GENETIC RELATIONSHIPS AMONG REPRESENTATIVE POPULATIONS OF FIVE *CHORISTONEURA* SPECIES: *C. OCCIDENTALIS*, *C. RETINIANA*, *C. BIENNIS*, *C. LAMBERTIANA*, AND *C. FUMIFERANA* (LEPIDOPTERA: TORTRICIDAE)[1]

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Abstract

The genetic make-up of representative populations of five *Choristoneura* species was compared using starch gel electrophoresis. Species included *C. occidentalis* Freeman from Idaho, *C. biennis* Freeman from British Columbia, *C. retiniana* (Walsingham) (= *C. viridis* Freeman) from Oregon, *C. lambertiana ponderosana* Obraztsov from Colorado, and *C. fumiferana* (Clemens) from Maine. When variation at individual gene loci was examined, intraspecific variation was often as great, and sometimes greater, than interspecific variation and few significant differences were noted among the species. The highest levels of overall genetic similarity occurred among *C. occidentalis*, *C. biennis*, and *C. retiniana*. Relatively greater genetic distances were found between this group and *C. lambertiana* and *C. fumiferana*. *C. fumiferana* was most distantly related to all other groups. Genetic identity values fell within the range more commonly associated with conspecific populations rather than with separate species.

Résumé

La constitution génétique de populations représentatives de cinq espèces de *Choristoneura* a été comparée par électrophorèse sur gel d'amidon. Les espèces étaient *C. occidentalis* Freeman de l'Idaho, *C. biennis* Freeman de Colombie Britannique, *C. retiniana* (Walsingham) (= *C. viridis* Freeman) d'Oregon, *C. lambertiana ponderosana* Obraztsov du Colorado, et *C. fumiferana* (Clemens) du Maine. L'examen de la variation à des loci géniques individuels a révélé que la variation intraspécifique est souvent aussi grande, et parfois plus grande, que la variation interspécifique, et peu de différences significatives ont été notées entre les espèces. Les niveaux de similarité génétique globale les plus élevés ont été observés entre *C. occidentalis*, *C. biennis* et *C. retiniana*. Des distances génétiques relativement grandes ont été observées entre ce groupe, et *C. lambertiana* ou *C. fumiferana*. *C. fumiferana* était l'espèce la plus distante de tous les autres groupes. Les valeurs d'identité génétique mesurées se répartissent sur un intervalle plus communément associé à des populations conspécifiques qu'à deo espèces discrètes.

The spruce budworm complex, initially considered a single species, *Choristoneura fumiferana*, was revised in 1967 with descriptions of several new species (Freeman 1967; Freeman and Stehr 1967). The spruce/fir-feeding "Fumiferana complex" includes the eastern species, *C. fumiferana* (Clemens), and four western species: *C. occidentalis* Freeman, *C. retiniana* (Walsingham) (= *C. viridis* Freeman), *C. orae* Freeman, and *C. biennis* Freeman. The pine-feeding "Lambertiana complex" consists of the eastern *C. pinus* Freeman (with 2 subspecies) and the western *C. lambertiana* (Busck) (with 3 subspecies). A third group, the "Carnana complex", is believed to be associated with Douglas-fir in California and is still of uncertain affinity (Powell 1980). Within the entire spruce budworm complex, however, disagreement still exists as to the number of species and subspecies, their

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levels of taxonomic distinctness, their geographic distributions, and the names applied to them. According to Powell (1980), the principal source of confusion among western *Choristoneura* groups lies in the extensive variation found among populations. In sympatric populations, the degree of reproductive isolation is essentially unknown and allopatric populations show much variation in size and color of adults, larvae, and pupae, and in host plant preferences. Both polymorphic and continuously variable features occur and characters conventionally used by taxonomists to define species of Lepidoptera (e.g., genitalic structures) are not sufficiently differentiated in this group to provide reliable morphological indicators of genetic and behavioral compatibility among populations.

