GENETIC RELATIONSHIPS AMONG *CHORISTONEURA* SPECIES (LEPIDOPTERA: TORTRICIDAE) IN NORTH AMERICA AS REVEALED BY ISOZYME STUDIES

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Abstract

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Allozymes at several polymorphic loci were assayed in larval collections of 12 recognized species and two possible new species of *Choristoneura* and two species of *Archips*. Most of the 48 collections came from high density populations, and those of *C. fumiferana*, *C. occidentalis*, and *C. pinus* represented much of the geographic range of these species. Mean percentage heterozygosity ranged from 2.0 to 18.6%, based on nine polymorphic loci. Three loci are sex-linked in *C. fumiferana*, two in *C. pinus* and *C. occidentalis* and probably in some other members of the group. Allozymes of aspartate transaminase (AAT-1) were most varied among the species and permit identification of individual *C. fumiferana* in better than 95% of cases. Among the group of coniferophagous *Choristoneura* species genetic distances were small (max. Nei = 0.232); *C. fumiferana* was the most distinct species. Wagner trees based on modified Rogers' distances supported the above conclusions but indicated that separations among *C. biennis*, *C. orae*, *C. occidentalis*, *C. carnana*, *C. subretiniana*, and the two new species of *Choristoneura* were very small and probably below the species level, based on the allozymes measured.

Harvey, G.T. 1996. Parenté génétique des espèces de *Choristoneura* (Lepidoptera: Tortricidae) d'Amérique du Nord selon les résultats de l'étude des isozymes. *The Canadian Entomologist* **128**: 245–262.

Résumé

Les allozymes de plusieurs locus polymorphes ont été analysés au sein d'échantillons de larves appartenant à 12 espèces connues et deux espèces peut-être nouvelles de *Choristoneura* et à deux espèces d'*Archips*. La majorité des 48 récoltes proviennent de populations très denses et les récoltes de *C. fumiferana*, *C. occidentalis* et *C. pinus* représentent une grande partie de la répartition géographique de ces espèces. Le pourcentage moyen d'hétérozygotie à neuf locus polymorphes a été évalué à 2,0–18,6%. Trois locus sont liés au sexe chez *C. fumiferana*, deux chez *C. pinus* et *C. occidentalis* et probablement aussi chez d'autres membres du groupe. Ce sont les allozymes de l'aspartate transaminase (AAT-1) qui varient le plus d'une espèce à l'autre et ils permettent de reconnaître les individus de *C. fumiferana* dans plus de 95% des cas. Chez les groupes d'espèces de *Choristoneura* des conifères, les distances génétiques sont faibles (max. Nei = 0,232); *C. fumiferana* est l'espèce la plus distinctive. Les arbres de Wagner basés sur les distances modifiés de Rogers confirment ces assertions mais indiquent que la séparation entre *C. biennis*, *C. orae*, *C. occidentalis*, *C. carnana*, *C. subretiniana* et les deux nouvelles espèces de *Choristoneura* est ténue et probablement sous le niveau spécifique d'après les allozymes mesurés.

[Traduit par la Rédaction]

Introduction

The genus *Choristoneura* includes eight species and eight subspecies that feed on foliage and cones of conifers in North America (Harvey 1985) and several other species that feed on deciduous foliage of trees and plants (Powell 1996). At least two of the coniferophagous species, *Choristoneura fumiferana* and *C. occidentalis*, are important pests of coniferous forests. The group is characterized by numerous polymorphisms and by extensive overlap of characters among species so that identification of individual insects in all developmental stages is almost impossible (Harvey 1985; Powell 1996).

Genetic relationships among five of these species based on isozyme studies were reported by Stock and Castrovillo (1981) and Castrovillo (1982). More recent studies of isozymes have explored the population genetics of the most important member of this group, *C. fumiferana* (Harvey 1996), in relation to geographic and population density differences. Herein, I compare other members of the genus, both the coniferous and broad-leaf feeders, as well as two *Archips* spp. These results extend our knowledge to include 14 *Choristoneura* and provide more information about genetic relationships in this group.

Methods

Insect Collections. Collections of late-instar larvae were obtained from many locations across Canada and in the western United States. Because of continuing difficulties with identification of many members of this group (Powell 1996) it is important to provide specific information about the date, location, and host of each collection, and the identity of collectors (Table 1, which shows species code names); Table 2 gives the code for host species. Most of the collections came from high density populations. However, collections of some of the less-common *Choristoneura* and both *Archips* spp. were not from recognized outbreak populations. Further information about the species and their population distribution and history can be obtained from various annual reports [Forest Insect and Disease Survey (Canada) 1970–1980, 1981–1989]. The European *C. murinana* was collected from a geographic outlier in the southern extension of white fir (*Abies alba* Mill.) (V. Nealis, personal communication).

Population records. Identities of the insects were determined as they were received, based on location, host, and information from the collectors. The *C. fumiferana* populations used in this analysis were from nine widely separated locations selected from the larger group described previously (Harvey 1996). Advantage was taken of the high populations of *C. pinus* (1983–1989) to obtain larval collections from several locations, including New Brunswick, Ontario, and Saskatchewan (with the assistance of W.J.A. Volney, NFC) so the data represent a broad coverage across the range of this species in Canada. Collections of all other species came from one to several different locations within their broader distribution (Harvey 1985; Powell 1996; Shepherd et al. 1995).

The larvae collected from Tehachapi, California, although originally identified as *C. retiniana*, are now known to be *C. retiniana spaldingiana* (Powell and DeBenedictis 1996). Our analyses also included two entities of unknown status collected by T. Gray (Pacific Forestry Centre) in British Columbia. One of these is a lodgepole-pine-feeding population from the area around Prince George, B.C., and is identified as "*CPG*" (Gray and Gries 1993). It resembles other pine-feeding species, including *C. pinus*, and is caught in traps baited with acetate pheromone, although the actual pheromone appears unique (Gray and Gries 1993). The other budworm, "*CR*", was reported from Scots pine in the region of Richmond (Gray and Slessor 1989) and may be close to the "Pacific Northwest Coastal Populations" of Powell (1996). The tested insects of that entity represented laboratory-reared larvae originating from the field collection (Table 1).

The deciduous tree feeders, *C. conflictana* and *C. rosaceana* and the two *Archips* species, were collected in Ontario and identified by the Forest Insect and Disease Survey in Sault Ste. Marie. One sample of *C. rosaceana* (ROL) came from a laboratory colony derived from collections in British Columbia, and was obtained through the help of J. Borden, Simon Fraser University.

