypes. Ann. Rev. Entomol. 29: 471-504.

Dirks, C.O. 1964. The white pine weevil in Maine: its biology and dispersal and the effect of prompt clipping of infested leaders on trunk form. Me. Agr. Exp. Sta. Bull. 625, 23 pp.

Falconer, D.S. 1981. Introduction to Quantitative Genetics. Longman, London and New York. 340 pp.

Fisher, R.A. 1930. The Genetic Theory of Natural Selection. Clarendon Press, Oxford. 272 pp.

Garrett, P.W. 1972. Resistance of eastern white pine (Pinus strobus L.) provenances to the white pine weevil (Pissodes strobi Peck). Silvae Genetica. 21(3-4): 119-121.

Graham, S.A. 1926. Biology and control of the white pine weevil, Pissodes strobi (Peck). Cornell Univ. Agr. Exp. Sta. Ithaca, N.Y. Bull. 449, 32 pp.

Howard, D.J. 1982. Speciation and Coexistence in a Group of Closely Related Groun Crickets. Ph.D. Thesis, Yale University, New Haven. 186 pp.

Laven, H. 1959. Speciation by cytoplasmic isolation in the Culex pipiens complex. Cold Spring Harbor Symp. Quant. Biol. 24: 166-173.

Levene, H. 1949. A new measure of sexual isolation. Evolution 3: 315-321.

Li, C.C. 1955. Population Genetics. University of Chicago Press, Chicago. 366 pp.

Maynard-Smith, J. 1966. Sympatric speciation. Am. Nat. 100: 637-650.

Maynard-Smith, J. 1970. Population size, polymorphism, and the rate of non-Darwinian evolution. Am. Nat. 104: 231-237.

Mayr, E. 1963. Animal Species and Evolution. Cambridge, Mass. Harvard Univ. Press. 797 pp.

McMullen, L.H., and S.F. Condrashoff. 1973. Notes on dispersal, longevity, and overwintering of adult Pissodes strobi (Peck) (Coleoptera: Curculionidae) on Vancouver Island. J. Entomol. Soc. B.C. 70: 22-26.

Morse, F.S. 1958. The White Pine Weevil in Wisconsin Jack Pine Plantations. Master of Science (Entomology) Thesis, Univ. of Wisconsin, Madison Wisc. 40 pp.

Mosher, D.G., et al. 1979. Michigan Forest Pest Report. Mich. Cooperative For. Pest Management Program. 32 pp.

Mosher, D.G., et al. 1980. Michigan Forest Pest Report. Mich. Cooperative For. Pest Management Program. 33 pp.

Nei, M. 1973. Analysis of gene diversity in subdivided populations. Proc. Natl. Acad. Sci. U.S. 70: 3321-3323.

Nei, M. 1975. Molecular Population Genetics and Evolution. North-Holland American Elseview, New York. 288 pp.

Nei, M. 1977. F-statistics and analysis of gene diversity in subdivided populations. Ann. Hum. Genet., Lond. 41: 225-233.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics. 89: 583-590.

Nei, M., and Y. Imaizumi. 1966. Genetic structure of human populations. I. Local differentiation of blood gene frequencies in Japan. Heredity 21: 9-35.

Phillips, T.W. 1985. Ecology and Systematics of Pissodes Sibling Species (Coleoptera: Curculionidae). Ph.D. Thesis, S. Univ. N.Y., Syracuse, N.Y. 204 pp.

Pimentel, D.,G.J.C. Smith, and J. Soans. 1967. A population model of sympatric speciation. Am. Nat. 101: 493-504.

Plummer, C.C., and A.E. Pillsbury. 1929. The white pine weevil in New Hampshire. N.H. Exp. Sta. Bull. 247, 32 pp.

Prentice, R.M., and V. Hildahl. 1957. Report of the forest insect survey, Manitoba and Saskatchewan. Ann. Rept. For. Ins. Sur., Dept. Agr. Can.. Div. Biol. Sci. Ser. p. 61.

Rice, W.R. 1984. Disruptive selection on habitat preference and the evolution of reproductive isolation: A simulation study. Evolution 38(6): 1251-1260.

Selander, R.K., and D.W. Kaufman. 1973. Genic variability and strategies of adaptation in animals. Proc. Nat. Acad. Sci. U.S. 70: 1875-1877.

Smith, S.G., and B.A. Sugden. 1969. Host trees and breeding sites of native North American Pissodes bark weevils, with a note on synonomy. Ann. Entomol. Soc. Amer. 62: 146-148.

Soans, A.B., D. Pimentel, and J.S. Soans. 1974. Evolution of reproductive isolation in allopatric and sympatric populations. Am. Nat. 108: 117-124.

Spurr, S.H., and R.B. Friend. 1941. Compression wood in weeviled northern white pine. J. For. 39: 1005-1006.

Sullivan, C.R. 1959. Effect of light and temperature on the behavior of adults of the white pine weevil, Pissodes strobi Peck. Can. Entomol. 91: 213-232.

Sullivan, C.R. 1960. The effect of physical factors on the activity and development of adults and larvae of the white pine weevil, Pissodes strobi (Peck). Can. Entomol. 92: 732-745.

Sullivan, C.R. 1961. The effect of weather and the physical attributes of white pine leaders on the behaviour and survival of the white pine weevil, Pissodes strobi Peck, in mixed stands. Can. Entomol. 93: 721-741.

Swofford, D.L., and R.B. Selander. 1981. BIOSYS-1: A Computer Program for the Analysis of Allelic Variation in Genetics. Users Manual. Univ. of Illinois, Urbana, Ill. 65 pp.

Tauber, C.A., and M.J. Tauber. 1977. A genetic model for sympatric speciation through habitat diversification and seasonal isolation. Nature 268: 702-705.

Tavormina, S.J. 1982. Sympatric genetic divergence in the leaf-mining insect Liriomyza brassicae (Diptera: Agromyzidae). Evolution 36: 523-534.

Thoday, J.M. 1972. Disruptive selection. Proc. R. Soc. London Ser. B. 182: 109-143.

Walsh, B. 1864. On phytophagic varieties and phytophagic species. Proc. Entomol. Soc. Philadelphia 3: 403-430.

Ward, R.H., and C.F. Sing. 1970. A consideration of the power of the χ^2 test to detect inbreeding effects in natural populations. Am. Nat. 104: 355-366.

Wright, S. 1965. The interpretation of population structure by F-statistics with special regard to systems of mating. Evolution 19: 395-420.

Wright, S. 1978. Evolution and the Genetics of Populations, vol. 4. Variability Within and Among Natural Populations. Univ. of Chicago Press, Chicago.