POPULATION GENETICS OF THE SPRUCE BUDWORM, *CHORISTONEURA FUMIFERANA* (CLEM.) FREEMAN (LEPIDOPTERA: TORTRICIDAE), IN RELATION TO GEOGRAPHICAL AND POPULATION DENSITY DIFFERENCES

G.T. HARVEY

Natural Resources Canada, Canadian Forest Service - Sault Ste. Marie, PO Box 490, Sault Ste. Marie, Ontario, Canada P6A 5M7

Abstract

The Canadian Entomologist **128**: 219–243 (1996)

Collections (68) of spruce budworm from 33 locations from Newfoundland to Alaska were analysed for isozyme frequencies using horizontal starch gels. Collections represented pre-, early-, mid-, late-, and post-outbreak stages of several populations in balsam fir, white spruce, and mixed host forests, as well as successive annual collections at several locations. Isozymes were measured at 11 loci in mature larvae and at six loci in pheromone-trapped males; frequencies were essentially the same in both stages, and from all host species. Three loci (IDH-2, LDH-1, and AAT-1) were found to be sex-linked, with no heterozygotes in females. Mean percentage heterozygosity ranged from 13.2 to 23.1; at individual locations it tended to decrease over successive years of outbreak and over successive collections in the same year. Contingency chi-square analysis indicated small differences related to location and outbreak history but all populations were generally homogeneous over the entire range. Nevertheless, one allozyme of AAT-1 exhibited a significant cline in frequency from the southeast to the northwest. Gene flow across the entire range appeared to be appreciable.

Harvey, G.T. 1996. Génétique des populations de la Tordeuse des bourgeons de l'épinette, *Choristoneura fuimiferana* (Clem.) Freeman (Lepidoptera: Tortricidae), en fonction des variations géographiques et démographiques. *The Canadian Entomologist* **28**: 219–243.

Résumé

Des récoltes (68) de Tordeuses des bourgeons de l'épinette en 33 localités, de Terre-Neuve à l'Alaska, ont été analysées par électrophorèse horizontale sur gel d'amidon quant à la fréquence des isozymes. Les échantillons représentaient divers stades épidémiques, pré-épidémie, début d'épidémie, milieu d'épidémie, fin d'épidémie, post-épidémie, de plusieurs populations, dans des forêts de sapins baumiers, d'épinettes blanches et dans des forêts mixtes, de même que des récoltes annuelles successives à différents endroits. Les isozymes ont été mesurés à 11 locus chez les larves à maturité et à six locus chez les mâles capturés dans des pièges à phéromones; la fréquence était essentiellement la même aux deux stades chez toutes les espèces d'hôtes. Trois locus (IDH-2, LDH-1 et AAT-1) se sont révélés liés aux chromosomes sexuels, mais il n'y avait pas d'hétérozygotes chez les femelles. Le pourcentage moyen d'hétérozygotie allait de 13,2 à 23,1; à certains endroits particuliers, ce pourcentage avait tendance à diminuer au cours des années successives d'épidémie et au cours des récoltes successives d'une même année. Un tableau de contingence (chi carré) a mis en lumière de petites différences reliées à la localité et au déroulement de l'épidémie, mais toutes les populations étaient généralement homogènes dans toute l'étendue de la répartition. Néanmoins, la fréquence d'un allozyme d'AAT-1 suivant une tendance significative du sud-est au nord-ouest. Le passage des gènes semble important dans toute d'étendue du territoire étudié.

[Traduit par la Rédaction]

Introduction

One of the most important insect pests of coniferous trees of North America, the spruce budworm, *Choristoneura fumiferana* (Clem.) Freeman, feeds on and kills many spruce and fir trees over millions of hectares. During outbreaks the number of insects may be five orders of magnitude greater than between outbreaks. The mechanisms of these oscillations have long attracted the attention of entomologists and foresters, and have important implications for pest management (Royama 1984; Berryman 1987). Research over many years

(summarized in Sanders et al. 1985) has not made it possible to predict outbreaks well enough to identify where high insect densities are imminent. Many factors undoubtedly interact to produce cyclic population oscillations; these factors must include the population genetics of the insects themselves (Carson 1968; Wallner 1987).

The spruce budworm is a highly variable species in all stages (Harvey 1985). The genetic basis for some of these polymorphisms has been established (Stehr 1959); expression of some of the polymorphisms may also be dependent on temperature (Volney et al. 1983). The technique of characterizing individual insects and populations by isozyme frequencies offered a method to quantify the genetic variation and to search for differences related to geography, host, population history, dispersal, etc., as found for southern pine beetle populations (Namkoong et al. 1979). Differences in insects among population cycle phases might be exploited for early detection and control of outbreaks.

Materials and Methods

Insect Collections. Larval collections. To measure isozyme frequencies adequately throughout all phases of a population cycle, sampling should be done annually at several locations for one or more complete cycles (Volney 1989). This poses major difficulties, not least of which is knowing where an outbreak will develop. To shorten the time span involved, sampling was initiated in several locations at low and high populations densities, and continued for several years.

Collections of late-instar larvae were obtained from balsam fir, *Abies balsamea* (L.) Mill. (Bf), white spruce, *Picea glauca* (Moench) Voss (Sw), or both hosts from 1980 to 1988 from budworm infestations across Canada (Table 1; Fig. 1). The first collections provided samples from several populations causing moderate and severe defoliation in the recent infestation in eastern and central Canada, and from one low density population in central Ontario (GRA). In following years, collections were obtained from some of the same locations and at new locations in the enlarging infestation in northwestern Ontario (Fig. 1). These included successive annual collections (1980–1988) from a study of outbreak populations at Black Sturgeon Lake, Ont. (BSL). Collections were also obtained from areas of moderate to severe defoliation near Fort Nelson, B.C. (NEL6, NEL7, and NEL8), as part of a cooperative study of budworms in western Canada (Shepherd et al. 1995). Thus, although the coverage was most intensive in Ontario, the larval samples represent almost the entire range of this species in North America (Harvey 1985; Shepherd et al. 1995) (Fig. 1).

Larval collections of spruce budworm were allowed to feed on foliage of their host or, when necessary, on artificial diet (Grisdale 1970). Frequencies of studied isozymes in diet-reared insects are the same as in larvae fed on fresh foliage. Larvae in the latter half of the sixth instar (last) were frozen immediately and stored at −80°C until analysis. Some pupae and adults were also frozen to augment small samples.

Population records. Collections were made by the author or by Forest Insect and Disease Survey (FIDS) personnel who identified the location by a Universal Transverse Mercator (UTM) number (Anonymous 1969) and reported the defoliation level (Morris 1954). The selection of collection locations was based on annual insect distribution and abundance records [Forest Insect and Disease Survey (Canada) 1970–1980, 1981–1989] and from unpublished reports by FIDS personnel in several regions, including Wood and Van Sickle (1989) and Wood et al. (1987). For each collection, identified by a letter code and year, the UTM grid number was used (Tables 1 and 2) for analysis of trends related to geographical location.

Outbreak status at the time of sampling was assigned a code based on a best estimate of the start of population increase recorded in FIDS records. Starting with 1970 data, the 1st year that the egg-mass (e.m.) density surpassed 30 e.m./10 m^2 of foliage, which causes moderate-to-severe (M-S) defoliation (Morris 1954), was assigned the code number of 3

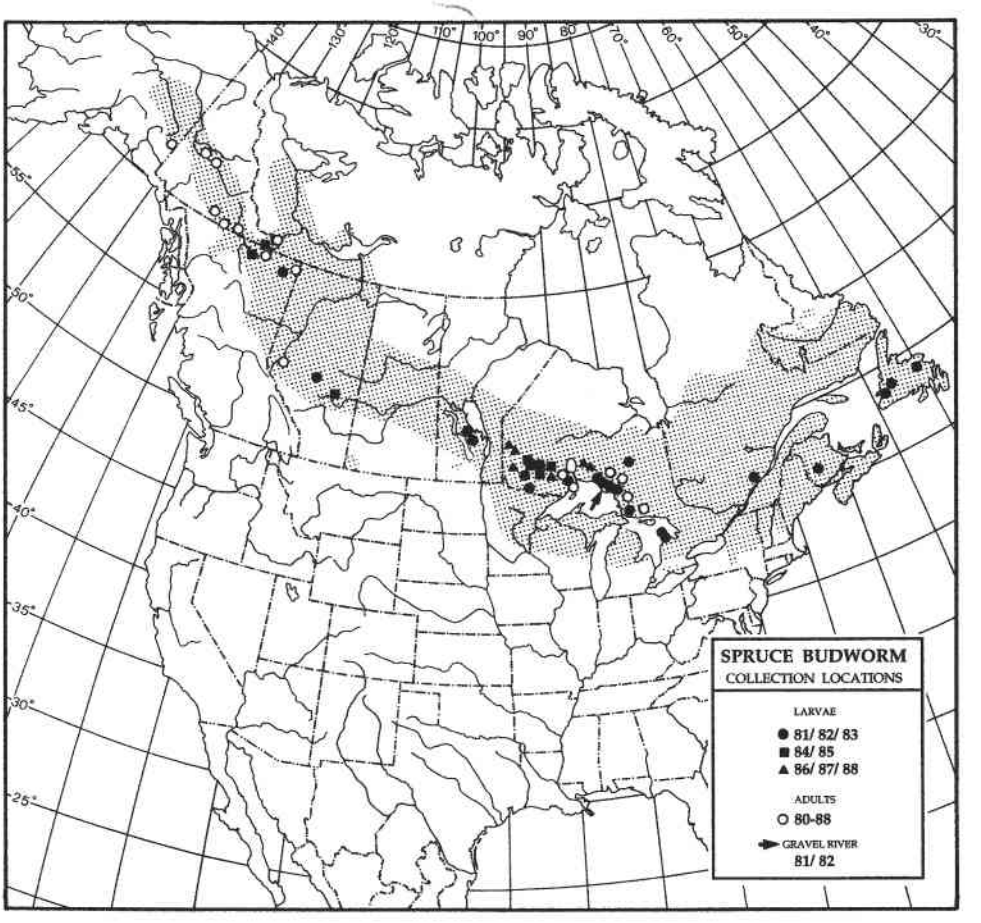

FIG. 1. Map showing locations of larval and adult collections and the distribution of *Choristoneura fumiferana*.

(Table 1). The infestation code for each sample, INF, equals 3 + 'number of years M-S defoliation on the year of collection' (Tables 1 and 2).

The 1981 collection at Gravel River (GRA, Wiggins Twp, Ont.) was obtained by intensive collection in a 'non-infested' area 3 years before moderate defoliation levels were recorded in 1984; it has been assigned an INF of 1. There was no noticeable defoliation in 1981 or 1982 but 28 e.m./10 m^2 were recorded in 1982 and the first signs of defoliation were noted in 1983. Approximately 70 km to the east (FOX, MAN) populations were causing M-S defoliation as early as 1975 (Fig. 1). Defoliation west of Gravel River did not reach M-S levels until 1981. This first collection at Gravel River, then, appears to be from an early stage of an outbreak. The 1983–1985 collections from Desbarats, Ont. (DES) came from an area of light defoliation following collapse of the outbreak there and represent a post-outbreak stage. All other larval collections were obtained from populations in the M-S defoliation category, where adequate samples of large larvae are easily obtained.