Larvae received in the laboratory were allowed to continue feeding on the foliage of the host from which they were collected or, when necessary, on artificial diet (Grisdale 1970), until harvested for freezing in the latter half of the sixth instar. The sex of all larvae, pupae, and adults was recorded (Harvey and Stehr 1967), except for the two *Archips* species which are not readily sexed as larvae. Treatment of insects and preparation of extracts for

TABLE 1. Sources, identities, and codes for larval collections

Location*	Code †	UTM grid			Host*	Date	Collector
Choristoneura fumiferana **(Clemens) (*fum*)**							
Cornerbrook, Nfld.	COR	21	43	542	1	05/7/83	W.J. Sutton
Fredericton, N.B.	FED	19	65	506	1	12/6/81	F. Titus
Valcartier, P.Q.	VAC	19	33	520	1,4	17/6/81	J. McNeil
Desbarats, Ont.	DES*	17	27	513	4	11/6/84	P.M. Roden
Terrace Bay, Ont.	TER	16	49	540	4	24/6/85	V. Jansons
Black Sturgeon L., Ont.	BSL*	16	36	546	1,4	18/6/84	J. Regniere
Ear Falls, Ont.	EAR	15	48	560	1	02/6/87	C. Jones
Edmonton, Alta.	EON	12	36	595	4	11/6/81	H. Cerezke
Liard R. Hotspr., B.C.	LID*	09	65	659	4	23/6/88	R. Turnquist
C. occidentalis **Freeman (*occ*)**							
Clinton, B.C.	HAR	10	60	565	8	16/6/80	S.J. Allen
Kamloops, B.C.	HAR	10	70	562	8	09/7/85	R.O. Erickson
Savona, B.C.	SAV	10	64	561	8	'81, 85, 86	Several
Ashcroft, B.C.	ASH	10	62	559	8	23/6/82	P.T. Dang
Pimainis Hills, B.C.	PIM	10	62	559	8	25/6/82	P.T. Dang
Johnstone Cr., B.C.	JON	11	35	543	8	18/6/86	J. Vallentgoed
Osoyoos, B.C.	OSO	11	31	543	8	04/6/86	J. Loranger
Lyons, Boulder Co., CO	COL	13	63	444	8	28/6/85	R.E. Stevens
C. carnana californica **Powell (*car*)**							
Trinity Co., CA	CAR	10	53	450	8	10/5/85	W.J.A. Volney
C. retiniana spaldingiana **Obraztsov (*ret*)**							
Tehachapi, Kern Co., CA	REK	11	38	390	2	18/5/85	W.J.A. Volney
C. biennis **Freeman (*bie*)**							
McMurdo Cr., B.C.	WAS	11	49	566	5	29/6/79	C. Wood
Pinkerton Mt., B.C.	BOP	10	61	594	8	11/6/86	R. Garbutt
Mackay R., B.C.	MAC	10	65	580	8	21/6/88	R.O. Erichson
Tumerich Rd., B.C.	TUM	10	59	595	6	25/6/88	R. Ferris
C. orae **Freeman (*ora*)**							
Kispiox, B.C.	KIS	09	56	615	7	29/6/82	P.T. Dang
Dahl Cr. Kitimat, B.C.	KIT	09	51	600	6	05/7/84	N Humphreys
Kenny L., AK	KEN	07	40	675	4	18/5/84	A.G. Gordon
Anchorage, AK	COP	06	38	668	4	17/7/85	E. Holsten
C. pinus **Freeman (*pin*)**							
Cains River, N.B.	CAJ	19	72	514	9	23/6/87	B. Pendrel
Parry Sound, Ont. (2)	SNU	17	57	501	9	17/6/83	C.J. Sanders
Owen Sound, Ont.	OWE	17	45	499	9	15/6/83	H. Evans
Kirkwood Twp., Ont.	KIR	17	30	513	9	03/7/84	P. Roden
Sault Ste. Marie, Ont.	HIA	16	70	515	9	07/7/83	C.J. Sanders
Raco, MI	RAJ	16	67	522	9	28/6/85	G.T. Harvey
Ignace, Ont.	SUJ	15	61	546	9	23/6/87	C. Jones
Prince Albert, Sask.	PAJ	13	45	595	9	25/6/87	W.J.A. Volney
English R., Ont.	ENJ	15	61	549	9	07/7/89	D. Lawrence
C. lambertiana ponderosana **Obraztsov (*pon*)**							
Lyons, Boulder Co., CO	POB	13	63	444	10	21/6/85	R.E. Stevens
Larimer Co., CO	POL	13	63	444	10	21/6/85	R.E. Stevens
Boulder Co., CO	LAP	13	63	444	10	'85, 86	D.A. Leatherman

TABLE 1. (*Concluded*)

Location*	Code †	UTM grid			Host*	Date	Collector
***C. lambertiana subretiniana* Obraztsov (*sub*)**							
Burns, OR (Lab col.)	BUR	11	38	490	10	(15/8/87)	(R. Beckwith)
Unidentified *Choristoneura* "*CPG*" (Gray and Gries 1993)							
McLeod Lake, B.C.	WIJ	10	50	600	11	29/6/89	T. Gray
Unidentified *Choristoneura* "*CR*" (Gray and Slessor 1989)							
Ladner, B.C. (Lab col.)	TOM	10	49	544	12	14/2/84	(T. Gray)
***C. murinana* Hübner (*mur*)**							
Sion, Switzerland	SIN		—		3	10/6/88	N. Mills
***C. conflictana* (Walker) (*con*)**							
York Region For., Ont.	PEF	17	64	490	13	17/6/83	H. Evans
Webbwood, Ont.	WEB	17	43	512	13	06/6/85	E. Czerwinski
Several Locations, Ont.	CON		—		13	5&6/85	FIDS
Blind R., Ont.	NOU	17	33	514	14	01/6/88	D. Constable
Mountain L. Access, Ont.	MTN	15	70	533	13	29/5/86	H. Evans
***C. rosaceana* (Harris) (*ros*)**							
Maple, Ont.	ROS1	17	64	488	14	17/6/83	FIDS
Sault St. Marie, Ont.	ROS2+	16	70	515	13,14	'83,84,85	FIDS
Pembroke, Ont.	ROS5	18	31	507	14	5&6/86	B. Smith
Lab Colony, UBC, B.C.	ROL		—		—	(24/11/88)	J. Borden
***Archips cerasivorana* (Fitch) (arc)**							
Point Pelee, Ont.	ARC	17	37	464	16	04/7/83	C. Barnes
***A. argyrospila* (Walker) (*arg*)**							
Pembroke, Ont.		18	31	507	14	'84,85	B. Smith
Kemptville, Ont.		18	40	496	14	07/6/84	W. Ingram
Espanola, Ont.		17	36	507	13	31/5/85	E. Czerwinski
Sault Ste. Marie, Ont.		16	70	515	15	15/5/84	G.T. Harvey
Petroglyphs Park, Ont.		17	73	494	14	31/5/88	B. Smith

* Grouped by species, with species code; host code in Table 2.
† Three-letter code to identify collection locations.

electrophoresis followed procedures described previously (Harvey and Sohi 1985; Harvey 1996).

