INTERSPECIFIC CROSSES AND FERTILE HYBRIDS AMONG THE CONIFEROPHAGOUS *CHORISTONEURA* (LEPIDOPTERA: TORTRICIDAE)

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Abstract

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Laboratory studies of seven North American *Choristoneura* species and two subspecies from widely distributed locations demonstrate the low level of genetic separation among them. No differences were found in numbers ($n = 30$) or morphology of chromosomes among members of the group or in any of the hybrids examined. High levels of mating success were obtained in all inter se matings as well as in crosses and back-crosses. However, mating success was greater for crosses within host type and within pheromone type than for crosses between types. Viability and fertility were similar in all the hybrids and close to those of the inter se progenies. Mean weights of initial eggs varied by a factor of 2 from the lowest (*C. pinus* Freeman) to the highest (*C. lambertiana ponderosana* Obraztsov). Mean weights of initial eggs produced by hybrids were generally close to those of the parental species. However, when one parent was *C. fumiferana* (Clem.), mean weights of initial eggs were either much larger (male *C. fumiferana*) or much smaller (female *C. fumiferana*) than either parental type. These differences could affect survival of some progeny under harsh conditions. This evidence supports other studies in indicating that *C. fumiferana* is genetically distinct from other species in this group.

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Résumé

Des études en laboratoire sur sept espèces et deux sous-espèces nord-américaines de *Choristoneura* réparties en des localités dispersées ont démontré que la variation génétique entre elles est faible. Aucune différence n'a été constatée dans le nombre ($n = 30$) ou la morphologie des chromosomes entre les membres du groupe ou les hybrides examinés. Une grande proportion des accouplements se sont effectués avec succès aussi bien dans les accouplements inter se que dans les croisements et les rétro-croisements. Cependant, le succès des accouplements s'est avéré plus élevé dans le cas de croisements entre individus du même type d'hôte ou du même type de phéromone que dans le cas de croisements entre différents types. La viabilité et la fertilité étaient équivalentes chez tous les hybrides et très semblables à celles qui prévalaient au sein de la progéniture issue d'accouplements inter se. La masse moyenne des premiers oeufs variait par un facteur de 2, des moins lourds (*C. pinus* Freeman) aux plus lourds (*C. lambertiana ponderosana* Obraztsov). La masse moyenne des premiers oeufs produits par les hybrides était généralement semblable à celle des premiers oeufs des espèces parentales. Cependant, dans les cas où l'un des parents appartenait à l'espèce *C. fumiferana* (Clem.), la masse moyenne des premiers oeufs était ou beaucoup plus élevée (mâle *C. fumiferana*) ou beaucoup moins élevée (femelle *C. fumiferana*) que l'un ou l'autre des types parentaux. Ces différences pourraient affecter la survie de certains rejetons dans des conditions rigoureuses. Ces données appuient les résultats d'autres études qui indiquent que *C. fumiferana* est une espèce génétiquement distincte des autres espèces du groupe.

[Traduit par la Rédaction]

Introduction

Morphological differences that permit clear separation of all of the North American conifer-feeding *Choristoneura* have not been found (Harvey 1985*a*; Powell 1995*a*). Present taxa are based on host, distribution, and biological as well as morphological characteristics.

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Although some species are geographically separated, a number of sympatries have been demonstrated (Harvey 1985*a*; Powell and De Benedictis 1995*b*; Shepherd et al. 1995). There is evidence of natural hybridization between *C. occidentalis* and *C. retiniana* in south-central Oregon (Volney et al. 1984), between *C. carnana* and *C. occidentalis* in California, and possibly between other entities in the western United States (Powell 1995*a*). For other sympatries, such as *C. fumiferana* and *C. pinus*, there appears to be effective reproductive isolation.