Defoliation in the Liard River area of northern British Columbia, for which no e.m. data were available, was rated M-S from 1970 to 1974 and levels of defoliation increased again in 1984 (Shore and Alfaro 1986). However, defoliation returned to low-to-moderate levels

TABLE 1. Location, host, and infestation codes and statistics of genetic variability at 11 loci for 68 larval collections of *Choristoneura fumiferana*

Location	Code[1]	UTM grid	Date	A[2]	Size[3]	All[3]	Poly[4]	Het[4]	Host[5]	Inf[6]
Gander, Nfld.	GAN1	2168 541	30 June 1981	1	39.0	3.3	90.0	0.200	1	07
Pasadena, Nfld.	PAS1	2145 542	29 June 1981	1	23.5	2.7	81.8	0.172	2	10
Cornerbrook, Nfld.	COR3	2143 542	05 July 1983	1	46.0	3.0	81.8	0.184	1	12
Fredericton, N.B.	FED1	1965 506	12 June 1981	2	64.8	3.6	100.0	0.171	1	15
Valcartier, P.Q.	VAC1	1933 520	17 June 1981	2	70.4	3.6	100.0	0.183	1, 2	11
Owen Sound, Ont.	CRA1	1746 500	20 June 1981	3	62.7	3.5	100.0	0.169	2	14
McMurrick Twp, Ont.	MUR2	1746 503	09 June 1982	3	69.2	3.7	100.0	0.200	2	09
Desbarats, Ont.	DES1*	1727 513	11 June 1981	3	59.6	3.3	90.0	0.156	1, 2	15
Desbarats, Ont.[7]	DES2		11 June 1982	3	63.7	3.6	90.0	0.210	1, 2	16
Desbarats, Ont.	DES3		23 June 1983	3	70.4	3.6	100.0	0.176	1, 2	17
Desbarats, Ont.	DES4		11 June 1984	3	83.3	3.8	100.0	0.181	2	18
Desbarats, Ont.	DES5		20 June 1985	3	72.4	3.6	90.0	0.176	1, 2	19
Kipling Dam, Ont.	KIP1	1741 555	29 June 1981	4	72.1	3.5	81.8	0.181	1	08
Kipling Dam, Ont.	KIP2		28 June 1982	4	70.4	3.8	100.0	0.200	1	09
Kipling Dam, Ont.	KIP3	1741 554	23 June 1983	4	70.0	3.9	100.0	0.218	1	10
Manitouwadge, Ont.	MAN1*	1658 539	24 June 1981	4	62.7	3.5	100.0	0.216	1, 2	09
Manitouwadge, Ont.	MAN2		28 June 1982	4	63.7	3.5	90.0	0.200	1, 2	10
Manitouwadge, Ont.	MAN3		28 June 1983	4	68.5	3.5	100.0	0.194	1	11
Manitouwadge, Ont.	MAN4		28 June 1984	4	69.5	3.8	100.0	0.209	1	12
Manitouwadge, Ont.	MAN5		24 June 1985	4	65.5	3.4	81.8	0.172	1	13
Foxxe Lake, Ont.	FOX1	1650 540	24 June 1981	4	91.1	3.8	100.0	0.212	1, 2	04
Foxxe Lake, Ont.	FOX2		02 June 1982	4	73.6	3.7	100.0	0.188	1, 2	05
Terrace Bay, Ont.	TER1	1649 540	10 June 1981	4	63.5	3.5	100.0	0.213	1, 2	01
Terrace Bay, Ont.	TER2		28 June 1982	4	65.9	3.8	100.0	0.209	1	02
Terrace Bay, Ont.	TER3		28 June 1983	4	67.3	3.5	100.0	0.187	2	03
Terrace Bay, Ont.	TER4		28 June 1984	4	8.6	2.3	81.8	0.273	2	04
Terrace Bay, Ont.	TER5		24 June 1985	4	85.5	4.2	100.0	0.205	2	05
Terrace Bay, Ont.	TER6		18 June 1986	4	70.5	3.2	90.9	0.163	1	06
Gravel R., Ont.	GRA1*	1644 541	10 July 1981	4	25.5	3.1	90.9	0.194	1, 2	01
Gravel R., Ont.	GRA2		02 July 1982	4	52.2	3.4	100.0	0.200	1, 2	02
Beardmore, Ont.	SUS6	1642 549	19 June 1986	4	68.3	3.4	100.0	0.185	1	05
Patience Twp, Ont.	PAT6	1642 543	19 June 1986	4	25.0	2.7	81.8	0.132	1, 2	06
Patience Twp, Ont.	PAT7	1642 542	16 June 1987	4	42.2	3.1	90.9	0.149	2	07
Patience Twp, Ont.	PAT7		23 June 1987	4	42.1	2.6	72.7	0.126	2	06
Bl. Sturgeon L., Ont.	BSL1*	1636 546	23 June 1981	5	62.9	3.6	100.0	0.189	1, 2	01
Bl. Sturgeon L., Ont.	BSL2		28 June 1982	5	73.4	3.3	100.0	0.196	1	02
Bl. Sturgeon L., Ont.	BSL3		24 June 1983	5	66.8	3.9	90.9	0.219	1, 2	03
Bl. Sturgeon L., Ont.	BSL4		18 June 1984	5	120.7	4.0	100.0	0.174	1, 2	04
Bl. Sturgeon L., Ont.	BSL5		11 June 1985	5	76.1	3.7	100.0	0.158	1	05
Bl. Sturgeon L., Ont.	BSL6		11 June 1986	5	67.4	3.6	90.9	0.153	1, 2	06
Bl. Sturgeon L., Ont.	BSL7		19 June 1987	5	68.8	3.2	100.0	0.140	2	07
Cecil L. Ignace, Ont.	CEC5	1561 549	14 June 1985	6	80.2	3.6	90.9	0.176	1	06
Cecil L. Ignace, Ont.	CEC6	1561 548	11 June 1986	6	66.1	3.3	90.9	0.152	1, 2	07
Sanbar P. Pk., Ont.	SAB4	1560 548	28 June 1984	6	68.5	3.8	100.0	0.175	1	04
Ojibway Park, Ont.	OJIB	1556 553	18 June 1985	6	77.3	3.6	90.9	0.180	1	05
Aaron P. Pk., Ont.	ARN4	1552 551	27 June 1984	6	79.3	3.6	100.0	0.153	1	04
Ingall Lake, Ont.	ING3	1551 549	22 June 1983	6	80.2	3.5	100.0	0.174	1	04
Ingall Lake, Ont.	ING4		28 June 1984	6	91.9	3.6	100.0	0.167	1	05
Bear Pass, Ont.	BER1	1549 539	11 June 1981	6	65.7	3.4	90.9	0.159	1	10
Bear Pass, Ont.	BER2		05 June 1982	6	67.4	3.8	100.0	0.231	2	11
Bear Pass, Ont.	BER3		18 June 1983	6	71.1	3.5	90.9	0.179	1	12
Ear Falls, Ont.	EAR6	1548 560	11 June 1986	6	40.7	3.1	81.8	0.160	1	04
Ear Falls, Ont.	EAR7		02 June 1987	6	66.5	3.4	100.0	0.184	1	05
Ear Falls, Ont.	EAR8		10 June 1988	6	33.8	2.7	63.6	0.158	1	06
Vermillion Bay, Ont.	VER5	1547 552	04 July 1985	6	55.7	3.4	90.9	0.171	1	06
Red Lake, Ont.	RED7	1543 564	03 June 1987	6	52.2	3.0	81.8	0.147	1	05

TABLE 1. (*Concluded*)

Location	Code[1]	UTM grid	Date	A[2]	Size[3]	All[3]	Poly[4]	Het[4]	Host[5]	Inf[6]
Kirkup, Kenora, Ont.	KUP6	1540 550	09 June 1986	6	76.2	3.6	90.9	0.185	1	04
Moosehorn, Man.	MOS1	1454 568	19 June 1981	7	66.5	3.6	100.0	0.185	2	12
Gypsumville, Man.	GYP1	1453 573	17 June 1981	7	43.8	3.2	90.9	0.197	2	12
Castor, Alta.	EDM6	1247 578	12 June 1986	8	47.7	3.2	90.9	0.147	2	08
Edmonton, Alta.	EON1	1236 595	11 June 1981	8	61.8	3.7	100.0	0.201	2	03
Fort Nelson, B.C.	NEL6*	1048 653	24 June 1986	9	56.7	3.5	90.9	0.144	2	03
Fort Nelson, B.C.	NEL7	1048 658	16 June 1987	9	43.6	3.0	90.9	0.145	3	04
Fort Nelson, B.C.	NEL1*	1048 655	07 June 1987	9	43.8	3.6	90.9	0.173	2	04
Fort Nelson, B.C.[8]	NEL8*	1048 657	21 June 1988	9	25.6	2.9	90.9	0.193	2	05
Liard Hotspr., B.C.[8]	LID4*	0965 659	28 June 1984	9	50.8	3.6	100.0	0.202	2	02
Liard Hotspr., B.C.	LID8*	0967 659	23 June 1988	9	87.9	3.5	72.7	0.139	2	05
Smith River, B.C.	SIT4*	0960 662	23 June 1984	9	33.0	3.3	90.9	0.205	2	02
Means					62.9	3.4	93.5	0.181		
s_x					2.27	0.04	0.94	0.003		

[1] Population code for allele frequencies (Table 3). *Data for trapped males also at these locations.
[2] Geographic area number (Table 9).
[3] Average number of individuals (females removed from sex-linked loci), and number of alleles per locus.
[4] Percentage polymorphism (when frequency of most common allele does not exceed 0.95); percentage heterozygosity (by direct count).
[5] Host: 1, white spruce (Sw); 2, balsam fir (Bf); 3, alpine fir (Af); 4, lodgepole pine.
[6] Infestation code.
[7] Population sampled following collapse.
[8] Defoliation only reached M-S in local spots, and then not on successive years (see Shore and Alfaro 1986).

during the period of collection, except in understory trees (Turnquist and Ferris 1989). Nevertheless an INF value of 3 has been assigned for the 1986 collection which is considered to be roughly comparable to early phases of other outbreaks.

In summary, the larval collections included a wide representation of phases (stages) of outbreaks: early (1–2), rapid growth (3–5), peak population levels (6–9), final stages (10–15), and post-outbreak (16–19) as shown by the INF values in Table 1. No data were obtained from inter-outbreak populations. Nevertheless, these samples should provide a basis for detection of genetic changes or differences that may be associated with population cycles.

Moth collections. Pheromone traps were used to obtain samples of male moths from several very low density populations (Sanders 1992). Pherocon 1C sticky traps (Zoecon Corp., Palo Alto, CA) were baited with a synthetic bait consisting of 5% by weight 95:5 *E/Z* 11-14 tetradecenal in a polyvinyl carrier (Sanders 1978). Several different baits and virgin females were used for trapping (multiple entries in Table 2; Fig. 3) but no associated allozyme frequency differences were detected among the *C. fumiferana* moths. Most moths caught in sticky traps remain alive overnight and contain active enzymes (Harvey 1980). After one night of exposure, living moths were removed from the traps, placed in small plastic vials, and stored in liquid nitrogen. Upon return to the laboratory, the vials were held at −80°C until use.

Adults were collected from several locations in Ontario in 1980 and in 1988 using this method. Trapping locations were at least 100 km apart and were located in areas where no defoliation was detectable, across the outbreak and into adjoining non-outbreak areas (Table 2; Fig. 1). Moths from low density populations included those from Black Sturgeon Lake (PBS2) in 1980, which represented the early stages of the outbreak there, and from Kirkwood (PKI1) in 1988, at a late stage of that outbreak. Moths were also collected in 1988

TABLE 2. Location and other details of collections of trapped *Choristoneura fumiferana* moths

Location	Code[1]	UTM grid	Host[2]	Dates	Level[3]
Kirkwood, Ont.	PKI1	1730 513	1	04–08 July 1988	1
Desbarats, Ont.	PDE2*	1729 515	1, 2	3 July 1980	3
McCron Twp, Ont.	PMC2	1661 539	1, 2	11 July 1980	3
Manitouwadge, Ont.	PMA2*	1658 539	1, 2	8 July 1980	3
Black R., Ont.	PBL2	1655 539	1, 2	8 July 1980	3
Gravel R., Ont.	PGR2*	1644 541	1, 2	10 July 1980	2
Gravel R., Ont.	PGR3			24 July 1981	2
Sibley Penn., Ont.	PSI2	1637 537	1, 2	8 July 1980	2
Black Sturgeon L., Ont.	PBS2*	1636 546	1, 2	9 July 1980	1
Moberly Lake, B.C.	BMO1	1058 612	1	16–18 July 1988	1
Ft. Nelson 1, B.C.	BNE1*	1050 651	1	12–13 July 1988	3
Ft. Nelson 2, B.C.	BNO2	1050 650	4	13–14 July 1988	3
Ft. Nelson 2, B.C.	BNO4			14 July 1988	3
Liard River, B.C.	BLA1*	1036 657	4	9–11 July 1988	3
Smith R. Cross., B.C.	BSR1*	963 653	1	9–11 July 1988	3
Watson Lake, Y.T.	YWA1	951 661	1	10–11 July 1986	2
Watson Lake, Y.T.	YWA3			11 July 1986	2
Swift River, Y.T.	YSW1	943 663	1, 3	18 July 1986	2
Swift River, Y.T.	YSW3			18 July 1986	2
Carmacks, Y.T.	YCA1	845 686	1	14 July 1986	1
Carmacks, Y.T.	YCA3			9 July 1987	1
Carmacks, Y.T.	YCA4			19 July 1987	1
Carmacks, Y.T.	YCA5			19 July 1987	1
Flower Mtn., Y.T.	YFL1	843 688	1	14 July 1986	1
Moose Cr., Y.T.	YMO1	843 700	1	11 July 1987	2
Dawson, Y.T.	YDA1	756 708	1	12 July 1987	2
Dawson, Y.T.	YDA2			12 July 1987	2
Tok, AK	YTO2	747 692	1	14 July 1987	1
Tok, AK	YTO3			14 July 1987	1

[1] Population code for allele frequencies (Table 4). *Locations with data for both trapped males and larvae.
[2] Host, see Table 1.
[3] Population level: 1 = very light; 2 = light; 3 = moderate-to-severe.

from four areas of severe defoliation for comparison with larval data from the same areas (Table 2). Some moths trapped during a study of very low density populations of *C. fumiferana* in parts of Yukon, Alaska, and northeastern British Columbia in 1986–1988 (Shepherd et al. 1995) were also included in the analysis; they may represent authentic inter-outbreak populations.