Electrophoresis. Up to 80 individual insects from each collection were assayed 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). Insects were analysed by horizontal starch gel electrophoresis using two buffer systems: Poulik buffer, pH 8.0 (Poulik 1957); and tris-citrate, pH 7.1 (Ayala et al. 1972). Further details about biochemical and analytical methods are described by Harvey and Sohi (1985) and Harvey (1996). Isozyme bands were identified by letters, with the fastest band usually being 'A', etc. Travel distances

TABLE 2. Names and common names of hosts of collected insects*

1. *Abies balsamea* (L.) Mill.	Balsam fir
2. *Abies concolor* (Gord. & Glend.) Lindl	White fir
3. *Abies alba* Mill.	European white fir
4. *Picea glauca* (Moench) Voss	White spruce
5. *Picea engelmanii* Parry	Engelman spruce
6. *Larix lyallii* Parl.	Alpine larch
7. *Tsuga heterophylla* (Raf.) Sarg.	Western hemlock
8. *Pseudotsuga menziesii* (Mirb.) Franco	Douglas fir
9. *Pinus banksiana* Lamb.	Jack pine
10. *Pinus ponderosa* Laws.	Ponderosa pine
11. *Pinus contorta* Dougl.	Lodgepole pine
12. *Pinus resinosa* Ait.	Red pine
13. *Populus tremuloides* Michx.	Trembling aspen
14. *Quercus rubra* L.	Red oak
15. *Acer rubrum* L.	Red maple
16. *Prunus virginiana* L.	Choke cherry

* Showing code numbers used in Table 1.

were expressed relative to the most common band (1.00; Table 3). Isozyme identification and band positions were compared with those of Stock and Castrovillo (1981) in similar collections of *C. fumiferana*, and a similar scoring system was used.

Enzymes selected for measurement were dependable and showed good activity in budworm extracts. Only loci that showed significant levels of polymorphism were used for this study. In all of the *Choristoneura* species, 12 loci were measured; but in *Archips*, EST-2, EST-3, and EST-6 were appreciably different and consequently only nine loci could be used.

Data Analysis. Allozyme data were obtained for nine loci in 48 collections and for an additional three loci for the 41 of these collections that were North American conifer feeders. Identification of bands followed procedures reported previously; genotype designations were supported by breeding studies in *C. fumiferana* (Harvey 1996). Data were entered as individual genotypes for calculation of allozyme frequencies using BIOSYS-1 (Swofford and Selander 1981). For testing expected genotype frequences (Hardy-Weinburg), Levene's (1949) correction for small sample size was used (Swofford and Selander 1981). For loci shown to be sex-linked, females were removed from the analysis; this applied to three species only: *C. fumiferana* (three loci), *C. occidentalis* (two loci), and *C. pinus* (two loci) (Harvey 1996).

Relationships among the collections and entities based on allozyme frequencies were explored using genetic distances ("*D*", Nei 1978), cluster analysis (UPGMA), and Wagner trees based on Rogers' distance, Prevosti distance (Wright 1978), Edwards' "*E*" distance, and Cavalli-Sforza and Edwards' (1967) chord distance, all available on BIOSYS-1 (Swofford and Selander 1981). Each collection was treated as a unit in the analyses. Collections were also grouped by species, region, and host. Separate data analyses were carried out for the 41 collections of *Choristoneura* species for which 12 loci were measured.

The extent of genetic variance within and between individuals and groups among the coniferophagous *Choristoneura* was tested for each locus using *F*-statistics (Wright 1978; Jennings and Philipp 1992) and contingency chi-square tests. To meet the requirements for class size in Hardy-Weinburg and contingency chi-square tests, allelic classes with frequency less than 1 were pooled with the nearest mobility classes (Pashley et al. 1985). For these analyses, data were entered as allele frequencies (BIOSYS DATYP=3).

TABLE 3. Allele frequencies in taxa of *Choristoneura* and *Archips*

	Taxon†																
Locus*	*fum*	*bie*	*occ*	*ora*	*car*	*ret*	*pin*	*pon*	*sub*	"*CR*"	"*CPG*"	*mur*	*ros*	*ros*‡	*con*	*arg*	*arc*
PGI-1																	
(*N*)	799	264	546	231	98	45	486	136	51	48	43	71	38	35	153	15	9
A 2.57	0	0	0	0	0	0	0.007	0	0	0	0	0	0.079	0	0	0.033	0
B 2.00	0.001	0	0.004	0.004	0	0.011	0.011	0.004	0	0	0	0	0.632	0	0	0.800	1.000
C 1.43	0.033	0.074	0.069	0.093	0.066	0	0.353	0.007	0.284	0.030	0.047	1.000	0.171	1.000	0.029	0.167	0
D 1.00	0.904	0.888	0.906	0.874	0.903	0.944	0.582	0.967	0.716	0.958	0.953	0	0.118	0	0.928	0	0
E 0.57	0.058	0.030	0.021	0.026	0.020	0.044	0.041	0.022	0	0.010	0	0	0	0	0.033	0	0
F 0.29	0.004	0.008	0.001	0.002	0.010	0	0.005	0	0	0	0	0	0	0	0.010	0	0
PGM-1																	
(*N*)	797	265	543	231	96	45	484	136	51	48	43	71	35	35	149	15	6
A 1.39	0	0	0	0	0	0	0	0	0	0	0	0	0.157	0	0	0	0
B 1.30	0.001	0.002	0.002	0	0	0	0.010	0	0	0	0	0	0.700	1.000	0	0	0
C 1.16	0.003	0.004	0.006	0.006	0.021	0	0.019	0.184	0	0.021	0	0.993	0.143	0	0	0	0
D 1.08	0.101	0.091	0.165	0.102	0.167	0.033	0.813	0.699	0.216	0.135	0.128	0.007	0	0	0.030	0	0
E 1.00	0.875	0.853	0.777	0.833	0.766	0.711	0.152	0.118	0.755	0.656	0.872	0	0	0	0.003	0	0
F 0.95	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.960	0	0
G 0.91	0.019	0.043	0.048	0.052	0.047	0.256	0.006	0	0.029	0.188	0	0	0	0	0.033	0	0
H 0.82	0.001	0.008	0.003	0.006	0	0	0	0	0	0	0	0	0	0	0.007	0.700	0.667
I 0.68	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.267	0.333
MDH-1																	
(*N*)	701	263	463	176	97	45	442	122	48	40	43	51	31	19	141	9	9
A 1.37	0.017	0.004	0.013	0.014	0	0	0.027	0.012	0.063	0.038	0.035	0.039	0.048	0.105	0.096	0.111	0
B 1.00	0.970	0.996	0.981	0.986	0.990	1.000	0.943	0.984	0.896	0.962	0.953	0.961	0.919	0.895	0.897	0.722	0
C 0.61	0.013	0	0.006	0	0.010	0	0.029	0.004	0.042	0	0.012	0	0.032	0	0.007	0.167	1.000
IDH-1																	
(*N*)	797	265	546	227	98	45	486	136	51	48	43	71	38	35	159	15	9
A 1.14	0.055	0.002	0.009	0.015	0.005	0	0.004	0	0	0.021	0.151	1.000	0.132	0	0.003	0.733	0
B 1.00	0.917	0.989	0.974	0.978	0.974	0.989	0.989	0.996	1.000	0.979	0.814	0	0.737	1.000	0.959	0.267	0
C 0.84	0.001	0.008	0.011	0.002	0.015	0.011	0.002	0.004	0	0	0.012	0	0.132	0	0.003	0	0