However, both inter- and intraspecific matings are easily obtained under laboratory conditions for all combinations. Successful crosses have been reported for several combinations among *C. fumiferana, C. biennis, C. occidentalis, C. orae, C. pinus*, and *C. retiniana* (Smith 1953; Campbell 1967; Harvey 1967). Sanders et al. (1977) reported successful crosses and back-crosses among additional species and Volney et al. (1984) and Liebhold (1986) reported successful crosses and back-crosses between *C. occidentalis* and *C. retiniana* in the laboratory. Other successful crosses have been reported also (Harvey and Roden 1979; Harvey 1985*a*). However, evidence on comparative mating success has not been reported.

By making collections when population levels permitted and assembling these stocks in the laboratory, it was possible to conduct mating experiments among species normally separated by geographical and phenological differences and to assess mating success in most of the combinations among the species. Crosses leading to the second generation depend on success in the first generation but are readily obtained. Sufficient results from experiments by G.W. Stehr (deceased) and myself have now accumulated for analysis. These results are reported here.

Materials and Methods

Insect Collections. Collections of feeding, late-instar larvae were obtained from various field locations in Canada and the United States over the past 35 years (Stehr 1967; Harvey and Stehr 1967; Harvey 1983*a*). Sources and identities of the insects used in mating experiments summarized herein were mostly those in the reports identified above or in Harvey and Roden (1979) . Collections used for determination of egg weights of the different species and those used for the mating experiments are listed in Table 1. [For full names, code names, and authorities of insects see Table 1; for hosts see Table 2.] Adults from some of these collections and some of the hybrids have been deposited in the Canadian National Collection, Ottawa (Harvey and Roden 1979; Harvey 1991). Sources of *C. fumiferana* have been described previously (Harvey and Roden 1979; Harvey 1983*a*). The European *C. murinana* was collected from a geographic outlier in the southern extension of white fir (*Abies alba* Mill.) (V. Nealis, pers. comm.).

Methods used to obtain these collections and the possible sources of misidentification were discussed by Stehr (1967). Identifications were based on larval, pupal, and adult characters as well as on host and collection location; for most collections additional confirmation was provided by characters in progeny for one or more generations. For most of the species studied, collections originated from outbreak populations. For the sympatric species *C. fumiferana* and *C. pinus*, identifications were based on appearance and host and the differences are clear. For several western species, however, distribution and sympatries are less clearly established and further comments about identification are necessary.

Comparison of the identifications of the western species in Table 1 with the distributions shown by Powell and De Benedictis (1995*b*) indicates that most collections of all entities were obtained from areas where there was little evidence of uncertainty about the identities, or likelihood of the presence of hybrids. Most of the collections of *C. occidentalis* were from British Columbia and not in question. The collections from Montana (ROK) and Idaho (WIL) were both beyond the recognized zone of hybridization (Powell and De Benedictis 1995*b*)

TABLE 1. Sources of collections of *Choristoneura* spp. for mating studies and E_i measurements