Recent developments in molecular genetics offer useful techniques for resolving systematic problems of this type. Of the various methods for determining levels of genetic differentiation among taxa, with respect to the degree of sensitivity and ease of analysis, gel electrophoresis is ideal for comparing subspecies, species, and closely related genera (Avise 1974; Bush and Kitto 1978; Berlocher 1979). Because there is usually a one-to-one relationship between an individual's electrophoretic phenotype and its genotype, the capability of this method to detect variation at single gene loci removes ambiguity inherent in other, more traditional, approaches. Electrophoresis also permits comparisons of gene frequencies between populations or species at a large number of loci, a direct though conservative estimation of genetic differentiation, and often provides insights that extend beyond those obtained by more traditional criteria.

In the study reported here, the genetic make-up of representative populations of five *Choristoneura* species was examined using starch gel electrophoresis. Our aim was to estimate and compare levels of genetic differentiation among the species. In addition to specimens of the western spruce budworm (*C. occidentalis*) and the eastern spruce budworm (*C. fumiferana*), we also examined specimens of *C. biennis*, *C. retiniana*, and *C. lambertiana ponderosana* Obraztsov. A summary of the distinguishing features of these species is given by Freeman and Stehr (1967) and a distribution map is shown in Fig. 1. Of these five species, the first two are the most widespread and most destructive. *C. biennis*, the 2-year budworm, occurs at high elevations on alpine fir and Engelmann spruce in SE British Columbia and SW Alberta, areas where *C. occidentalis* is found at low elevations on Douglas-fir (Shepherd and Dolph 1979). Host preference, dark coloration of larvae and pupae, and the occurrence of a second diapause in the fourth instar in *C. biennis* distinguish the two species. The Modoc budworm, *C. retiniana*, has been reported predominantly on white fir in south-central Oregon and in NE California although phenotypically similar fir-feeding populations occur along the length of the Sierra Nevada, Tehachapi, and Transverse ranges in California (Powell 1980). The primary distinguishing feature between *C. retiniana* and *C. occidentalis* is the distinct green hemolymph coloration of *C. retiniana* which is readily seen through the unpigmented cuticle of larvae and pupae. Sanders (1974) found differences between the sex pheromone of *C. retiniana* and that of *C. fumiferana*, *C. occidentalis*, and *C. biennis*. The latter three species share a very similar sex pheromone.

C. lambertiana ponderosana is found in Colorado, Wyoming, and Montana. It is distinguished from other western species primarily by feeding habit; its host is ponderosa pine. It is also distinctive in having forewings more diffusely marked and lacking rust or reddish markings compared with other subspecies of *C. lambertiana* (Stevens *et al.* 1977; Powell 1980).

Methods

Samples of late-stage instar larvae were obtained from the following sites (Fig. 1) and subjected to electrophoretic analysis:

(1) *C. occidentalis* — 300+ from Payette National Forest, Valley County, Idaho, on Douglas-fir and grand fir.

(2) *C. biennis* — 600+ from Cariboo Mts., SE British Columbia, on subalpine fir.

(3) *C. retiniana* — ca. 50 from Fremont National Forest, Lake County, Oregon, on white fir.

(4) *C. lambertiana ponderosana* — ca. 90 from Lyons, Boulder County, Colorado, on ponderosa pine.

(5) *C. fumiferana* — 200+ from Washington County, Maine, on balsam fir.

Techniques used for electrophoresis of spruce budworms are described by Willhite (1979). Isozyme banding patterns were converted to genotype frequencies for tests of Mendelian inheritance and to allele (allozyme) frequencies for comparison of species. Contingency tests, based on the observed number of each allele present, were used to detect differences in gene frequencies at single loci. Loci differing significantly between species with respect to the distribution of particular gene types were considered for use as markers or distinguishing features of species. Data from all loci were used to calculate Nei's (1972) genetic identity and genetic distance values. Genetic identity values vary from zero to one where one indicates total genetic similarity. Nei's genetic distance is interpreted as a measure of the average number of electrophoretically detectable allelic substitutions per locus which have accumulated since two populations or species separated from a common ancestor. These values are useful estimates of genetic relationships when used at the species and population levels (Ayala 1975). Using the genetic identity values, a dendrogram was constructed, using the complete linkage clustering method (Sneath and Sokal 1973), to aid visualization of genetic relationships among species.