Electrophoresis. The sex of all larvae, pupae, and adults (all males) was recorded. Freshly thawed larvae were decapitated (to facilitate homogenization) and homogenized individually in 0.25 mL Tris-citrate buffer (0.09 *M* Tris, 0.0029 *M* citrate, ph 7.1) (Harvey and Sohi 1985). Pupae and adults (dewinged) were thawed and homogenized directly. After centrifugation the supernatant was used to load wicks. Extracts were kept on ice to prevent degradation of the enzymes.

Insects were analysed individually using horizontal starch gel electrophoresis (Electrostarch, Madison, WI). Methods of electrophoresis and staining followed, or were modified from Ayala et al. (1972), Hudson and Lefkovitch (1982), Shaw and Prasad (1970), and Wilhite and Stock (1983). Two buffer systems were used: Poulik buffer, pH 8.0 (Poulik 1957); and Tris-citrate, pH 7.1 (Ayala et al. 1972) as described by Harvey and Sohi (1985). By using two different gels and slicing before staining it was possible to measure 15 loci on

up to 26 insects per run. Isozyme bands were identified by letters (fastest band = 'A', etc.; Table 3); internal standards based on larvae of *C. fumiferana* were used. Travel distances were expressed relative to the most common band (1.00; Tables 3 and 4). Data from larvae with bands attributable to parasitoids (Castrovillo and Stock 1981) were discarded.

Enzymes selected for measurement were dependable and showed good activity in extracts of larvae and adults. For a better assessment of genetic variability in relation to collection location and outbreak history, only loci that showed significant levels of polymorphism were studied. Approximately 80 individual insects were assayed from each collection for the following enzymes (EEC numbers from Nomenclature Committee of the International Union of Biochemistry 1984): aspartate transaminase (AAT, EC 2.6.11), esterases (EST, EC 3.1.1.2), isocitrate dehydrogenase (IDH, EC 1.1.1.42), lactate dehydrogenase (LDH, EC 1.1.1.27), malate dehydrogenase (MDH, EC 1.1.137), phosphoglucoisomerase (PGI, EC 5.3.1.9), and phosphoglucomutase (PGM, EC 2.7.5.1). The biochemical basis for each enzyme system was validated by component deletion tests which demonstrated that no bands were produced unless substrate, cofactors, and other components were present.

Breeding Studies. Genotypes were determined in parents and their progenies to establish the inheritance pattern of the enzyme loci. Mating pairs (mostly *inter se*) were frozen for electrophoresis after 4–5 days, when 50–60% of the eggs had been laid. Following diapause, their progenies were reared to the late-instar stage when 10–20 larvae were frozen; remaining larvae were reared to adulthood for additional matings. Frequently other sibs (larvae or adults) were also used for confirmation of genotypes. Genotypes of some parents were estimated from their sibs or their progeny. The genetics of the esterases, which become unmeasurable in adults soon after eclosion, had to be studied in larvae using data from sibs to determine the parental genotypes. These genetic studies provide a firm base for the identification of bands for seven loci, and moderate support for the other four loci; they also support use of the terms 'allozyme' or 'alleles' for the bands measured.

Data Analysis. Allozyme frequencies were obtained for 11 loci in 68 collections from 33 locations across Canada from 1980 to 1988. The band for each allozyme was found in at least one of the 33 populations, even though not all were present in the collections shown in Table 3. At some locations there are data for several outbreak years; among these, data from four locations for 5 or more years were analysed as a separate subset (see Tables 10 and 11). Collections of trapped adults provided measurements at only six loci and were analysed separately (see Table 8). Genotype scores were analysed using the BIOSYS software for VAX (Swofford and Selander 1981). Data were entered as individual genotypes and allozyme frequencies were calculated. For testing expected genotype frequencies Levene's (1949) correction for small sample size was used (Swofford and Selander 1981). For the three sex-linked loci (IDH-2, LDH-1, and AAT-1) only males were used in the analyses.

Relationships among the collections based on allozyme frequencies were explored using contingency chi-square and cluster analysis (UPGMA). To meet the requirements for class size in contingency chi-square tests, allelic classes with frequency below 1% were pooled with the nearest mobility classes (Pashley et al. 1985). For these analyses, data were entered as allele frequencies (BIOSYS DATYP = 3). In the initial analysis each collection was treated as a unit; consequently collections over several seasons from one location, or even consecutive collections the same year at a location, were treated as separate collections. In subsequent analyses the replicate samples were pooled to evaluate differences among the 33 locations. The extent of genetic variance among individuals, subpopulations, and populations was tested for each locus using F statistics (Wright 1978; Jennings and Philipp 1992) and gene flow was estimated by the methods of Slatkin (1985) and Slatkin and Barton (1989). Larval collections were also grouped by geographic area as identified in Table 1 (see Table 9) and by region (Maritimes, areas 1 and 2; Ontario, areas 3–6; west, areas 7–9) (see Table 7).

TABLE 3. Allele frequencies in larvae from selected populations throughout the range of the spruce budworm[1]

	Population[2]												
Locus[3]	GAN1	FED1	VAC1	DES4	MAN2	GRA2	BSL2	CEC5	ING4	EAR7	MOS1	NEL6	LID4
PGI-1													
(*n*)	53	80	81	96	80	65	90	97	113	80	83	70	63
C 1.43	0.038	0.044	0.031	0.042	0.038	0.046	0.022	0.036	0.035	0.038	0.024	0.029	0.024
D 1.00	0.887	0.931	0.889	0.906	0.856	0.946	0.889	0.923	0.898	0.900	0.910	0.914	0.889
E 0.57	0.075	0.019	0.068	0.052	0.075	0.008	0.072	0.036	0.058	0.063	0.060	0.050	0.079
F 0.29	0	0.006	0.012	0	0.031	0	0.017	0.005	0.009	0	0.006	0.007	0.008
PGM-1													
(*n*)	53	80	91	96	80	65	90	97	113	78	83	70	63
B 1.30	0	0	0	0	0	0	0	0	0	0	0	0	0.008
C 1.16*	0	0.006	0.005	0.005	0.006	0	0	0.005	0.004	0	0	0	0.008
D 1.08	0.104	0.094	0.093	0.141	0.075	0.023	0.089	0.057	0.102	0.083	0.072	0.071	0.079
E 1.00	0.887	0.875	0.879	0.833	0.906	0.962	0.889	0.918	0.876	0.904	0.849	0.900	0.857
G 0.91	0.009	0.025	0.022	0.016	0.013	0.015	0.022	0.021	0.009	0.013	0.078	0.029	0.048
H 0.82*	0	0	0	0.005	0	0	0	0	0.009	0	0	0	0
MDH-1													
(*n*)	27	62	35	90	35	48	39	97	106	80	22	63	40
A 1.37	0.037	0.040	0.029	0.039	0	0.010	0.051	0	0.014	0.006	0.023	0.008	0.025
B 1.00	0.926	0.944	0.971	0.944	1.000	0.990	0.936	1.000	0.986	0.981	0.977	0.992	0.962
C 0.61*	0.037	0.016	0	0.017	0	0	0.013	0	0	0.013	0	0	0.013
IDH-1													
(*n*)	53	80	91	96	80	65	90	97	110	80	83	70	63
A 1.14	0.066	0.069	0.044	0.068	0.075	0.023	0.056	0.046	0.023	0.069	0.036	0.064	0.056
B 1.00	0.915	0.925	0.934	0.917	0.894	0.915	0.928	0.918	0.955	0.881	0.928	0.921	0.913
C 0.84*	0	0	0	0	0	0	0	0	0	0.006	0.006	0	0.016
D 0.64	0.019	0.006	0.022	0.016	0.031	0.062	0.017	0.036	0.023	0.044	0.030	0.014	0.016
IDH-2													
(*n*)	17	40	42	39	33	30	49	51	33	40	46	28	25
A 1.79	0.088	0.050	0.060	0.038	0.015	0.083	0.071	0.049	0.015	0.063	0.076	0	0.040
B 1.00	0.824	0.950	0.940	0.962	0.985	0.900	0.908	0.951	0.985	0.938	0.924	1.000	0.960
C 0.37*	0.088	0	0	0	0	0.017	0.020	0	0	0	0	0	0
LDH-1													
(*n*)	17	39	42	44	40	29	50	49	57	40	45	26	28
C 1.15	0	0.013	0.024	0.034	0.025	0.069	0	0.020	0.053	0.050	0.011	0.038	0.018
D 1.00*	1.000	0.987	0.976	0.966	0.975	0.931	0.990	0.980	0.947	0.950	0.989	0.962	0.982
E 0.84	0	0	0	0	0	0	0.010	0	0	0	0	0	0
AAT-1													
(*n*)	17	40	43	48	37	30	50	52	56	39	46	28	28
B 1.18*	0	0	0	0.010	0	0	0	0	0	0.013	0.011	0	0
C 1.00	0.441	0.738	0.605	0.719	0.689	0.583	0.610	0.615	0.714	0.551	0.652	0.679	0.607
D 0.81	0.441	0.237	0.314	0.229	0.257	0.300	0.350	0.346	0.250	0.397	0.315	0.304	0.357
E 0.62	0.118	0.025	0.081	0.042	0.054	0.117	0.040	0.038	0.036	0.038	0.022	0.018	0.036
EST-2													
(*n*)	53	80	90	102	80	65	90	97	113	79	82	70	63
A 1.09*	0.009	0	0	0	0	0	0.006	0	0.031	0	0	0.021	0.024
B 1.03	0	0	0	0.059	0.038	0.046	0.094	0.067	0.097	0.114	0.024	0.043	0.087
C 1.00	0.896	0.900	0.950	0.843	0.850	0.915	0.783	0.856	0.823	0.854	0.823	0.864	0.706
D 0.96	0.075	0.075	0.044	0.093	0.087	0.038	0.117	0.057	0.049	0.032	0.104	0.029	0.127
E 0.89*	0	0.025	0.006	0.005	0.019	0	0	0.021	0	0	0.049	0.043	0.056
F 0.86*	0.019	0	0	0	0.006	0	0	0	0	0	0	0	0

TABLE 3. (*Concluded*)