TABLE 3. (*Continued*)

	Taxon†																
Locus*	*fum*	*bie*	*occ*	*ora*	*car*	*ret*	*pin*	*pon*	*sub*	"*CR*"	"*CPG*"	*mur*	*ros*	*ros*‡	*con*	*arg*	*arc*
D 0.64	0.027	0.002	0.005	0.004	0.005	0	0.005	0	0	0	0.023	0	0	0	0.035	0	1.000
IDH-2																	
(*N*)	393	261	519	184	96	43	460	133	51	37	43	71	36	35	148	8	9
D 2.40	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.007	0.250	1.000
A 1.79	0.046	0	0.015	0.016	0	0.023	0.010	0.011	0.020	0.027	0	1.000	1.000	1.000	0.980	0.375	0
B 1.00	0.953	1.000	0.980	0.984	1.000	0.977	0.988	0.970	0.980	0.973	1.000	0	0	0	0.014	0.375	0
C 0.37	0.001	0	0.005	0	0	0	0.002	0.019	0	0	0	0	0	0	0	0	0
LDH-1																	
(*N*)	414	195	245	191	86	38	194	131	51	47	43	11	36	29	123	1	9
A1.50	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.472	0	0
B 1.26	0	0	0	0	0	0	0	0	0	0	0	0	0.083	0	0.455	1.000	0
C 1.15	0.022	0.010	0.006	0.003	0.012	0	0.015	0	0	0	0	0	0.917	1.000	0.073	0	0
D 1.00	0.977	0.990	0.990	0.997	0.983	1.000	0.966	1.000	1.000	1.000	1.000	1.000	0	0	0	0	1.000
E 0.84	0.001	0	0.004	0	0.006	0	0.018	0	0	0	0	0	0	0	0	0	0
F 0.67	0	0	0	0	0	0	0.001	0	0	0	0	0	0	0	0	0	0
AAT-1																	
(*N*)	428	239	256	219	94	45	213	136	38	34	43	71	38	34	159	8	9
A 1.32	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.125	0
B 1.18	0.006	0	0	0	0	0	0	0	0	0	0	0	0.013	0	0	0.875	0
C 1.00	0.651	0	0	0	0	0	0	0	0	0	0	0	0.974	1.000	0	0	0
D 0.81	0.296	0.044	0.016	0.005	0.005	0	0.002	0.004	0	0	0	0.007	0.013	0	0.003	0	1.000
E 0.62	0.048	0.956	0.979	0.995	0.984	1.000	0.993	0.996	1.000	1.000	1.000	0.986	0	0	0.997	0	0
F 0.41	0	0	0.006	0	0.011	0	0	0	0	0	0	0.007	0	0	0	0	0
G 0.22	0	0	0	0	0	0	0.005	0	0	0	0	0	0	0	0	0	0
AAT-2																	
(*N*)	596	215	328	184	81	39	420	131	51	45	43	71	38	34	148	7	9
A+2.00	0.013	0.009	0.012	0.003	0	0.026	0	0.004	0	0.111	0	0.993	0.013	0	0.010	0	0

TABLE 3. *(Concluded)*

Locus*	Taxon† *fum*	*bie*	*occ*	*ora*	*car*	*ret*	*pin*	*pon*	*sub*	"*CR*"	"*CPG*"	*mur*	*ros*	*ros*‡	*con*	*arg*	*arc*
B–1.00	0.966	0.986	0.983	0.984	0.994	0.962	0.971	0.989	1.000	0.889	1.000	0.007	0.987	1.000	0.980	1.000	1.000
C–3.00	0.021	0.005	0.005	0.014	0.006	0.013	0.029	0.008	0	0	0	0	0	0	0.010	0	0
EST-5																	
(*N*)	801	186	538	224	86	44	485	129	51	46	43	48	38	35	102	15	9
A 2.61	0.001	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.767	1.000
B 2.36	0.009	0	0	0	0	0.023	0.006	0	0	0	0	0	0	0	0	0.233	0
C 2.11	0.061	0.019	0.003	0.040	0.006	0.205	0.075	0	0	0	0.035	0	0	0	0	0	0
D 1.90	0.047	0.008	0	0.025	0.006	0.693	0.028	0.008	0	0.054	0.035	0	0.039	0	0	0	0
E 1.69	0.506	0.110	0.059	0.141	0.087	0	0.143	0.035	0.108	0.043	0.116	0	0.039	0	0	0	0
F 1.42	0.041	0.051	0.063	0.047	0.081	0	0.034	0.136	0.157	0	0.105	0	0.184	0.500	0	0	0
G 1.22	0.043	0.022	0.027	0.031	0.041	0.034	0.037	0.376	0.088	0.033	0.012	0.073	0.605	0.500	0.809	0	0
H 1.00	0.277	0.755	0.804	0.676	0.744	0.034	0.632	0.368	0.627	0.870	0.698	0.927	0.066	0	0.157	0	0
I 0.63	0.015	0.035	0.044	0.040	0.035	0.011	0.044	0.078	0.020	0	0	0	0.066	0	0.034	0	0

* Locus, allele letter, and travel distance. (*N*) = number of insects.
† Taxon identified in Table 1.
‡ ROL of Table 1.

Results

Enzymes present in *C. fumiferana* have been described previously (Stock and Castrovillo 1981; Harvey 1996). All loci measured were similar to *C. fumiferana* in all taxa tested, except for the two *Archips*, where three esterase loci were considerably different and could not be scored in the same systems. The principal allozymes were common to all the *Choristoneura* (Table 3), although there were marked differences in frequencies, particularly in the broad-leaf feeders (*C. rosaceana*, *C. conflictana*). Differences in AAT-1 between *Choristoneura* species appear to have taxonomic value. Several allozymes were unique to the *Archips* species.