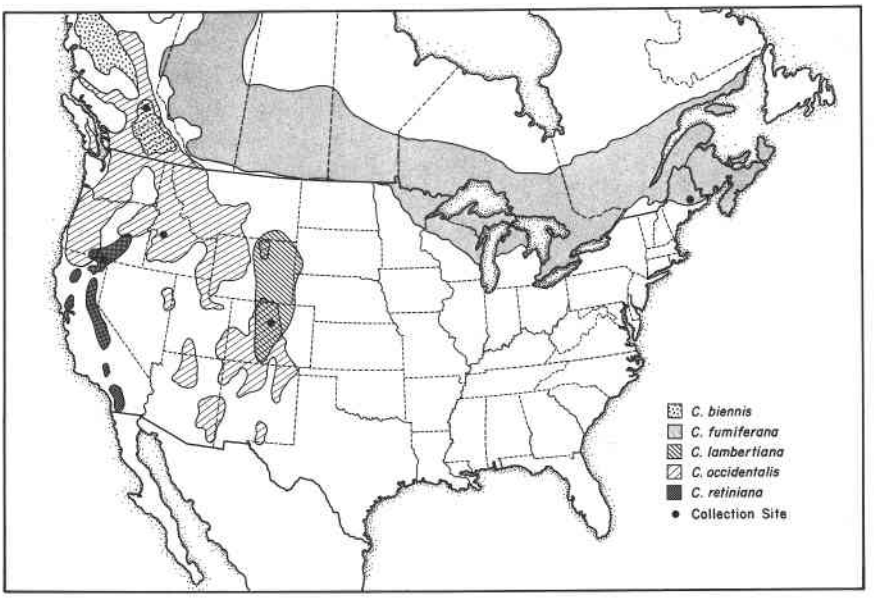

FIG. 1. Approximate distribution of five *Choristoneura* species in North America (modified from Shepherd and Dolph 1979, Fellin 1980, Powell 1980, and Prentice 1966).

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Results were compared with those reported for *Choristoneura* species by Stock and Robertson (1980) and for *C. occidentalis* in Idaho and Montana by Willhite (1979).

Results

Data were obtained on 18 gene loci representing 12 enzyme systems in the five *Choristoneura* species (Table I; Fig. 2). These loci gave consistent, interpretable banding patterns conforming to patterns of Mendelian inheritance. Assays of three other loci (EST4, LAP1, MDH1) gave inconsistent results so are not reported here. When products of more than one locus occurred for a single enzyme type, the loci were numbered from an anodal to cathodal direction. Fourteen (AAT1; CK; EST1, 2, 3, 5, and 6; IDH; LDH; LAP2 and 3; ME; MDH2; PGI) of the 18 loci were polymorphic in at least one species, with esterases being most highly polymorphic. AAT2, AGP, 6PGDH, and XDH were monomorphic in all groups.

When variation at individual loci was compared among species (Table I) and among samples of a single species, *C. occidentalis* (Willhite 1979), intraspecific variation was often as great, and sometimes greater, than interspecific variation. However, some significant differences in gene frequencies were observed among the *Choristoneura* species. At AAT1 and EST5, *C. fumiferana* was distinguished by a different common allozyme from the other species. At LAP2, *C. fumiferana* and *C. retiniana* both had a different common allozyme than the other species. At all three of these loci, *C. occidentalis* and *C. biennis* were most similar.