	Population[2]												
Locus[3]	GAN1	FED1	VAC1	DES4	MAN2	GRA2	BSL2	CEC5	ING4	EAR7	MOS1	NEL6	LID4
EST-3													
(*n*)	53	79	86	104	78	64	86	95	112	72	83	67	63
G 1.09*	0	0.006	0.006	0	0	0	0	0	0	0	0.030	0	0
A 1.04	0.057	0.044	0	0.034	0.013	0.016	0	0.016	0.013	0.035	0.030	0.015	0
B 1.00	0.868	0.880	0.837	0.865	0.840	0.797	0.959	0.837	0.938	0.840	0.837	0.955	0.905
C 0.95	0.075	0.044	0.128	0.082	0.135	0.172	0.041	0.105	0.027	0.076	0.072	0.022	0.079
D 0.89	0	0.019	0.012	0.010	0.013	0.016	0	0.011	0.009	0.049	0.030	0.007	0
E 0.84*	0	0.006	0.017	0.010	0	0	0	0.021	0.013	0	0	0	0.016
F 0.75	0	0	0	0	0	0	0	0.011	0	0	0	0	0
EST-5													
(*n*)	52	80	91	103	80	65	90	97	112	80	83	69	63
A 2.61	0	0	0.005	0	0	0	0	0	0	0	0	0	0
B 2.36	0.019	0	0.016	0.005	0.019	0.008	0	0	0	0.006	0	0.007	0
C 2.11	0.087	0.038	0.049	0.083	0.144	0.038	0.072	0.077	0.067	0.087	0.036	0.014	0.032
D 1.90	0.077	0.056	0.049	0.044	0.044	0.031	0.056	0.082	0.058	0.063	0.072	0.065	0.048
E 1.69	0.529	0.531	0.522	0.529	0.475	0.485	0.439	0.474	0.464	0.463	0.494	0.449	0.492
F 1.42	0.029	0.063	0.033	0.039	0.031	0.054	0.033	0.098	0.080	0.031	0.127	0.043	0.079
G 1.22	0.010	0.038	0.044	0.029	0.044	0.031	0.061	0.010	0.054	0.044	0.030	0.014	0.087
H 1.00	0.240	0.256	0.242	0.257	0.219	0.346	0.311	0.253	0.263	0.306	0.217	0.406	0.254
I 0.63	0.010	0.019	0.038	0.015	0.025	0.008	0.028	0.005	0.013	0	0.024	0	0.008
EST-6													
(*n*)	34	53	72	98	56	48	83	53	86	64	75	3	60
A 0.08*	0	0	0	0	0	0	0	0.009	0	0	0	0.016	0
B 0.06	0.029	0.038	0.076	0.071	0.080	0.115	0.060	0.075	0.047	0.008	0.040	0.008	0.067
C 0.03	0.971	0.953	0.903	0.908	0.911	0.854	0.940	0.906	0.948	0.992	0.947	0.960	0.933
D–0.03	0	0.009	0.021	0.020	0.009	0.031	0	0.009	0.006	0	0.013	0	0

[1] Full table for 68 collections available from the author.
[2] Population code in Table 1.
[3] Letter designation and travel distance. Males only for IDH-2, LDH-1, and AAT-1. *Rare alleles.

For study of geographical differences at individual loci, allele frequencies (Table 3) were converted to arcsine square-root percentage values (Snedecor 1946). For these analyses locations were described by UTM numbers (Tables 1 and 2); the 'zone' designation was combined with the 'easting' value to give a four-digit code related to longitude (UTM1), but decreasing toward the west. 'Northings' were used as a three-digit code (UTM2) which increased toward the north, to evaluate changes related to latitude. Multiple samples at the same locations were treated as replicates. Correlation analyses were performed between converted allozyme frequencies at each locus and the location and infestation codes. Where significant R values were detected, regression analyses were also performed.

Data for the trapped males were analysed separately and were grouped by geographic area (east, collections 1–9; west, 10–29) and by defoliation level in the area (Table 2). Defoliation level designations used were similar to those used for larval collections but another level, 'very light', was added to describe locations without visible defoliation; the moderate and severe levels were pooled because of low numbers of locations in both categories.

The data were analysed for the presence of rare alleles (Pashley and Bush 1979) at the 33 locations. Wilhite and Stock (1983) defined rare alleles in *C. occidentalis* Freeman as 'alleles not detected in at least four collections (29%) where $n \geq 50$ but present in more than one collection'. Because of the larger number of collections in my study this definition was modified to 'alleles not detected in at least 25% of the locations where $n \geq 50$ (eight out of

TABLE 4. Allele frequencies in trapped males from selected populations throughout the range of the spruce budworm

	Population[1]										
Locus[2]	PKI1	PDE2	PMA2	PGR2	PBS2	BNO2	BSR1	YCA1	YMO1	YDA1	YTO3
PGI-1											
(*n*)	58	60	36	80	78	22	40	35	65	38	37
1.43	0.017	0.033	0.083	0.044	0.026	0.045	0.013	0	0.038	0.026	0.081
1.00	0.914	0.908	0.861	0.894	0.904	0.886	0.900	0.943	0.877	0.934	0.730
0.57	0.069	0.058	0.056	0.063	0.071	0.068	0.087	0.057	0.077	0.039	0.189
0.29	0	0	0	0	0	0	0	0	0.008	0	0
PGM-1											
(*n*)	58	60	36	80	78	22	40	41	65	38	36
1.30	0	0	0	0	0	0	0	0	0	0	0
1.16*	0	0	0	0	0	0	0	0	0	0	0
1.08	0.043	0.092	0.069	0.069	0.064	0.045	0.063	0.037	0.085	0.079	0.083
1.00	0.931	0.867	0.931	0.913	0.923	0.955	0.925	0.927	0.815	0.908	0.819
0.91	0.017	0.042	0	0.019	0.013	0	0.013	0.024	0.046	0.013	0.042
0.82*	0.009	0	0	0	0	0	0	0.012	0.038	0	0.014
MDH-1											
(*n*)	58	39	26	80	78	22	40	40	64	38	37
1.37	0.009	0.013	0	0.025	0	0.023	0.025	0	0.008	0	0
1.00	0.974	0.962	1.000	0.944	1.000	0.977	0.962	1.000	0.992	1.000	1.000
0.61*	0.017	0.026	0	0.031	0	0	0.013	0	0	0	0
IDH-1											
(*n*)	58	60	36	80	78	22	40	40	65	38	36
1.14	0.017	0.025	0.042	0.031	0.038	0.023	0.038	0	0.023	0	0
1.00	0.948	0.958	0.917	0.944	0.929	0.955	0.950	0.837	0.738	0.816	0.819
0.84*	0	0	0	0	0	0	0	0	0.008	0	0
0.64	0.034	0.017	0.042	0.025	0.032	0.023	0.013	0.162	0.231	0.184	0.181
IDH-2											
(*n*)	57	60	36	79	77	22	40	40	65	38	36
1.79	0.026	0.042	0.069	0.057	0.065	0	0.025	0.013	0.008	0	0.014
1.00	0.974	0.950	0.917	0.943	0.935	1.000	0.975	0.988	0.977	1.000	0.986
0.37*	0	0.008	0.014	0	0	0	0	0	0.015	0	0
AAT-1											
(*n*)	55	49	19	10	10	22	40	50	65	38	37
1.18*	0	0.010	0	0	0	0	0	0	0	0	0
1.00	0.700	0.633	0.895	0.600	0.750	0.682	0.712	0.810	0.723	0.803	0.865
0.81	0.245	0.306	0.105	0.350	0.100	0.318	0.237	0.140	0.262	0.158	0.122
0.62	0.055	0.051	0	0.050	0.150	0	0.050	0.050	0.015	0.039	0.014

[1] Population code in Table 2.
[2] Letter designations shown in Table 3. *Rare alleles.

30) but present in two or more'. Sixteen alleles were identified that fulfilled this definition (Tables 3 and 4).

Results

Allozymes in *Choristoneura*. PGM, PGI, and LDH had one locus; AAT, IDH, and MDH had two loci but only for IDH was the second locus routinely measurable. The esterases had at least five loci; EST-5 was dependably scored, EST-2, EST-3, and EST-6 were less

TABLE 5. Evidence of sex-linkage at four loci in progenies of *Choristoneura fumiferana*

Locus Mating[1]	n[1]	Genotype numbers, F/M						df	Chi-square[4]
AAT-1		**CC**	**CD**[2]	**CE**[2]	**DD**	**DE**	**EE**		
CC × DD	2		0/16		19/0			1	0
DD × CC	2	13/0	0/14					1	0
CC × CD	6	23/22	0/19		22/0			3	0.242
DD × CD	2	13/0	0/11		5/6			3	5.026
EE × CC	1	6/0		0/5				1	0
CC × CE	2	8/6		0/9			7/0	3	0.666
LDH-1		**CC**	**CD**[2]	**CE**	**DD**	**DE**[2]	**EE**		
CC × DD	1		0/10		9/10			1	0
DD × CD	4	17/0	0/15		21/28			3	4.35
DD × DE	1				2/2	0/4	1/0	3	1.00
IDH-2		**AA**	**AB**[2]	**BB**					
AA × BB	1		0/8	8/0				1	0
BB × AB	3	6/0	0/4	10/10				3	3.57
EST-2[3]		**BB**	**BC**[2]	**BD**[2]	**CC**	**CD**[2]	**DD**		
BB × BC	1	3/0	0/5		1/0			3	6.00
CC × BC	2	8/0	0/8		9/13			3	1.25
CC × CD[5]	2				18/6	0/20	3/1	3	(18.01**)
DD × BC[5]	1	6/0		0/8	3/1	0/4		3	(2.31)

[1] Parental genotypes (F × M) (see Table 3) and number of families (n).
[2] Heterozygous progeny showing absence of females.
[3] Parents not known; based on progeny analysis (parents known from other loci).
[4] Chi-square tested for X-linkage model; data show χ^2 values; none significant except those marked with ** where $p < 0.001$.
[5] Presence of homozygous C or D male indicates a difficulty in scoring, sexing, or interpretation in these two families.

dependable (cf. Wilhite 1979) but were recorded for most insects. Larvae, pupae, and adults were all suitable for isozyme analysis but only six of the enzymes surveyed were measurable in field-collected males (esterases have too low activity). There was no evidence of differences in allozyme frequencies among the three stages. Allozyme identification and band positions are in good agreement with those of Stock and Castrovillo (1981) for *C. fumiferana*.

Analysis of breeding results revealed the expected Mendelian ratios for four loci (with types of matings and number of families) as follows: PGI (6; 36), PGM (10; 47), MDH (7; 50), and IDH-1 (6; 36). Three loci were found to be clearly sex-linked: IDH-2 (3; 35), AAT-1 (11; 84), and LDH (4; 46) (Table 5). AAT is produced by two loci in *Choristoneura* species (Stock and Castrovillo 1981). The anodal locus, AAT-1, is dimeric and is sex-linked, hemizygotic in females (May et al. 1977). The sex-linked nature of IDH-2 and LDH-1 has not been reported previously. From the breeding studies, EST-2 (6; 36) appeared to be sex-linked.

Only four of the esterase loci were useable: EST-2, 3, 5, and 6. Est-2 has six alleles; although it appeared to be sex-linked, 32 heterozygous females (out of 1069) were encountered in field collections compared with 79 (of 1078) heterozygous males. However, because evidence from sexing records and other sex-linked loci failed to reveal sexing errors, EST-2 has not been treated as a sex-linked locus. Est-5 has nine alleles and there is evidence for one or more null alleles (Stock and Castrovillo 1981). Among the 41 progenies analysed,

32 different types (of the possible 6000) were recognized and genotype ratios in all but two conformed to expected Mendelian ratios with no evidence of sex-linkage. EST-3 is monomeric; all 35 progenies showed shortages of heterozygotes and the less common homozygotes. There was no evidence of sex-linkage and although genotype ratios in all progenies were consistent with expectations, analysis of pooled families showed highly significant deficiencies (chi-square) of heterozygotes (percentage of expected: females, 50; males, 33), suggesting the presence of null alleles. There are no data from known parental genotypes for EST-6. Of 13 progenies analysed, 10 were homozygous for the major allele. The other three families, although small, had two or three genotypes and conformed to standard genetic ratios with no evidence of sex-linkage.

Sixteen rare alleles were identified in larvae (Table 3) using the modified definition. In addition to those alleles identified in Table 3, PGI-1:2.00 and MDH-1:0.47 were also classified as rare alleles. An additional six alleles occurred only once and were too rare to be used. Fourteen of these alleles appeared to be the same alleles identified as rare in *C. occidentalis* (Wilhite and Stock 1983); however, only nine could be classified as rare in *C. fumiferana*. Of the remaining five, two were too rare to meet the criteria and three were common to more than 74% of the collections. One of the latter, AAT-1:C [equivalent to AAT:160 of Wilhite and Stock (1983)] is the most common allele in *C. fumiferana*, as reported previously (Stock and Castrovillo 1981). PGI:E [PGI:50 of Wilhite and Stock (1983)] was also present in all collections.