Because of the presence of three sex-linked loci in *C. fumiferana* (Harvey 1996), it was of interest to determine whether these or other loci were sex-linked in any of the other species studied. The two *Archips* species were not sexed. Most loci were not sex-linked in any of the other species tested: PGI-1, PGM-1, MDH-1, IDH-1, AAT-2, and EST-5. Among the *Choristoneura* species, there was no evidence of sex-linkage among the loci tested in *C. conflictana*, *C. rosaceana*, or *C. murinana*. Numbers were too low to demonstrate sex-linkage in *C. carnana*, *C.r. spaldingiana*, *C.l. ponderosana*, *C.l. subretiniana*, or the undescribed species "*CR*" and "*CPG*". Among the coniferophagous species, sex-linkage at two loci (AAT-1 and LDH-1) was clearly present in *C. occidentalis* and *C. pinus* as well as *C. fumiferana*, and may be expected in all members of this group. However, it could not be proven for the other *Choristoneura* species because of very low numbers (numbers of heterozygotes at either locus were not greater than 1% of the total insect numbers in that species). Sex-linkage at IDH-2 was present in *C. fumiferana*, but not in *C. occidentalis* or *C. pinus* and probably not in any other members of the group, although for several of the entities sample sizes were very low. Consequently, for the analyses, females were removed from the designated sex-linked loci only from *C. fumiferana*, *C. occidentalis*, and *C. pinus*.

Harvey (1996) demonstrated that most of the loci measured in *C. fumiferana* conform to Hardy-Weinberg expectations (HWE). The same was true for the enlarged species group in this study. Although there were a few exceptions for seven loci (PGI-1, PGM-1, MDH-1, IDH-1, LDH-1, AAT-1, AAT-2), *P* values were below the 1% level for only 4.2% (14/336) of the samples. Frequencies of allozymes of IDH-2, one of the sex-linked loci, failed to conform to HWE in about 25% of the population samples. In spite of removal of females from *fum*, *occ*, and *pin*, most of the exceptions to HWE (8/48) were still in these three species. EST-5 also failed to conform to HWE in about 23% of the cases; these exceptions were distributed over all the species studied. Nevertheless, these two loci were retained in the analyses. The three additional loci measured in the North American coniferophagous *Choristoneura* (EST-2, EST-3, and EST-6) all had numerous deviations from HWE that were distributed fairly evenly over the different entities. The EST-2, in fact, deviated in 75% of the cases. (Data for EST-2, EST-3, and EST-6 are not presented in Table 3, but can be obtained from the author.)

There were notable differences in genetic variability among the 17 taxa (Table 4). Among the North American conifer feeders, polymorphism of loci ranged from 22.2 to 44.4%, with the exception of *C. fumiferana* at 55.6% (Taxa 1–11, Table 4). Mean heterozygosity was highest in the two eastern species (*C. fumiferana* and *C. pinus*), likely the result of the larger numbers of sites represented in these two species. There was also considerable variation in percentage polymorphism among the broad-leaf feeders (11.1–77.8%). The colony of western *C. rosaceana* (ROL) was noticeably more uniform (seven loci monomorphic) than the field-collected material from Ontario (one locus monomorphic) (22.2 vs. 66.7% loci polymorphic). The low variability of some of these samples may be a result of the small number and size of the collections on which they are based.

Although loci chosen for measurement had a high level of polymorphism in *fumiferana*, this was not true for all of the species. PGI-1, PGM-1, MDH-1, IDH-1, AAT-2, and EST-5

TABLE 4. Genetic variability at nine loci in 17 taxa

Taxon (*N*)*	Mean sample size per locus	Mean no. alleles per locus	Loci polymorphic,† %	Mean heterozygosity,‡ %
1. *Choristoneura fumiferana* (9)	636.2	4.4	55.6	18.6
2. *C. biennis* (4)	239.2	3.4	33.3	9.1
3. *C. occidentalis* (7)	442.7	4.0	33.3	10.5
4. *C. orae* (4)	207.4	3.6	33.3	10.7
5. *C. carnana*	92.4	3.3	33.3	10.3
6. *C.r. spaldingiana*	43.2	2.4	33.3	7.3
7. *C. pinus* (9)	408.7	4.1	44.4	16.2
8. *C.l. ponderosana* (3)	132.2	3.0	22.2	10.6
9. *C.l. subretiniana*	49.2	2.1	44.4	13.6
10. "*CR*"	43.7	2.3	33.3	12.6
11. "*CPG*"	43.0	2.3	33.3	9.3
12. *C. murinana*	59.6	1.7	11.1	2.0
13. *C. rosaceana*, Ont.	36.4	3.0	66.7	18.6
14. *C. rosaceana*, B.C.	32.3	1.2	22.2	7.7
15. *C. conflictana* (2)	142.4	3.2	44.4	6.3
16. *Archips argyrospila*	10.3	2.2	77.8	9.9
17. *A. cerasivorana*	8.7	1.1	11.1	3.7

* Number of populations if more than 1; populations pooled.
† A locus is considered polymorphic if the frequency of the most common allele does not exceed 0.95.
‡ Mean percentage heterozygosity by direct count.

were generally polymorphic in all the coniferophagous *Choristoneura*. Other loci were monomorphic in several species: IDH-1 in *sub*, *mur*; IDH-2 in *bie*, *car*, "*CPG*", *mur*, *ros*; LDH-1 in *ret*, *pon*, *sub*, "*CPG*", "*CR*", and *mur*; AAT-1 and AAT-2 in *sub*, "*CPG*", and "*CR*". All loci were polymorphic in *C. conflictana*. In the *Archips* spp. all but two loci were polymorphic in *A. argyrospila* but only one was polymorphic in *A. cerasivorana*.

Genetic distances (Nei 1978) were small among populations within the eight species where more than one population was sampled. *D* values among four or more separate collections of *C. fumiferana*, *C. biennis*, *C. occidentalis*, *C. orae*, *C. pinus*, and *C.l. ponderosana* were all very small (max. $D = 0.004$; diagonal in Table 5). Genetic distances between populations of *C. rosaceana* and *C. conflictana* were appreciably greater but were based on only two populations each.

Genetic distances between the species varied considerably (Table 5). The maximum value among the North American coniferophagous group of *Choristoneura* ($D = 0.233$) was between *C. fumiferana* and *C. pinus*, the only two fully sympatric species considered; the next highest values were for comparisons between *C. fumiferana* and *C.l. ponderosana* and *C.r. spaldingiana*. Values for other comparisons between conifer-feeding species were smaller, ranging from 0.001 to 0.150 (Table 5). *D* values among *C. murinana*, *C. rosaceana*, *C. conflictana*, and the two *Archips* species were all markedly greater, ranging from 0.419 to 2.140 (below the line in Table 5), as expected between full species or genera.