Name, Code	Source	Host[1]	Lat °N	Long °W	Date (d/m/y)	Collector
***C. biennis* Freeman (*bie*)**						
F′2 LAK	Lake Louise, Banff N.P., AB	1	52	116.4	/06/52	J.Petty
F′4 SKY	Koidern (mi 1061 Alaska Hwy), YT	1	62	140.5	24/06/64	R. Wood
F′6 NUK	Numa Creek, Kootenay N.P., BC	1	51.2	116.4	27/06/66	V.B. Patterson
F′6 RIK	Saskatchewan R. Crossing, AB	1	52	116.4	26/06/66	V.B. Patterson
F′0 KAN (3)	White R., Canal Flats, BC	3,4	50.5	115.6	11/07/70	Vanderwals
F′6 KAR (3)	Hendrix Lake, BC	4	51.8	121.5	05/07/76	S.J. Allen
F′7 SIK (2,3)	McMurdo Cr., Golden, BC	1,4	51.2	121.5	21/06/77	C.B. Cottrell
F′9 WAS (2,3)	McMurdo Cr., Golden, BC	3	51.2	116.7	29/06/79	C. Wood
***C. orae* Freeman (*ora*)**						
F′1 TIM	Kitimat Stn., BC	2,5	54.0	128.7	21/06/61	K. Jardine
F′2 TAM,MIT	Kitimat Stn., BC	2,5	54.0	128.7	14/05/62	E.G. Harvey
F′3 TAT	Kitimat, BC	5	54.0	128.7	/06/63	E.G. Harvey
F′1 RAE	Kitimat, BC	5	54.0	128.7	06/07/70	E.V. Morris
F′9 ANA (3)	Anchorage, AK	1	62.0	150	20/06/79	E. Holsten
F′9 ANK (3)	Anchorage, AK	1	60	150	04/06/79	E. Holsten
F′4 KEN (3)	Kenny L., Copper R. Basin, AK	1	61.8	145	07/05/84	A.G. Gordon
F′5 COP (3)	Copper Basin, AK	1	61.8	145	17/06/85	E. Holsten
***C. occidentalis* Freeman (*occ*)**						
F′3 ROK	Slide Rock Mt., Lolo N.F., MT	14	46.6	113.5	29/07/63	D.G. Fellin
F′4 WIL	Williams Cr., Salmon N.F., ID	8,9	45.1	114	30/07/64	K. Lister
H′7 HOP	Hope, BC	8	49.4	121.4	12/06/67	C.B. Cottrell
F′0 VEN	Mission Mt., Shalath, BC	8	50.7	122.2	25/06/70	Doidge
F′6 BOS (3)	Kwoiek Cr., Boston Bar, BC	4	50.1	121.6	29/06/76	J. Mowts
F′6 HUP (3)	Silver-Skagit Rd., Mi. 28, Hope, BC	8	49.2	121.4	30/06/76	E.V. Morris
F′6 SUM (3)	Adams Lake, BC	8	51.4	119.6	17/06/76	Andrews
F′5 HAR (3)	Harper Mt., Kamloops, BC	8	50.7	120.8	07/07/85	S.J. Allen
F′5 COL (3)	Lyons, Boulder Co., CO	8	40.3	105.4	26/06/85	R.E. Stevens
***C. carnana californica* Powell (*car*)**						
F′5 CAR (3)	Trinity Co., CA	8	41	122.7	10/05/85	W.J.A. Volney
***C. retiniana* (Walsingham, 1879) (*ret*)**						
F′0 VID	4 mi NE Lakeview, OR	6	42.2	120.3	09/07/70	F.H. Schmidt
F′5 VEM (3)	Benton Meadows, OR	6	41.5	120.6	24/07/75	G. Daterman
F′6 VIS (3)	Lakeview, OR	7	42.2	120.3	22/07/76	G. Daterman
***C. pinus pinus* Freeman (*pin*)**						
2′6 JUS	Spooner, WI	10	45.7	91.8	18/06/62	I.M. Campbell
S′65-1954	Murtrie Co., Kenora, ON	10	49.8	94.4	26/06/65	G.G. Jackson
S′65-2201	Hanmer, ON	10	46.5	81	01/07/65	J.R. McPhee
S′65-1759	Nairn Centre, ON	10	46.3	81.4	22/06/65	J.R. McPhee
F′6 FAJ	Sault Ste. Marie, ON	13	46.5	84.4	28/06/66	G.T. Harvey