Population Genetics. In the 68 samples the great majority of values for five loci conformed to Hardy-Weinburg expectations (HWE). The number of significant deviations from HWE at these loci, where $P < 0.01$ based on exact probabilities, were as follows: PGI, 1; PGM, 3; EST-5, 9; MDH-1 and IDH-1, none. The three sex-linked loci also conformed to HWE when analysed for males only. Exceptions at these loci were AAT-1, 1; LDH-1, 4; and IDH-2, 20. On pooled results for eight loci, only 4.0% (22 of 544) exceeded HWE at the 1% level of probability. However, by itself, IDH-2 had a 29% level of nonconformity to HWE, which suggests some residual problem with its genetics or scoring. Three esterase loci frequently failed to meet HWE ratios: EST-2, 94%; EST-3, 43%; and EST-6, 42%. In field-collected larvae EST-6 showed substantial heterozygote deficiencies (percentage of expected: females, 59.8; males, 59.7). Although justification for the continued inclusion of EST-2, EST-3, and EST-6 in the tests was not strong, they were used by Stock and Robertson (1980) and have been retained here.

Allozyme frequencies in 13 locations are shown in Table 3. The full data set (68 samples) was used in all analyses. Mean values were as follows: sample size, 62.9; alleles/locus, 3.4 ± 0.04; and polymorphism, 93.5% (after removal of females from sex-linked loci; Table 1). In *C. occidentalis* polymorphism ranged from 31 to 52% and averaged 49% for all larvae from 15 locations (Wilhite and Stock 1983). Average heterozygosity in the present study was $18.1 \pm 0.30\%$ ($17.9 \pm 0.3\%$ based on 33 locations). This was below the expected level if Hardy-Weinberg ratios prevail at all loci, but close to the value of 16.8 reported by May et al. (1977) and within the range of 9–21% reported for *C. occidentalis* by Wilhite and Stock (1983).

Data from both sexes were pooled in these analyses (except for the sex-linked loci) because differences, tested in the data set for four populations × 5 years, were negligible. Based on all 11 loci and including females in the sex-linked loci, the percentage of polymorphic loci averaged the same in both sexes (72.7), and the genetic distance (D) value between sexes (0.004) was the same as the within sex distances. In the eight non-sex-linked loci the mean heterozygosity in males was not significantly greater than that of females (0.189 ± 0.059 cf. 0.176 ± 0.062).

Geographic differences. From samples collected over 8 years, *C. fumiferana* was found to be generally uniform in allozyme complement at all loci tested, across its extensive

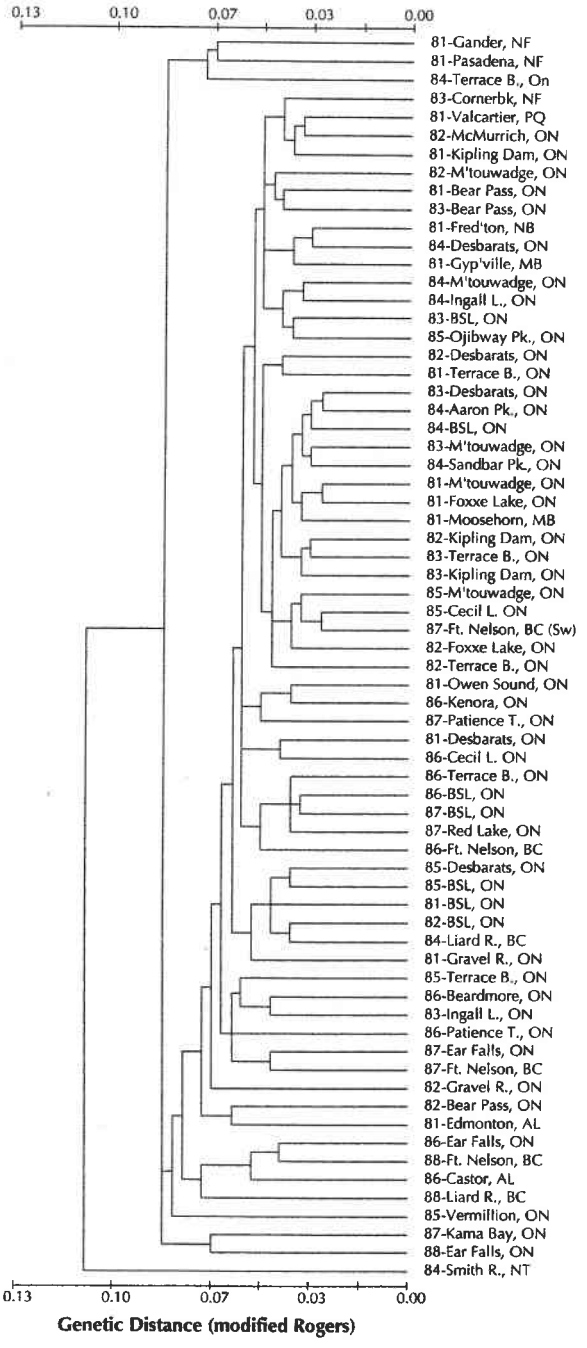

FIG. 2. Phenogram of genetic relationships [UPGMA clustering of Rogers' (modified) distance coefficients] among larval collections of *Choristoneura fumiferana* (Wright 1978). Cophenetic correlation = 0.822.

https://doi.org/10.4039/Ent128219-2 Published online by Cambridge University Press

TABLE 6. Summary of F statistics[1] at 11 loci for larvae from all locations[2]

Locus	F(IS)	F(IT)	F(ST)
PGI-1	0.044	0.052	0.009
PGM-1	0.024	0.037	0.012
MDH-1	−0.023	0.006	0.029
IDH-1	0.015	0.023	0.007
IDH-2	0.633	0.644	0.032
LDH-1	0.235	0.254	0.025
AAT-1	0.026	0.047	0.021
EST-2	0.791	0.799	0.039
EST-3	0.312	0.329	0.025
EST-5	0.137	0.147	0.012
EST-6	0.398	0.414	0.025
Mean	0.202	0.217	0.019

[1] Wright's F statistics (1978).
[2] Mean insects/locus/collection = 62.0 ± 2.3; 68 collections.

range from New Brunswick to British Columbia (Fig. 2). Genetic differentiation among populations (F_{ST}) was low for all individual loci and for the 11 loci together (mean F_{ST} = 0.019, Table 6) (Wright 1978). Most of the total genetic differentiation was within populations (F_{IS} = 0.202), particularly in IDH-2 and EST-2. F_{XY} values for the variance in populations compared with area, region, and total (Jennings and Philipp 1992) were all 0.020. Thus the proportion of allelic variation among individuals within populations ($1 - F_{XY}$) is 98% of the total variation (Preziosi and Fairbairn 1992). There was, nevertheless, significant heterogeneity among populations within all regions, as shown by chi-square values for most loci within regions, but particularly in Ontario and the west (Table 7). Loci showing significant P values in all three regions include IDH-2, EST-2, and EST-3. When grouped together there were significant differences across all regions at all loci except IDH-1.

Genetic uniformity among trapped males (six loci) was also high (Fig. 3). Population levels at several of these locations (Table 2) were too low for defoliation to be detected and mean sample size was only 23.1. F statistics (F_{ST} = 0.038) and F_{XY} values indicated only small differences among regions and populations; 96.2% of the variance was within populations. Contingency chi-square analyses confirmed the high degree of homogeneity among populations in the east but not in the west (Table 8A), where P values were highly significant for all six loci, showing an important differentiation between these regions. Chi-square values were also significant for several loci among populations classified as 'very light' and 'light' but only for one locus among those classified as 'moderate-to-severe'. Mean percentage heterozygosity was about the same in all three groups.

In larvae, genetic distances among the sample locations grouped into nine areas (Table 1) were very small (Table 9), as expected from Figure 2. The greatest individual genetic distances, those between the most distant collections (Newfoundland and British Columbia), and among collections from British Columbia, were still very low. Differences related to population density were equally small. Allozyme frequencies among trapped males (Table 4) were similar and average genetic distances among regions were all small (0.003–0.005). Allozyme frequencies in larvae and trapped males from nine locations from Ontario to northern British Columbia (not always in the same season) were almost identical (D = 0.002); percentage heterozygosity was slightly but not significantly greater in the larvae (17.4 ± 0.122 vs. 16.0 ± 0.007%). Because genetic distances throughout the matrix of

TABLE 7. Heterogeneity among larval populations grouped by region and all together

	Maritimes		Ontario		West		All	
Locus	df	*P*	df	*P*	df	*P*	df	*P*
PGI-1	8	0.120	255	0.000	30	0.000	335	0.000
PGM-1	8	0.592	255	0.000	40	0.000	402	0.000
MDH-1	8	0.177	102	0.000	20	0.000	201	0.000
IDH-1	8	0.504	153	0.000	30	0.300	201	0.088
IDH-2	8	0.002	102	0.000	20	0.004	134	0.000
LDH-1	4	0.389	50	0.000	10	0.000	134	0.002
AAT-1	8	0.058	153	0.024	30	0.029	201	0.002
EST-2	16	0.000	204	0.000	40	0.000	335	0.000
EST-3	20	0.000	306	0.000	50	0.000	402	0.000
EST-5	28	0.209	357	0.000	70	0.000	536	0.000
EST-6	8	0.191	153	0.000	30	0.000	268	0.000
Totals	124	0.000	2091	0.000	360	0.000	3149	0.000

[1] Based on 68 larval collections (33 locations) 1981–1988. (Constitution of regions described in text).

locations were so small no attempt was made to analyse relationships between *D* values and inter-location distances (Richardson et al. 1986).

Correlation analysis, however, showed that the frequencies of AAT-1 (two alleles: C, E), EST-2 (three alleles: B, C, and D), and EST-5 (one allele: I) were related to location (UTM values). The regressions for frequencies of AAT-1 alleles had the highest R^2 values; frequencies of alleles C and E were related to each other and to collection location. Allozyme E was significantly regressed on UTM1 (≈ longitude) ($R^2 = 27.9\%$, $P \leq 0.0001$) but not on UTM2 (≈ latitude), however the multiple regression of E on both grid values gave the highest R^2 values ($R^2 = 30.4$, $P \leq 0.0001$). The frequency of E in eastern collections is 4.9 times that at the northwestern limits of the distribution. Frequencies of AAT-1:C showed a weak trend with UTM1 and UTM2 ($R^2 = 18.3$, $P = 0.001$), with the slope opposite to that for AAT-1:E, frequencies in the east being 0.88 times those in the northwest. There was also a weak cline in EST-2:B ($R^2 = 16.4$, $P \leq 0.006$) from a frequency of 0.010 in the northwest to almost 0 in the east. EST-2 allozymes C and D had trends in the opposite direction and regression values were also significant. In EST-5 only the uncommon allele I showed an association between allele frequency and location, ranging from 0 in the west to 0.043 in eastern locations ($R^2 = 8.5$, $P \leq 0.0001$).

The distribution of the 16 rare alleles over the 33 larval collections sites did not reveal any trends related to geographical location. The number of rare alleles per location ranged from 0 to 15 (mean 5.9 over 30 locations where $n > 50$). The number of rare alleles per area ranged from 5 to 15 (Table 9); eight were found in >40% of the samples, three were present in only two locations each. Four of the rare alleles (IDH-1:C, AAT-1:B, EST-6:A, and PGM-1:H) were not found east of central Ontario (area 4, Table 9) (out of a total of 485 insects measured) but were generally present west of that. EST-3:E, IDH-2:C, and PGM-1:C were more common in the east and central areas (areas 3, 4, and 5) but less so west of Manitoba (from 593 insects). One rare allele, PGI:B (2.00), was found only in areas 3, 4, and 5 and was totally absent outside that region. However, the remainder of the rare alleles (seven) were scattered across the entire range of collection points and there were no relationships that allowed grouping into similarity units.