At EST2, *C. retiniana* had a lower frequency of the common allozyme than other species. However, the range of variation in frequency of the common EST2 allozyme (.66–.96) reported among *C. occidentalis* samples (Willhite 1979) came close, at its lower limit, to the frequency of the common allozyme recorded for *C. retiniana* in this study (.58). At LAP3, *C. retiniana* was more polymorphic than other groups. *C. lambertiana* had a higher frequency of EST6 allozyme 2 than the other species and a different common allozyme at EST1. Again, however, the range of *C. occidentalis* intraspecific variation at these loci encompassed these differences. *C. lambertiana* was the only species polymorphic at MDH2. At EST3, *C. lambertiana* had a lower frequency of the common allozyme than the other species, but high levels of intraspecific variation noted at this locus among *C. occidentalis* samples suggest that this is not a significant distinguishing feature between *C. occidentalis* and *C. lambertiana*. Over all loci, no significant differences in gene frequency were seen between *C. occidentalis* and *C. biennis*.

Greater insight into species relationships was obtained when data from all loci were compared simultaneously. Genetic identity values (Table II) ranged from .99 (between *C. occidentalis* and *C. biennis*) to .84 (between *C. fumiferana* and *C. lambertiana*). Genetic distances (Table II) showed that, between *C. occidentalis* and *C. biennis*, only an estimated 0.7 electrophoretically detectable allelic substitutions for every 100 loci have occurred since the divergence of these two groups from a common ancestral population. Between *C. fumiferana* and *C. lambertiana*, an estimated 17 allelic substitutions for every 100 loci have occurred since their divergence from a common ancestor. *C.retiniana* was most closely related to *C. occidentalis/biennis* and, of the four western species, *C. lambertiana* was most distantly related (Fig. 3).

Differences among species noted in this study are consistent, in most cases, with differences noted among species by Stock and Robertson (1980). However, *C. retiniana* (= *viridis*) from the Berkeley laboratory colony was significantly different from field-collected *C. retiniana* at both EST2 and 3. Thus, results of the earlier study suggested a higher level of genetic divergence between *C. occidentalis* and

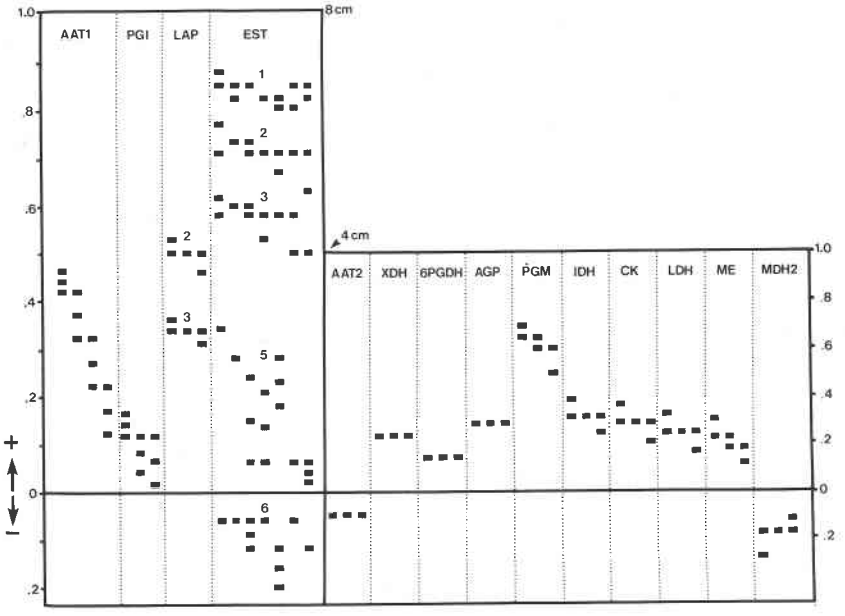

FIG. 2. Representative genotypes and relative mobilities of *Choristoneura* isozymes. Isozymes best resolved on an 8 cm RW gel are shown on the left; those best resolved on a 4 cm AC gel are shown on the right.

C. retiniana than was the case when field populations were compared. Also, an appreciably higher frequency of silent alleles at EST2 was found in the 1977-collected *C. occidentalis* from McCall, Idaho (Stock and Robertson 1980). In neither the 1978 (Willhite 1979) or 1979 (reported here) Idaho collections of *C. occidentalis* were silent alleles detected at the EST2 locus. This difference could reflect a temporal shift in frequencies at this locus, since recent studies have shown that frequencies of the EST2 silent allele are highly labile (Stock and Robertson, in prep.).