In the separate analysis of the 41 populations of North American coniferophagous *Choristoneura*, for which 12 loci could be used, genetic distances (Nei's "*D*") were generally similar to those of Table 4. However, the highest *D* values were for *C.r. spaldingiana* compared with all the other species, followed by *C. pinus* and *C.l. ponderosana* compared with *C. fumiferana*. That there is still considerable differentiation within the remaining group of entities is shown by the mean F_{ST} value of 0.250 and by the presence of significant chi-square values. Principal loci contributing were AAT-1, PGM-1, and the four esterase loci.

TABLE 5. Matrix of genetic distance coefficients (Nei 1978) averaged by species*

Species	No. of Pops	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1. *Choristoneura fumiferana*	9	0.002															
2. *C. biennis*	4	0.122	0.001														
3. *C. occidentalis*	7	0.131	0.002	0.002													
4. *C. orae*	4	0.125	0.002	0.003	0.004												
5. *C. carnana*	1	0.128	0.001	0.001	0.002	†											
6. *C.r. spaldingiana*	1	0.163	0.074	0.078	0.068	0.072	†										
7. *C. pinus*	9	0.233	0.087	0.074	0.081	0.074	0.151	0.004									
8. *C.l. ponderosana*	3	0.210	0.085	0.071	0.078	0.069	0.115	0.039	0.001								
9. *C.l. subretiniana*	1	0.137	0.008	0.008	0.008	0.006	0.076	0.057	0.069	†							
10. *"CR"*	1	0.150	0.007	0.004	0.007	0.005	0.077	0.080	0.074	0.017	†						
11. *"CPG"*	1	0.126	0.004	0.005	0.004	0.004	0.070	0.094	0.086	0.012	0.011	†					
12. *C. murinana*	1	1.197	0.811	0.792	0.799	0.806	1.002	0.725	0.835	0.763	0.739	0.786	†				
13. *C. rosaceana*	2	0.731	0.972	0.964	0.966	0.960	0.985	0.885	0.883	0.892	1.009	1.021	1.100	0.095			
14. *C. conflictana*	2	0.696	0.515	0.501	0.501	0.500	0.510	0.532	0.419	0.504	0.499	0.527	0.993	0.596	0.098		
15. *Archips argyrospila*	1	1.022	1.076	1.073	1.067	1.070	1.076	1.030	1.067	1.045	1.114	1.047	1.299	0.904	0.952	†	
16. *A. cerasivorana*	1	1.226	1.407	1.402	1.412	1.410	1.421	1.378	1.391	1.358	1.461	1.387	2.140	1.816	2.070	0.706	†

* Analysis based on nine loci.

† Only one population included.

However, most of the variation was within and between populations (means: $F_{IS} = 0.320$; $F_{IT} = 0.490$); IDH-2 and the esterases were the principal loci involved.

Relationships among the taxa were also explored by cluster analysis and distance Wagner procedures (Swofford and Selander 1981; Swofford 1981; and others). All phenograms were generally similar to the one produced by cluster analysis (UPGMA) using modified Rogers' distance (Cophenetic correlation = 0.987) (Fig. 1). Phenograms based on 12 loci generally agreed with the results in Figure 1. More details of the relationships among the closely related members of the *Choristoneura* group were explored through Wagner trees using several distance measures, including modified Rogers' (Fig. 2).

Discussion

The North American coniferophagous species are of interest because of their economic importance and because of the difficulties in separating them morphologically. These studies included all the members of this complex group of coniferophagous *Choristoneura* listed by Powell (1983) except *C. carnana californica*, *C. pinus maritima*, and the nominate subspecies of *C. lambertiana*. Earlier studies of allozymes were limited to five entities (Stock and Castrovillo 1981), so the opportunity to evaluate differences in the larger group has proved useful.

The first sex-linked characters reported in *Choristoneura* were in adult wing scale colour (Stehr 1955) and in haemolymph colour (Stehr 1959) of several species. Sex-linkage in the allozymes of AAT-1 was first reported by May et al. (1977). Results reported here and elsewhere (Harvey 1996) confirm the sex-linked nature of AAT-1 in three species (*C. fumiferana*, *C. occidentalis*, and *C. pinus*) and suggest it is also true of the remaining members of this group of species. Two other loci (IDH-2 and LDH-1) are sex-linked in *C. fumiferana* (Harvey 1996); current studies indicate that LDH-1 is also sex-linked in *C. occidentalis* and *C. pinus*, but that IDH-2 is not sex-linked in other members of the group. Further work is needed on IDH-2 and on the esterases in several members of the group. The importance of sex-linkage and polymorphisms in physiologically significant processes in *Choristoneura* was discussed by Stehr (1964) and, more recently, by Sperling (1994). Further attention to sex-linkage may be expected to help unravel the nature and evolution of the members of this closely related group of species.

The most marked differences in allozyme frequencies among the species tested were in aspartate aminotransferase (AAT-1). Frequencies of this allozyme in several species of *Choristoneura* were reported by Stock and Castrovillo (1981), Castrovillo (1982), and Harvey (1985). The present studies extend these results to several additional species (Table 3). The most common allele in the conifer-feeding species was E (0.62) which was present in 98–100% of individuals of all species tested, including *C. murinana*, except *C. fumiferana* where it was present at only 4.8%. In contrast, the principal allozyme of *C. fumiferana*, C (1.00), found at a frequency of 65.1%, was unique and was not found in 1388 larvae of the other 11 species. The allele D (0.81), present in 29.6% of *C. fumiferana*, was not unique but was found, although at much lower frequencies, in several other conifer-feeding species (0–1.6%; 4.4% in *C. biennis*). In *C. rosaceana*, the principal allele was E as in *C. fumiferana*; alleles B and D were also present but rare and only in the Ontario collections. In *C. conflictana*, E was the most common allozyme, as in the large group of conifer feeders. The two *Archips* species differed from each other, and from the *Choristoneura*. In *A. argyrospila*, B (1.18) was the major allele; an allele unique to this species, A, was present at a frequency of 12.5%. The only allele in *A. cerasivorana* was D (0.81) which although present in several other species was most common (29.6%) in *C. fumiferana*.