Population cycle differences. Allozyme frequencies in the larval samples did not show any significant correlations with INF values, except for three of the 62 allozymes measured: AAT-1:D and AAT-1:E ($R^2 = 11.9$, $P = 0.006$) and EST-2:A; the overall significance was

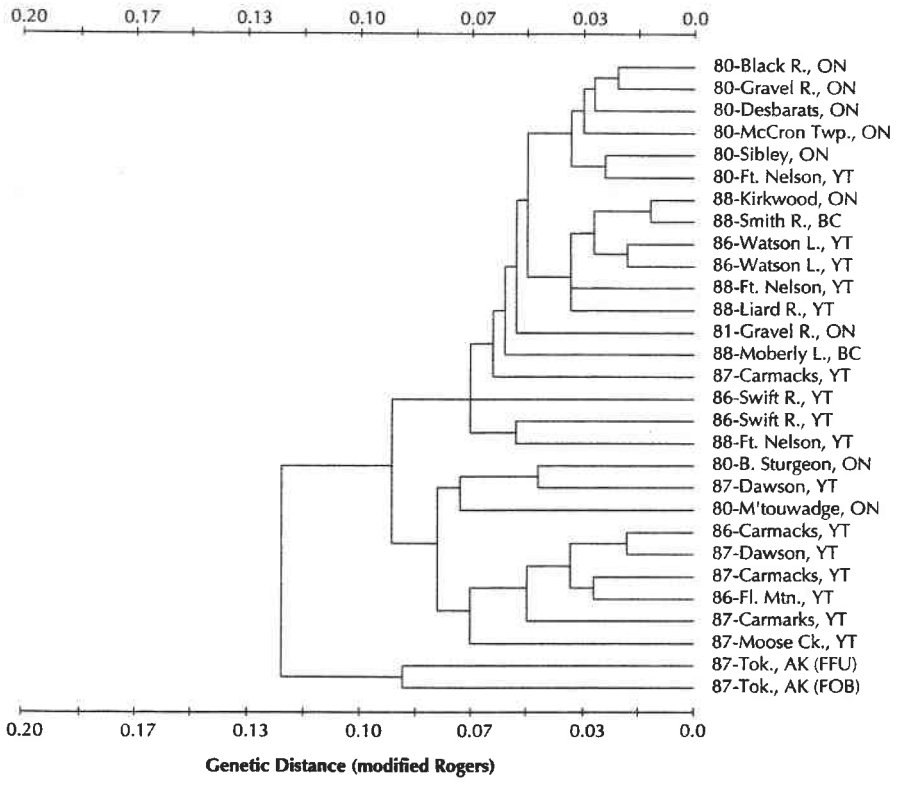

FIG. 3. Phenogram of genetic relationships [UPGMA clustering of Rogers' (modified) distance coefficients] among trapped males of *Choristoneura fumiferana* (Wright 1978). Cophenetic correlation = 0.836.

negligible. Furthermore, the weak relationship for AAT-1 allozymes appeared to be explained adequately by the reduced variability of frequencies of AAT-1:E in older populations [for INF 3–5, f(E) 0–0.085; for INF 14–19, f(E) 0.060–0.085]. In multiple regressions involving location as well as INF, the latter was no longer significant. Heterozygosity (based on transformed data), however, gradually decreased over successive years of the outbreaks (regression: $R^2 = 23.5\%$, $P = 0.002$). The declines appeared to occur together across the entire range, but some individual locations showed unexpectedly high values on some years. This could reflect an influx of moths from another area.

Among trapped males there were differences in allozyme frequencies (chi-square) related to defoliation level in the very light and light areas but only one among the moderate-to-severe areas (Table 8B). The significant value for AAT-1 in areas of moderate-to-severe defoliation probably arises from the geographic cline in allele frequencies discussed above. Among populations causing light and very light defoliation, values for all six loci except MDH-1 were significant in one or other group, and P values for totals were highly significant, indicating a lack of homogeneity among locations at low population levels.

In these results there was no evidence among larvae or trapped males of differences in allozyme constitution or frequencies between beginning or incipient outbreaks, populations causing severe defoliation over several years, or post-outbreak populations. There was no allozyme whose appearance or frequency could signal a change in the constitution of the population.

TABLE 8. Contingency chi-square analysis of six loci in trapped males averaged by region (A) and by defoliation level (B)

Table 8A[1]

	East		West	
Locus	df	*P*	df	*P*
PGI-1	24	0.06025	38	0.00156
PGM-1	16	0.42131	95	0.00000
MDH-1	16	0.14064	38	0.00001
IDH-1	16	0.53492	38	0.00000
IDH-2	8	0.76767	38	0.00000
AAT-1	16	0.03064	38	0.00000
Totals	96	0.03781	144	0.00000

[1] No. of collections: east, 9; west, 20.

Table 8B[2]

	Very light		Light		Moderate-to-severe	
Locus	df	*P*	df	*P*	df	*P*
PGI-1	18	0.00027	18	0.81212	24	0.14960
PGM-1	27	0.01136	26	0.00000	16	0.23162
MDH-1	9	0.37377	18	0.01662	16	0.39466
IDH-1	18	0.00003	18	0.00000	16	0.14689
IDH-2	9	0.01379	18	0.00016	8	0.16116
AAT-1	18	0.00094	18	0.01802	16	0.00006
Totals	99	0.00000	126	0.00000	96	0.27561

[2] No. of collections: very light, 10; light, 10; M-S, 9.

Larval data subset. Analysis of the subset of larval data from four locations for five to eight consecutive seasons confirmed the lack of correlation between heterozygosity and infestation status, host, or geographic location. There was, however, a reduction in heterozygosity in successive years of the outbreaks, although there were occasional surprisingly high values in the latter years (Table 10). Among successive larval collections in the same season at Black Sturgeon Lake a further decline in heterozygosity occurred in both 1986 and 1988 (Table 10), so that heterozygosity formed a weak but significant regression with a negative slope ($R^2 = 23.5\%$, $P = 0.002$). Where larvae and adult males were both collected at the same location heterozygosity was lower in adults, but the difference was not significant.

In this same data set there were also significant differences in allozyme frequencies with time for all of the loci except PGI-1 and AAT-1, in at least one of the four populations and in totals (chi-square, Table 11). However, only EST-2 was consistently different for all four locations. The largest number of significant values occurred in the Black Sturgeon Lake collections and least in the Desbarats collections.

Ten of the 17 rare alleles were present in all four populations of this data set and only seven were missing from one or more, but the differences were not consistent among the groups. The number of rare alleles per collection increased from four in year 1 to eight in years 5 and 6 and then decreased to 4.5 and 2.0 in the 7th and 8th years. This value reflected

TABLE 9. Matrix of genetic distances[1] among 11 loci in larvae averaged by area

Area[2]	No. of pops.	Area[2] 1	2	3	4	5	6	7	8	9	NR[3]
1 Nfld.	3	0.001									5
2 N.B./P.Q.	2	0.003	0.001								6
3 Ont. east	7	0.003	0.001	0.001							12
4 Ont. CE[2]	22	0.003	0.001	0.001	0.002						12
5 Ont. CW[2]	7	0.002	0.002	0.001	0.002	0.001					15
6 Ont. west	16	0.004	0.002	0.002	0.002	0.003	0.003				13
7 Manitoba	2	0.004	0.000	0.002	0.002	0.002	0.002	0.001			5
8 Alberta	2	0.009	0.003	0.004	0.004	0.004	0.004	0.004	0.005		10
9 B.C.	7	0.006	0.004	0.004	0.004	0.003	0.004	0.004	0.006	0.005	11

[1] Nei (1978) unbiased genetic distance.
[2] Area number (Table 1). In Ontario: CE = east-central; CW = west-central.
[3] NR = number of rare alleles.
[4] Mean range over all combinations 0.000–0.009.

positively the number of larvae sampled, especially in the early and late years (1980, 80; 1981–1985, 317; 1986, 154; 1987, 83). Some rare alleles were more common in the early years of the outbreak and others in the latter part, but several were found throughout the entire period. None of the differences appeared to be significant and there was no evidence of a trend during 8 successive years sampling from these four outbreaks.

Discussion

There were strong similarities in the genetic constitution of budworm from all locations, as clearly illustrated by the phenograms based on modified Rogers' distance coefficients (Wright 1978) (Figs. 2 and 3). With the exception of the collections from the ends of the distribution, all are very closely grouped together (Rogers' '*D*' = 0.08 or less) and there were no groupings related to year, location, or region (Fig. 2). For example, Gravel River, Ont. (82), Fort Nelson, B.C. (87, 88), Ingall Lake, Ont. (83), and Edmonton, Alta. (83) all fall

TABLE 10. Mean percentage heterozygosity at 11 loci in *Choristoneura fumiferana* larvae at four locations on successive years and successive collections the same year

Location[2]	Mean percentage heterozygosity[1] 1980	1981	1982	1983	1984	1985	1986	1987	1988
Desbarats (1970)	—	15.6	21.0	17.6	18.1	17.6	—	—	—
Manitouwadge (1975)	18.5	21.6	20.0	19.4	20.9	17.2	—	—	—
Terrace Bay (1982)	—	21.3	20.9	18.7	27.3	20.5	16.3	—	—
Black Sturgeon (1981)[3]	—	19.9	19.6	21.9	15.7	16.5	21.3	14.0	16.2
	—	16.7	—	—	19.7	13.9	19.2	—	17.1
	—	—	—	—	—	16.3	15.9	—	15.7
	—	—	—	—	—	—	11.8	—	11.2

[1] Mean sample size per locus: 50.9 (SD 22.5), range 6.1–85.5. Mean Standard Error for % heterozygosity: 0.52%.
[2] Location and date of 1st year of M-S defoliation.
[3] Successive values in some years represent different collection dates during the larval feeding period.

TABLE 11. Contingency chi-square analysis at 11 loci in larvae from four populations[1] over 8 years

Locus	No. of alleles	*P* values for locations			
		DES	MAN	TER	BSL
PGI-1	5,6,4,4	0.186	0.169	0.032	0.045
PGM-1	4,4,5,3	0.001	0.000	0.049	0.000
MDH-1	3,3,3,3	0.033	0.002	0.000	0.016
IDH-1	4,3,3,4	0.105	0.349	0.376	0.000
IDH-2	2,2,2,3	0.233	0.002	0.233	0.089
LDH-1	2,2,2,2	0.029	0.271	0.857	0.000
AAT-1	3,3,3,3	0.131	0.239	0.214	0.332
EST-2	4,4,5,5	0.000	0.006	0.000	0.000
EST-3	6,5,5,6	0.111	0.000	0.000	0.000
EST-5	8,8,8,8	0.010	0.032	0.017	0.000
EST-6	3,3,4,4	0.311	0.054	0.000	0.000
Totals		0.000	0.000	0.000	0.000

[1] Data subset of Table 10 (1981–1988); for codes see Table 1.

closely together (Fig. 2). And the seven annual collections from Black Sturgeon Lake, Ont. (BSL) are spread across almost the entire phenogram. Furthermore, the one larval collection with the lowest population density that could represent an inter-outbreak population (Gravel River, 1982) is not appreciably different from other neighbouring samples nor is it separated by itself in the phenogram. There was, nevertheless, a concentration of eastern collections in the upper half of the phenogram and of western collections in the lower part.

Phenograms produced by the Distance Wagner procedure (Swofford 1981) were little different from those produced by cluster analysis, although the Cophenetic correlation was somewhat higher (based on modified Rogers' distance: coefficient = 0.902). There was some variation between phenograms in the composition of the subgroups but there was no common pattern or grouping related to location or outbreak status. Similarly, May et al. (1977) found no significant differences in genetic distance at 10 loci among budworm from seven sites spanning a 250-km distance in Maine.

The phenograms based on the trapped males were similar (Fig. 3), with collections from Ontario and the Yukon generally intermixed and with no order related to population level. As noted for the larvae, however, there was a weak east–west grouping of collections. The two collections from Tok, AK, are grouped at some distance from the other samples. These two collections are the farthest west and their genetic makeup could show the influence of hybridization with other taxa that may be present in Alaska.