Discussion

The ecological division between the spruce/fir-feeding Fumiferana complex and the pine-feeding Lambertiana complex is considered quite distinct (Stehr 1967; Powell

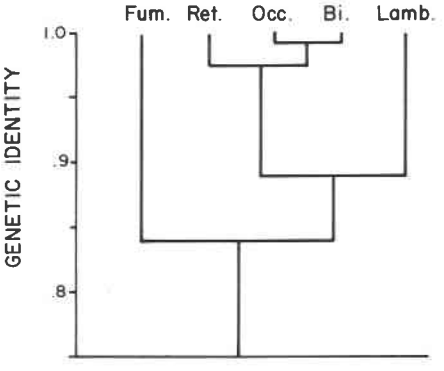

FIG. 3. Dendrogram showing genetic relationships among representative populations of five *Choristoneura* species.

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Table I. Isozyme frequencies in samples of five *Choristoneura* species

Enzyme	Abbrev.	Locus	Allozyme	Relative mobility	*occidentalis*	*retiniana*	*fumiferana*	*biennis*	*lambertiana*
Aspartate aminotransferase	AAT	1	1*	.46	0	0	0	0	0
			2	.42	0	0	.68	0	.02
			3	.32	0	0	.25	.04	.95
			4	.22	1.0	1.0	.07	.94	.03
			5	.12	0	0	0	.02	1.0
		2	1	.10	1.0	1.0	1.0	1.0	
Creatine kinase	CK	1	1	.36	.01	0	0	.01	0
			2	.28	.97	1.0	.96	.98	1.0
			3	.20	.02	0	.04	.01	0
Esterase	EST	1	1	.88	0	.08	0	0	0
			2	.85	.69	.59	.56	.57	.39
			3	.83	.23	.34	.38	.34	.61
			4	.81	.08	0	.06	.09	0
		2	1	.77	.01	0	0	0	0
			2	.73	.01	.07	.005	.10	.09
			3	.71	.82	.58	.95	.84	.89
			4	.67	.14	.33	.04	.04	.02
			5	.63	.02	.02	.005	.02	0
		3	1*	.62	0	0	0	0	0
			2	.60	.28	0	.13	.06	.01
			3	.58	.67	.92	.85	.84	.74
			4	.53	.05	.06	.02	.08	.17
			5	.50	0	.02	0	.02	.08
		5	1	.34	0	0	.05	.02	0
			2	.28	.01	0	.06	.01	.03
			3	.24	.03	.02	.46	.09	0
			4	.21	0	.10	.07	.04	.08
			5	.18	.07	.17	.12	.04	.34
			6	.06	.86	.69	.23	.75	.46
			7	.02	.01	.02	.02	.04	.09
		6	1	−.20	0	0	0	.01	0
			2	−.12	.07	.04	.02	.04	.18
			3	−.06	.93	.96	.98	.95	.82

α-Glycerophosphate dehydrogenase	AGPDH	1	1	.28	1.0	1.0	1.0	1.0	1.0
Isocitrate dehydrogenase	IDH	1	1*	.38	0	0	0	0	0
			2	.31	1.0	.93	.98	1.0	1.0
			3	.24	0	.07	.02	0	0
Lactate dehydrogenase	LDH	1	1	.32	0	0	.03	0	0
			2	.24	.97	1.0	.97	.97	1.0
			3	.16	.03	0	0	.03	0
Leucyl aminopeptidase	LAP	2	1	.53	.27	.59	.72	.12	.10
			2	.50	.73	.41	.27	.88	.90
			3	.46	0	0	.01	0	0
		3	1	.36	0	.12	.04	.01	.01
			2	.34	1.0	.83	.96	.99	.99
			3	.31	0	.05	0	0	0
Malic enzyme	ME	1	1	.29	0	.08	.02	0	.20
			2	.22	.92	.88	.94	.98	.70
			3	.18	.04	.02	.02	.02	.10
			4	.16	.04	.02	.02	0	0
Malate dehydrogenase	MDH	2	1	−.30	0	0	0	0	.10
			2	−.24	1.0	1.0	1.0	1.0	.90
6-Phosphoglucose dehydrogenase	6PGDH	1	1	.14	1.0	1.0	1.0	1.0	—
Phosphoglucose isomerase	PGI	1	1	.16	.04	.01	.04	.09	—
			2	.12	.93	.98	.90	.83	—
			3	.04	.02	.01	.03	.05	—
			4	.01	.01	0	.03	.03	—
Xanthine dehydrogenase	XDH	1	1	.23	1.0	1.0	1.0	1.0	1.0