Genetic distances in populations of *C. fumiferana* from 33 locations across Canada were found to be very small (max. $D = 0.008$), indicating a single species with apparent high levels of gene flow and insignificant differences related to population history (Harvey 1996). The

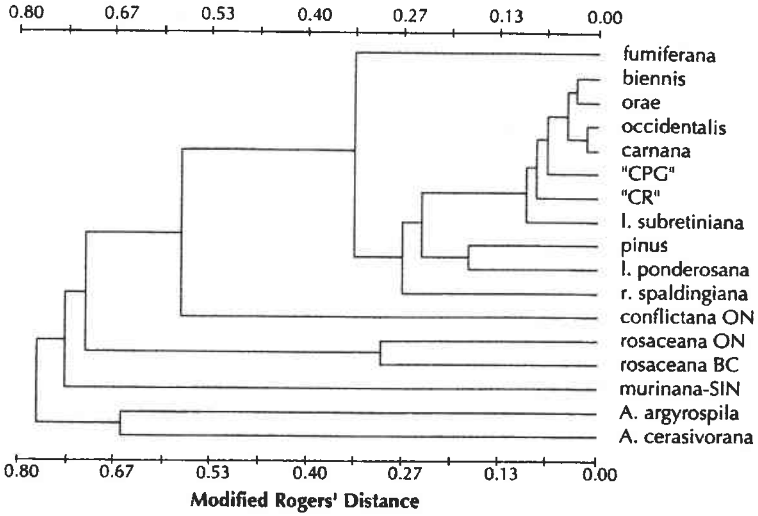

FIG. 1. Phenogram of genetic relationships based on nine loci [UPGMA clustering of Rogers' (modifed) distance coefficients] among pooled larval collections of *Choristoneura* and two *Archips* species (Wright 1978). Cophenetic correlation = 0.987.

data reported here for widely separated populations of *C. pinus* show that it is also genetically uniform across its range, with the mean genetic distance among nine locations very small and similar to *C. fumiferana* (0.004; Table 5). There were no significant differences among the collections related to location or population density, and *C. pinus* can be confirmed as a single species across its entire range. As with *C. fumiferana*, however, chi-square analysis suggests differences between locations at some loci. The small genetic distances among collections of other conifer-feeding species (Table 5) suggest similar homogeneity within them; however, in no case does the number and geographic distribution of population samples validate such conclusions.

Genetic identities (I, Nei 1978) among the North American coniferophagous species were all high ($I \geq 0.797$). D values (max. = 0.233; Table 5) were generally below values associated with the species level of distances (Brussard et al. 1985; Gooding et al. 1992). The species that was most different is *C. fumiferana* (D = 0.122–0.233; Table 5). The principal source of this difference is at locus AAT-1, as discussed above, but there are also differences in PGM, PGI (particularly with *C. pinus*), and EST-5. D values for *C.r. spaldingiana*, *C. pinus*, and *C.l. ponderosana* were also greater than those among other coniferophagous *Choristoneura* species.

Genetic relationships among all 16 taxa are illustrated by the phenogram (UPGMA) based on modified Rogers' distances (Fig. 1). As expected from Table 5, the two *Archips* species, the broad-leaf-feeding *Choristoneura* (*C. rosaceana* and *C. conflictana*), and the European *C. murinana* were separated at genetic distances in excess of 0.47, consistent with their status as full species. Furthermore the phenogram confirms the grouping of the North American coniferophagous *Choristoneura* as a closely related complex separate from the other species. The Wagner tree using modified Rogers' distances was slightly different, and the Cophenetic correlation increased to 0.994 after optimization. However, the relationships among the entities were virtually unchanged and the branch lengths closely similar.

The separate analysis of 41 populations of the 11 coniferophagous *Choristoneura* species using 12 loci allowed more detailed examination of the interrelationships among the

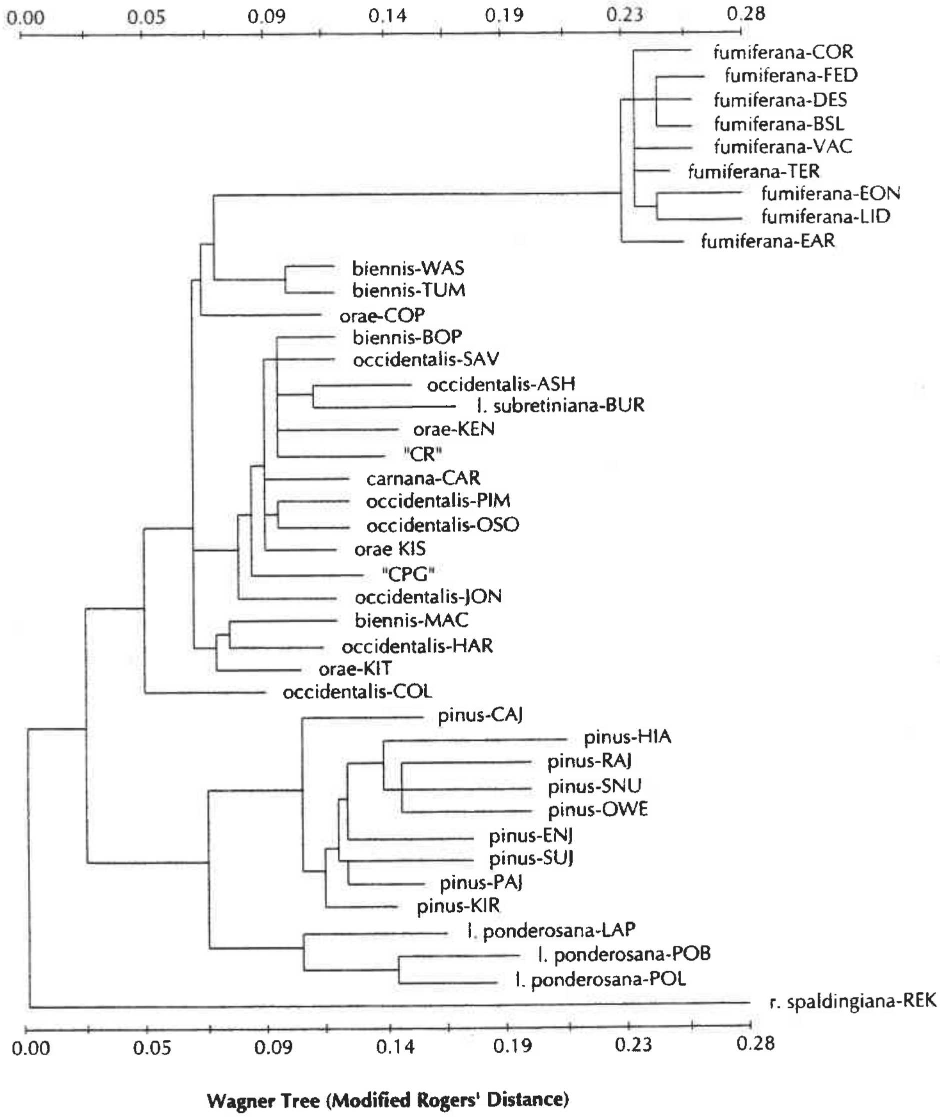

FIG. 2. Optimized Wagner tree of modified Rogers' distances (Wright 1978) based on 12 loci among 41 larval collections of *Choristoneura* species. Cophenetic correlation = 0.970.

populations. In general the Wagner trees based on different distance measures were quite similar. Modified Rogers' distances (Fig. 2) provided the simplest tree, although the Cophenetic correlation of 0.970 was slightly below that of the other trees tested. In all trees, *C.r. spaldingiana* separated first at the greatest distance from the root. The second group to separate contained all the *C. pinus* and *C.l. ponderosana* collections which formed a separate group in all trees. All the remaining entities formed a large group without clear species groupings, except for *C. fumiferana* (Fig. 2). Trees based on Edwards', Cavalli-Sforza-Edwards', and Prevosti distances were similar except that the large remaining group was divided into two sections, surrounding the *C. pinus* and *C.l. ponderosana* group. However, *C. occidentalis*, *C. biennis*, and *C. orae* were present in both sections of this divided group.