There are some limitations to the study that may affect the validity or applicability of the results. Larval collections may be subject to bias related to the location of sampled trees, etc., but they are generally unbiased by attributes of the insects themselves, which may not be true for trapped moths (Löfstedt 1990). If pheromone sensitivity exhibits inter-moth variation of the same order as colour and other attributes of this species, trapping may be selective, and population characteristics based on trapped moths not fully representative. However, Löfstedt (1990) found variance in male response to be low. In addition, because of moth movement, trapped moths may not represent all the moths at that location or may include moths from other locations. However, allozyme frequencies from trapped males and larvae from the same areas were the same; *D* values in these comparisons were close to *D* values within the two groups. Except for the reduced number of loci measurable, data from trapped males are, therefore, comparable to those for larvae, and appear to be valid for

representing genetic composition in areas of very low populations, where larvae cannot be obtained.

Other possible sources of differences among the insects may include host and sex. The larval collections originated from locations where either one or both principal hosts were present (Tables 1 and 2), but it was not possible to establish the host for moths caught in pheromone traps. However, small pilot analyses using larvae from locations where one host predominated showed no differences in allozyme frequencies associated with host or gender of the insects. The same results have also been reported for *C. occidentalis* (Wilhite and Stock 1983). From this evidence and because of difficulty in determining host at several locations, subsequent analyses were done without reference to host or gender.

The usefulness of the results may also be limited by the small number of loci and the function of those allozymes in the development of the insect. To measure samples from a large number of locations, polymorphic loci measurable in two buffer systems were selected. This choice undoubtedly explains the high percentage polymorphism (93.5 ± 0.94). Because of the fundamental role of these enzymes in assimilation and intermediary metabolism, their comparative uniformity in all samples is probably not surprising. However, the high level of allozyme polymorphism is consistent with the highly variable nature of this budworm, as demonstrated for several morphological characters, for its phenology, and for other attributes (Harvey 1985; Volney and Cerezke 1992; and others). Most of this variation in genetic constitution finds expression within populations, but there are also appreciable differences between areas and regions. The high level of variation within populations is supported by the allozyme data but the lack of greater differences on the larger scales is surprising.

The identification of populations or demes of spruce budworm presents considerable difficulty. In most outbreaks it may be possible to find, at least in the early stages, distinct and separate 'populations'. However, in large outbreaks, such as those affecting eastern North America in 1944 and 1975 (Kettela 1983; Hardy et al. 1986), the infestation stretched almost continuously for 3000 km or more. Although there seems to be a valid basis for identifying as separate demes, outbreak populations in northeastern British Columbia, Ontario, and Newfoundland, even these distinctions may break down. The separate treatment of collections in the analyses appeared to be justified by their distribution in the phenograms (Figs. 2 and 3) and the F_{ST} values (Table 6). Separate analyses with replicates pooled to focus on the 33 locations produced negligible change in the results.

The measures of population density and the categorization of outbreak stage may appear somewhat arbitrary. Population density, expressed as numbers of larvae per unit of foliage weight (Régnière et al. 1989), is the preferred measure for population analysis. Tree defoliation, although directly related to the numbers of insects, is a more subjective measure. For most locations annual monitoring data were available for e.m. density, larval–pupal density, defoliation (ground and/or aerial survey), and/or pheromone traps. However, for some of the early collections and those from remote areas, only defoliation estimates were available. Consequently, for comparisons of effects related to insect density, defoliation estimates had to be used. However, this information was available over several years and for an eruptive species such as the spruce budworm permitted accurate description of the course of the outbreak and assignation of an outbreak stage to all samples; the resulting INF values are probably accurate to ±1 year. The use of 3 years advance of reaching 30 e.m./10 m^2 for calculation of INF values is based on the interpretation that increases in population density leading to defoliation start at least 2–3 years before defoliation becomes detectable and before e.m. density reaches levels that are reliably measured. It represents an interpolation of the criteria proposed by Morris (1954) for the differences between light and moderate infestation levels.

Analyses of relationships between allozyme frequencies and independent variables were difficult because the variables themselves were not independent. Thus eastern

collections (high values for UTM1) were almost all at low latitudes (low UTM2 values) and western collections mostly at high latitudes. Further, most of the early collections came from the east and later collections from the northwest; consequently, population cycle effects overlaid any geographic relationships. Although not entirely successful, multiple regression techniques helped separate these factors and their influence on population differences. The progressive decrease in heterozygosity in successive years was independent of location, and appeared to be a real measure of change in the population with time.

The results, in spite of these limitations, provide quantitative data for evaluation of population genetics of *C. fumiferana*. All genetic distance values among populations were found to be small. Similarly, genetic identities (I, Nei 1978) among larvae from the 68 location/year values were all very high (mean $I = 0.997$, $s_x = 0.051$), indicating a very close similarity among all locations. Stock and Castrovillo (1981) also found budworm from Maine to be very similar genetically to those from the Great Lakes area. For comparison, the mean I among intraspecific populations of several species was 0.967, based on 14 taxa (Brussard et al. 1985; Gooding et al. 1992). The same authors reported I values for subspecies, sibling species, and non-sibling species to be 0.897, 0.778, and 0.608, respectively. However, these levels of identity vary among genera and families.

Although this homogeneity is not unexpected from a single species, D values were smaller than in many species and smaller than might be expected of a highly variable species with a range of about 6000 km across diverse climate zones and habitat types. In much of this range the two principal hosts are intermixed and the lack of host-related differences in the insects is not surprising, although host-related differences in allozymes have been reported for some forest insects (Namkoong et al. 1979; Sturgeon and Mitton 1986). However, balsam fir, the most common host in the east, is much less common west of Manitoba and is absent from northwest Alberta, Yukon, and Alaska. The small number of significant differences at individual loci between budworm in different regions and over all regions, and the lack of any significant differences for all loci together (Table 7), further emphasize the uniformity of their genetic constitution in spite of clear host and other environmental differences.

Further, based on the rare alleles identified, populations did not form similarity units that had geographical validity. Wilhite and Stock (1983) were able to group samples of *C. occidentalis* from 13 locations in Idaho and Montana into three similarity groups based on genetic distances (max. $D = 0.130$, Nei 1978) and rare alleles. The maximum interpopulation D value in the present study (Nei 1978, 0.023; modified Rogers', 0.12; Fig. 2) was much lower and, except for the collections from Newfoundland and Smith River, B.C., all samples were closely grouped in the phenogram at a Rogers' distance of about 0.088 and below (Fig. 2). The use of 16 loci by Wilhite and Stock may in part account for this difference in results but the rare alleles common to both studies did not lead to a grouping of locations in this species; the conclusion of greater homogeneity in *C. fumiferana* (Harvey 1985) is inescapable.

The groupings in *C. occidentalis* were attributed to reduced gene flow caused by physical barriers and by historical differences in outbreak development (Wilhite and Stock 1983). The apparent homogeneity among *C. fumiferana* populations suggests no such barriers to gene flow, which might otherwise be expected because of the large geographical distances and the differences in outbreak history. Estimated levels of gene flow based on F_{ST} (Table 6) confirmed that they are relatively high ($N_M = 12.9$) (Slatkin 1985). Although rare alleles can also be used to measure gene flow, only the F_{ST} method was used because it is "more useful under realistic conditions" (Slatkin and Barton 1989).

There was, nevertheless, clear evidence of spatial variation on a fine scale as shown by the chi-square and correlation analyses. Most marked of these was the cline in comparative frequency of AAT-1 alleles. Similar latitudinal clines have been reported at the ADH locus

in *Drosophila melanogaster* Meig. (Diptera: Drosophilidae) (David et al. 1989) and at several loci in *Rhagoletis pomonella* (Walsh) (Diptera: Tephritidae) (Feder and Bush 1989). It is of note that the most common alleles at this locus in *C. fumiferana* (C and D) are different from those of all other coniferophagous *Choristoneura* (Stock and Castrovillo 1981; Harvey 1985). Moreover, the cline in the frequency of AAT-1:E, which is the most common in western species, decreases to its lowest values in the western samples of *C. fumiferana*, where this species is close to or sympatric with western species. This argues against any gene flow from those western species. The presence and significance of the cline in *C. fumiferana* in spite of the evident exchange of other genes with eastern populations are at present unexplained.

No relationship was found between infestation status (INF) and frequency of any allozyme when multiple regressions were used to separate effects of between-year differences. Some allozymes of PGM-1, MDH-1, IDH-2, and AAT-1 and the four esterase loci did show changes related to collection year. These modest decreases were in one or more of the minor allozymes and were associated with increases in the major allozyme. In AAT-1 there was a decrease over time in *f* (E), even when geographic effects (i.e. more C in the northwest where E was lowest) and distribution of collections were taken into account. Although these differences were relatively small they appear to be reflected in the reduction of percentage heterozygosity in successive years and could be attributable to differential survival and moth dispersal.

Because there is a marked homogeneity in genetic constitution of budworm populations, it is probably not surprising that we did not find any qualitative differences to distinguish early stages of an outbreak from declining or post-outbreak populations. Although reductions in relative heterozygosity did occur in later stages of the outbreaks, there were no quantitative differences in allozyme frequencies that appeared to be related to outbreak phase. This may indicate that the very low density inter-outbreak populations are not genetically different from outbreak populations.

Summary

Starch gel electrophoresis revealed only small differences in genetically determined enzymes among populations throughout the range of *C. fumiferana*. There were no significant differences related to phases of outbreaks. These results may have been predictable in view of the lack of a sound theoretical basis for the genetics of insect outbreaks (Mitter and Schneider 1987). Although different loci might have shown different results, the high degree of uniformity among 11 variable loci has been established and constitutes a significant part of the *C. fumiferana* genome. This evidence from both larvae and trapped males confirms that we are dealing with a single species which, in spite of its high level of allozyme polymorphism, nevertheless has a high degree of homogeneity in genetic constitution throughout its range, at several outbreak phases and in successive years of outbreak.

The genetic uniformity in *C. fumiferana*, as expressed by the mean F_{ST} (0.019, Table 6), is similar to values reported for lepidopterous species where interbreeding occurs throughout the range of the species (Pashley et al. 1985) and where there are no signs of differentiation. This value also resembles that found in large continuous plant populations where "there is essentially no stochastic variation if the study area is sufficiently large relative to the underlying structure" (Heywood 1991). Gene flow among outbreak populations in Ontario, Quebec, and the Maritime provinces appears feasible from what is known about movement of moths (Greenbank et al. 1980) but it is more difficult to understand through the discontinuous and infrequent populations across the northwest and into Alaska. However, this uniformity could signify that the species is of relatively recent origin and still in the process of differentiating.

Acknowledgments

The many collections used in this study were obtained with the help of many co-operators, most of whom were members of the Forest Insect and Disease Survey of the Canadian Forest Service in its several regional laboratories across Canada. To all those who assisted in this way, I express my sincere thanks. The series of annual collections (1980–1988) from Black Sturgeon Lake, Ontario (BSL) were part of a study of outbreak populations conducted by V. Nealis, to whom I express thanks. The assistance of many others in Forestry Canada both in Ontario and in other regions is also appreciated, as is the assistance of J. McNeil (University of Laval). Special thanks to S.H. Berlocher and F. Sperling for manuscript review and advice on analytical procedures. I also thank W.J.A. Volney and colleagues in Sault Ste. Marie for helpful comments on the manuscript.

I thank Anne Hudson and Molly Stock for advice and assistance with electrophoresis techniques and data analysis. The processing of all the insects, their electrophoresis, and much of the scoring were skillfully performed by P.M. Roden, to whom I give special thanks.

References

Anonymous. 1969. The Universal Transverse Mercator Grid. Department of Energy, Mines and Resources, Ottawa, Canada. 13 pp.

Ayala, F.J., J.R. Powell, M.L. Tracey, C.A. Mourão, and S. Pérez-Salas. 1972. Enzyme variability in the *Drosophila willistoni* group. IV. Genic variation in natural populations of *Drosophila willistoni*. *Genetics* **70**: 113–139.

Berryman, A.A. 1987. The theory and classification of outbreaks. pp. 3–29 *in* Barbosa, P., and J.C. Schultz (Eds.), Insect Outbreaks. Academic Press, New York, NY. 578 pp.

Brussard, P.F., P.R. Ehrlich, D.D. Wilcox, and J. Wright. 1985. Genetic distances and the taxonomy of checkerspot butterflies (Nymphalidae: Nymphalininae). *Journal of the Kansas Entomological Society* **58**: 403–412.