*Very rare. Frequency less than 0.005 in all groups.

Table II. Nei's genetic identity (below diagonal) and genetic distance (above diagonal) values for pairwise comparison of 5 *Choristoneura* species

	occidentalis	*retiniana*	*fumiferana*	*biennis*	*lambertiana*
occidentalis	—	.020	.087	.007	.094
retiniana	.981	—	.075	.025	.113
fumiferana	.917	.928	—	.084	.173
biennis	.993	.975	.919	—	.077
lambertiana	.910	.893	.841	.926	—

1980). There are no confirmed records of oviposition by either of the two groups on reciprocal hosts leading to successful establishment of larvae. We therefore expected *C. lambertiana ponderosana* (the only pine-feeder of the five species compared) to be most distantly related to the others. However, these genetic data indicate that *C. fumiferana* is most distantly related to all other groups. This disparity could reflect the large geographic distance separating the *C. fumiferana* sample, taken in Maine, from the other groups. However, *C. fumiferana* from the Great Lakes area (Stock and Robertson 1980) were genetically very similar to the Maine population.

Problems associated with determining taxonomic relationships among the western spruce/fir-feeding species reflect the geographic complexity of western forest regions with their intermingling of tree species and their small-scale patterning of pure stands (Stehr 1967). Although host preferences generally differ among *C. occidentalis*, *C. biennis*, and *C. retiniana*, sympatry occurs in some areas of their distribution. The montane habitat of *C. occidentalis* is, at upper elevations, frequently in contact with the subalpine spruce/fir habitat of *C. biennis*, thus permitting contact between the two species (Shepherd and Dolph 1979). However, differences in life cycles between *C. occidentalis* and *C. biennis* effect temporal isolation even where their ranges overlap. The ranges of *C. occidentalis* and *C. retiniana* also overlap and morphotypes representative of these species may be found together in the same stands.[2] In south-central Oregon, populations containing larvae and adults showing a morphological and biological gradation between *C. occidentalis* and *C. retiniana* occur (Powell 1980).

These relationships are reflected in the high levels of genetic similarity noted between *C. occidentalis*, *C. biennis*, and *C. retiniana*, and their relatively greater genetic distance from the western pine-feeding *C. lambertiana* and the eastern population of *C. fumiferana*. Genetic identity values calculated among the five species ranged from .99 to .84. In other organisms, including many insect groups, values in this range are more commonly found between conspecific populations than between different species (Ayala 1975). Furthermore, fixation for different allozymes at a locus, an important indicator of reproductive and genetic divergence, was seen at no loci among the *Choristoneura* species studied here. These genetic data therefore suggest that reproductive isolation among at least some of these groups is not well established. This conclusion supports results of other investigations of biosystematic relationships within the genus *Choristoneura*. For example, in cross-mating experiments, Campbell (1967) found no evidence that *C. occidentalis* and *C. biennis*

[2]Personal communication from W. Waters, Division of Entomology and Parasitology, University of California, Berkeley, California 94720.

were reproductively isolated from each other. Sanders *et al.* (1977) found the *Choristoneura* species a convenient group for studies of the effects of hybridization on sex pheromone response because they readily hybridize in the laboratory.

These genetic data provide support to the view of Powell (1980) that definition of some of the *Choristoneura* species as reproductively isolated entities of comparable rank or genetic integrity is, at this point, artificial.

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