There was no evidence of relationships among species in the groupings in either tree that related to pheromone, host, or geography (Harvey 1985). Although *C. fumiferana*, *C. biennis*, and *C. occidentalis* are characterized by having an aldehyde pheromone, *C. fumiferana* is widely separated from the other two in the phenogram. Similarly, *C. orae*, *C. carnana*, and *C.r. spaldingiana* share an acetate pheromone, but are not grouped closely together. Of the species that feed on pine hosts, the eastern *C. pinus* and the western *C.l. ponderosana* were separated from *C.l. subretiniana*, "*CPG*", and "*CR*", which also show some separation in the phenogram. In all these species, collections from different locations (Table 1) appear to be randomly distributed in the groups and the affinities existing are not related to geographic location.

The early separation of *C.r. spaldingiana* was not expected from the results of Stock and Castrovillo (1981) who placed *C. retiniana* very close to *C. biennis*. However, their results were based on collections from the Warner Mountains (Modoc Co., CA), which is now recognized as a "hybrid zone" (Powell and DeBenedictis 1996). Nevertheless Castrovillo (1982) noted that the "retiniana" from the Tehachapi source differed markedly from collections from the Warner Mountains. It was, in fact, the most widely separated of all his collections (Nei's "*D*" = 0.179). The designation of the insects from Tehachapi and other areas east and south of the "hybrid zone" as the subspecies *C.r. spaldingiana* (Powell and DeBenedictis 1996) recognizes this difference. Thus the greater separation of *C.r. spaldingiana* in Figure 2 is probably a truer representation of the actual relationship than that shown by Castrovillo (1982) or Harvey (1985). Perhaps this entity should be separated at a greater than subspecific level.

Choristoneura pinus and *C.l. ponderosana* populations were separated at an early level in all the trees at an intermediate distance from the root. This separation and their subsequent separation into two subgroups appears consistent with their host and other affinities (Harvey 1985). However, this position is interesting because it separates the two subspecies of *C. lambertiana*, grouping *C.l. ponderosana* more closely to *C. pinus*, and *C.l. subretiniana* more closely to the remaining group. It is unfortunate that no samples of the nominate subspecies (*C.l. lambertiana*) could be obtained for testing.

The third group to separate contained all the *C. fumiferana* collections, which separated from the remaining species almost as far from the root as *C.r. spaldingiana*. The separation of *C. fumiferana* is caused in part by the uniqueness of its AAT-1 allozymes, particularly C, but also D. This uniqueness allows individual insects of this species to be distinguished from all other conifer-feeding *Choristoneura* in better than 95% of the cases. This character has been used to assist moth identification in a study of spicule numbers on several species in northwestern Canada (Gray et al. 1995). Allozymes of AAT-1 can also be used to assess hybridization between *C. fumiferana* and *C. biennis* in areas of sympatry (Shepherd et al. 1995).

The grouping of the remaining entities (*bie*, *ora*, *occ*, *car*, *sur*, "*CPG*", and "*CR*") into a single large cluster where all the species are intermixed together is consistent with their recognized closeness as entities and offers no assistance in their separation or identification. The group contains the spruce/Douglas fir/true fir feeders as well as species that feed on pines. The two pine feeders from Richmond and Prince George, B.C., and *C.l. subretiniana*, which also feeds on pine, were not grouped together or separated from the other entities. The closeness of *C. occidentalis*, *C. carnana*, and *C. biennis* in both figures is in good agreement with Powell (1996).

Positions of the species in the phenograms developed here are in general agreement with Stock and Castrovillo (1981) based on 18 loci, but differ somewhat from those reported by Castrovillo based on 21 loci (1982, fig. 4; Harvey 1985). Castrovillo found that

C. lambertiana was the most distant of the group studied, and that *C. fumiferana* was in a subgroup along with *C. pinus*, *C. orae*, and *C. occidentalis*, although somewhat apart from these three. Castrovillo's sample of *C.l. ponderosana* came from the same area as those used in this study. These differences between the separate studies can be accounted for, at least in part, by differences in the number and identity of the loci upon which separations are based. Sources and numbers of insects may also contribute.

The relationships among the species as depicted in Figures 1 and 2 are in good agreement with the recent work of Sperling and Hickey (1994) based on mitochondrial DNA sequencing, which shows *C. fumiferana* and then *C. pinus* as more distant from the group containing *C. occidentalis*, *C. orae*, *C. biennis*, and *C. occidentalis*. However, positions of the different entities are not in agreement with cladograms based on several characters developed by Powell and DeBenedictis (1996) in an attempt to understand pathways of speciational events.

The present results help to confirm the close relationships among the species forming the major group in Figure 1 and raise questions about the separate identities for *C. biennis*, *C. orae*, *C. occidentalis*, *C. carnana*, or *C.l. subretiniana*, and the status of the new "*CPG*" and "*CR*". They are consistent with the separate identities for *C. fumiferana*, *C. pinus*, and *C.l. ponderosana*, and possibly for *C.r. spaldingiana*. This conclusion supports that of Powell (1980), that not all of the entities are valid species at this stage in their evolution. Which ones of the present names can be retained and which entities should be given lower taxonomic rank requires further work. Nevertheless, the use of allozymes does provide another character that can be effective in establishing some separations, such as that of *C. fumiferana* from all the other tested entities (Harvey 1985; Shepherd et al. 1995).

Common to these several studies is the finding of the small level of differences among the coniferophagous *Choristoneura*. As found in other studies of this group no single character, be it morphological, physiological, or other, permits certain identification of individuals of all the various species and subspecies. However, Powell (1996) has utilized pheromone specificity, distribution, and morphology together to separate some of the taxonomic entities with considerable success. This process should be extended to include all the members of the group. For further progress in understanding the status and evolutionary development of these important entities additional characters, such as allozymes, DNA (Sperling and Hickey 1994), haemolymph polymorphism (Stehr 1964; Harvey and Stehr, 1967), and pre- and post-mating factors (Harvey in preparation) must all be considered.

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