Carson, H. 1968. The population flush and its genetic consequences. pp. 123–137 *in* Lewontin, R.C. (Ed.), Population Biology and Evolution: Proceedings of the International Symposium, June 7–9, 1967. Syracuse University Press, Syracuse, NY. 205 pp.

Castrovillo, P.J., and M.W. Stock. 1981. Electrophoretic techniques for detection of *Glypta fumiferanae*, an endoparasitoid of western spruce budworm. *Entomologia experimentalis et applicata* **30**: 176–180.

David, J.R., A. Alonso-Moraga, F. Borai, P. Capy, H. Merçot, S.F. McEvey, A. Munoz-Serrano, and S. Tsakas. 1989. Latitudinal variation of Adh gene frequencies in *Drosophila melanogaster*: A Mediterranean instability. *Heredity* **62**: 11–16.

Feder, J.L., and G.L. Bush. 1989. Gene frequency clines for host races of *Rhagoletis pomonella* in the midwestern United States. *Heredity* **63**: 245–266.

Forest Insect and Disease Survey (Canada). 1970–1980. Annual Report of the Forest Insect and Disease Survey. Canadian Forestry Service, Ottawa, Ont.

——— 1981–1989. Forest Insect and Disease Conditions in Canada. Canadian Forestry Service, Ottawa, Ont.

Gooding, R.H., B.M. Rolseth, J.R. Byers, and C.E. Herle. 1992. Electrophoretic comparisons of pheromotypes of the dingy cutworm, *Feltia jaculifera* (Gn.) (Lepidoptera: Noctuidae). *Canadian Journal of Zoology* **70**: 79–86.

Greenbank, D.O., G.W. Schaefer, and R.C. Rainey. 1980. Spruce Budworm Moth Flight and Dispersal: New Understanding from Canopy Observations, Radar, and Aircraft. *Memoirs of the Entomological Society of Canada* **110**: 49 pp.

Grisdale, D. 1970. An improved laboratory method for rearing large numbers of spruce budworm, *Choristoneura fumiferana* (Lepidoptera: Tortricidae). *The Canadian Entomologist* **102**: 1111–1117.

Hardy, Y., M. Mainville, and D.M. Schmidt. 1986. An Atlas of Spruce Budworm Defoliation in Eastern North America, 1938–80. Department of Agriculture, Forest Service, Cooperative State Research Service, Washington, DC. *Miscellaneous Publication* **1449**: 52 pp.

Harvey, G.T. 1980. Sampling low density spruce budworm populations for isozyme analysis. *The Canadian Entomologist* **112**: 969–970.

——— 1985. The taxonomy of coniferophagous *Choristoneura* (Lepidoptera: Tortricidae): A review. pp. 16–59 *in* Sanders, C.J., R.W. Stark, E.J. Mullins, and J. Murphy (Eds.), Recent Advances in Spruce Budworm Research: Proceedings of the CANUSA Spruce Budworms Research Symposium, 16–20 September 1984, Bangor, Maine. Supply and Services, Ottawa, Canada. 527 pp.

Harvey, G.T., and S.S. Sohi. 1985. Isozyme characterization of 28 cell lines from five insect species. *Canadian Journal of Zoology* **63**: 2270–2276.

Heywood, J.S. 1991. Spatial analysis of genetic variation in plant populations. *Annual Review of Ecology* and *Systematics* **22**: 335–355.

Hudson, A., and L.P. Lefkovitch. 1982. Allozyme variation in four Ontario populations of *Xestia adela* and *Xestia dolosa* and in a British population of *Xestia c-nigrum* (Lepidoptera: Noctuidae). *Annals of the Entomological Society of America* **75**: 250–256.

Jennings, M.J., and D.P. Philipp. 1992. Genetic variation in the longear sunfish (*Lepomis megalotis*). *Canadian Journal of Zoology* **70**: 1673–1680.

Kettela, E. 1983. A Cartographic History of Spruce Budworm Defoliation 1967 to 1981 in Eastern North America. *Canadian Forestry Service, Ottawa, Ontario, Information Report* **DPC-X-14**: 8 pp.

Levene, H. 1949. On a matching problem arising in genetics. *Annals of Mathamatical Statistics* **20**: 91–94.

Löfstedt, C. 1990. Population variation and genetic control of pheromone communication systems in moths. *Entomologia experimentalis et applicata* **54**: 199–218.

May, B., D.E. Leonard, and R.L. Vadas. 1977. Electophoretic variation and sex linkage in spruce budworm. *Journal of Heredity* **68**: 355–359.

Mitter, C., and J.C. Schneider. 1987. Genetic change and insect outbreaks. pp. 505–532 *in* Barbosa, P., and J.C. Schultz (Eds.), Insect Outbreaks. Academic Press, New York, NY. 578 pp.

Morris, R.F. 1954. A sequential sampling technique for spruce budworm egg surveys. *Canadian Journal of Zoology* **32**: 303–313.

Namkoong, G., J.H. Roberds, L.B. Nunnally, and H.A. Thomas. 1979. Isozyme variations in populations of southern pine beetles. *Forest Science* **25**: 197–203.

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

Pashley, D.P., and G.L. Bush. 1979. The use of allozymes in studying insect movement with special reference to the codling moth, *Laspyresia pomonella* (L.) (Olethreutidae). pp. 333–341 *in* Rabb, R.L., and G.G. Kennedy (Eds.), Movement of Highly Mobile Insects: Concepts and Methodology in Research. North Carolina State University, Raleigh, NC. 546 pp.

Pashley, D.P., S.J. Johnson, and A.N. Sparks. 1985. Genetic population structure of migratory moths: The fall armyworm (Lepidoptera: Noctuidae). *Annals of the Entomological Society of America* **78**: 756–762.

Poulik, M.D. 1957. Starch gel electrophoresis in a discontinuous system of buffers. *Nature* (London) **180**: 1477–1479.

Preziosi, R.F., and D.J. Fairbairn. 1992. Genetic population structure and levels of gene flow in the stream dwelling waterstrider, *Aquarius* (=*Gerris*) *remigis* (Hemiptera: Gerridae). *Evolution* **46**: 430–444.

Régnière, J., T.J. Lysyk, and M. Auger. 1989. Population density estimation of spruce budworm, *Choristoneura fumiferana* (Clem.) (Lepidoptera: Tortricidae) on balsam fir and white spruce from 45-cm mid-crown branch tips. *The Canadian Entomologist* **121**: 267–281.

Richardson, B.J., P.R. Baverstock, and M. Adams. 1986. Allozyme Electrophoresis. A Handbook for Animal Systematics and Population Studies. Academic Press, Sydney, Australia. 410 pp.

Royama, T. 1984. Population dynamics of spruce budworm *Choristoneura fumiferana*. *Ecological Monographs* **54**: 429–462.

Sanders, C.J. 1978. Evaluation of sex attractant traps for monitoring spruce budworm populations (Lepidoptera: Tortricidae). *The Canadian Entomologist* **110**: 43–50.

——— 1992. Sex Pheromone Traps for Monitoring Spruce Budworm Problems: Resolving Operational Problems. *Forestry Canada, Ontario Region, Sault Ste. Marie, Ontario, Information Report* **O-X-425**: 30 pp.

Sanders, C.J., R.W. Stark, E.J. Mullins, and J. Murphy (Eds.). 1985. Recent Advances in Spruce Budworm Research: Proceedings of the CANUSA Spruce Budworms Research Symposium, 16–20 September 1984, Bangor, Maine. Supply and Services, Ottawa, Canada. 527 pp.

Shaw, C.R., and R. Prasad. 1970. Starch gel electrophoresis of enzymes—a compilation of recipes. *Biochemical Genetics* **4**: 297–320.

Shepherd, R.F., T.G. Gray, and G.T. Harvey. 1995. Geographical distribution of *Choristoneura* species (Lepidoptera: Tortricidae) feeding on *Abies*, *Picea*, and *Pseudotsuga* in western Canada and Alaska. *The Canadian Entomologist* **127**: 813–830.

Shore, T.L., and R.I. Alfaro. 1986. The spruce budworm, *Choristoneura fumiferana* (Lepidoptera: Tortricidae), in British Columbia. *Journal of the Entomological Society of British Columbia* **83**: 31–38.

Slatkin, M. 1985. Rare alleles as indicators of gene flow. *Evolution* **39**: 53–65.

Slatkin, M., and N.H. Barton. 1989. A comparison of three indirect methods for estimating average levels of gene flow. *Evolution* **43**: 1349–1368.

Snedecor, G.W. 1946. Statistical Methods. The Iowa State College Press, Ames, IA. 485 pp.

Stehr, G. 1959. Hemolymph polymorphism in a moth and the nature of sex-controlled inheritance. *Evolution* **13**: 537–560.

Stock, M.W., and P.J. Castrovillo. 1981. Genetic relationships among representative populations of five *Choristoneura* species: *C. occidentalis*, *C. retiniana*, *C. biennis*, *C. lambertiana* and *C. fumiferana* (Lepidoptera: Tortricidae). *The Canadian Entomologist* **113**: 857–865.

Stock, M.W., and J.L. Robertson. 1980. Inter- and intra-specific variation in selected *Choristoneura* species (Lepidoptera: Tortricidae): A toxicological and genetic survey. *The Canadian Entomologist* **112**: 1019–1027.

Sturgeon, K.B., and J.B. Mitton. 1986. Allozyme and morphological differentiation of mountain pine beetles *Dendroctonus ponderosae* Hopkins (Coleoptera: Scolytidae) associated with host tree. *Evolution* **40**: 290–302.

Swofford, D.L. 1981. On the utility of the Distance Wagner Procedure. pp. 25–43 *in* Funk, V.A., and D.R. Brooks (Eds.), Advances in Cladistics. Proceedings of the First Meeting of the Willi Hennig Society. The New York Botanical Garden, Bronx, NY. 232 pp.

Swofford, D.L., and R.B. Selander. 1981. BIOSYS-1: A Fortran program for the comprehensive analysis of electrophoretic data in population genetics and systematics. *Journal of Heredity* **72**: 281–283.

Turnquist, R., and R. Ferris. 1989. Forest Insect and Disease Conditions. Prince George Region 1988. *Forestry Canada, Pacific Forestry Centre, Victoria, British Columbia, Report* **89-4**: 8 pp.

Volney, W.J.A. 1989. Biology and dynamics of North American coniferophagous *Choristoneura* populations. *Agricultural Zoology Reviews* **3**: 113–156.

Volney, W.J.A., and H.F. Cerezke. 1992. The phenology of white spruce and the spruce budworm in northern Alberta. *Canadian Journal of Forest Research* **22**: 198–205.

Volney, W.J.A., A.M. Liebhold, and W.E. Waters. 1983. Effects of temperature, sex and genetic background on coloration of *Choristoneura* spp. (Lepidoptera: Tortricidae) populations in south-central Oregon. *The Canadian Entomologist* **115**: 1583–1596.

Wallner, W.E. 1987. Factors affecting insect population dynamics: Differences between outbreak and non-outbreak species. *Annual Review of Entomology* **32**: 317–340.

Wilhite, E.A. 1979. Genetics of Outbreaking Western Spruce Budworm, *Choristoneura occidentalis* Freeman (Lepidoptera: Tortricidae), Populations in Idaho and Montana. M.S. thesis, University of Idaho, Moscow, ID. 106 pp.

Wilhite, E.A., and M.W. Stock, 1983. Genetic variation among western spruce budworm (*Choristoneura occidentalis*) (Lepidoptera: Tortricidae) outbreaks in Idaho and Montana. *The Canadian Entomologist* **115**: 41–54.

Wood, C.S., and G.A. Van Sickle. 1989. Forest Insect and Disease Conditions. British Columbia and Yukon 1988. *Forestry Canada, Pacific Forestry Centre, Victoria, British Columbia, Information Report* **BC-X-306**: 33 pp.

Wood, C.S., G.A. Van Sickle, and L.M. Humble. 1987. Forest Insect Disease Conditions. British Columbia and Yukon 1987. *Canadian Forestry Service, Pacific Forestry Centre, Victoria, British Columbia, Information Report* **BC-X-296**: 40 pp.

Wright, S. 1978. Evolution and the Genetics of Populations. Vol. 4. Variability Within and Among Natural Populations. University of Chicago Press, Chicago, IL. 580 pp.

(Date received: 26 June 1994; date accepted: 25 September 1995)