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***C. lambertiana subretiniana* Obraztsov (*sub*)**						
F′3 RET (3)	Washoe Co., NV	9	39.5	120	08/07/63	C. Wray
F′4 AOL, PUR	North Truckee, CA	6,9	39	120	04/07/64	A.E. Cameron
F′4 LAM	Benton Meadows, Modoc N.F., OR	9	41.5	120.6	02/07/64	G. Daterman
F′5 SUR (3)	Benton Meadows, OR	9	41.5	120.6	24/07/75	G. Daterman
***C. lambertiana ponderosana* Obraztsov (*pon*)**						
F′3 LAR	Poudre Canyon, Larimer Co., CO	12	40.7	105.5	11/07/63	C.J. Germain
F′4 CLP, RFL	Larimer Co., CO	12	40.8	105.7	06/07/64	M.E. McKnight
F′4 PON	Pondre Dist., CO	12	40.7	105.5	25/06/64	M.E. McKnight
F′5 SEV	Thousand L., Mt. Sevier CO., UT	4	39	112	12/07/65	M. McGregor
F′5 AVE	Beaver Co., UT	6	38	112	14/07/65	M. McGregor
F′ 7 PAN (3)	Larimer Co., CO	12	40.8	105.7	23/06/77	R.E. Stevens
F′5 POB (3)	Near Lyons, Boulder Co., CO	12	40.8	105.7	21/06/85	R.E. Stevens
F′6 LAP (3)	Poudre Canyon, Larimer Co., CO	12	40.8	105.7	12/06/86	D.A. Leatherman
***C. lambertiana* uncertain**						
F′4 MAM	Mammoth Hot Springs, WY	11	45	110	04/08//64	D.G. Fellin
F′4 BAN (3)	Near Burmis, AB	11	49.6	114.7	29/06/64	J. Petty
***C. murinana* Hubner (*mur*)**						
SIN	Sion, Switzerland	15	—	—	10/06/88	N. Mills

[1]Hosts identified in Table 2.
[2]Out-of-phase population.
[3]Sources of adults for crossing experiments.
[4]Other insect sources, including *C. fumiferana* (Clem.), listed in Stehr (1967), Harvey and Stehr (1967), Harvey (1983*a*), and Harvey and Roden (1979).
[5]Code and name, explained in Harvey and Roden (1979), is same as used for moths deposited in the Canadian National Collection.

TABLE 2. Host species of *Choristoneura* collections

Host species	Code[1]
Picea glauca (Moench) Voss	1
Picea sitchensis (Bong.) Carr	2
Picea engelmannii Parry	3
Abies lasiocarpa (Hook.) Nutt.	4
Abies amabilis (Dougl.) Forbes	5
Abies concolor (Gord. and Glend.) Hoopes	6
Abies grandis (Dougl.) Lindl.	7
Pseudotsuga menziesii (Mirb.) Franco	8
Pinus contorta Dougl.	9
Pinus banksiana Lamb.	10
Pinus flexilis James	11
Pinus ponderosa Laws.	12
Pinus sylvestris L.	13
Pinus albicaulis Engelm.	14
Abies alba Mill.	15

[1]Host code for Table 1.

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and would appear to be correctly identified, but the limits of the area of hybridization are not well known.

The single collection of *C. carnana* from Trinity Co., CA, was identified by W.J.A. Volney, and is within the known range of *C. carnana californica* (Powell and De Benedictis 1995*b*).

The *C. retiniana* collections were from *Abies* in south-central Oregon and probably came from the 'area of hybridization' between *C. occidentalis* and *C. retiniana* (Powell and De Benedictis 1995*b*). All were received as larvae, and only moths from green larvae were used in matings. The identification as *C. retiniana* was confirmed by the colour of the larval progeny (Liebhold 1986).

Western pine-feeding forms used in these studies included the two subspecies of *C. lambertiana*, which for clarity here are also described. Adults of *C. ponderosana* were consistently smaller than those of *C. l. subretiniana*, had cream and red-brown forewings and 'creamy or light gray to white hind wings', and included both of the 'morphs' described by Stehr (1967). Most of these insects were collected from *Pinus ponderosae* in Larimer Co., CO, and all would conform to *C. ponderosana* of Powell and De Benedictis (1995*b*) . The collections from Utah (SEV on *Abies lasiocarpa*) and Nevada (AVE on *Abies concolor*) were from unusual hosts but in larval and adult appearance closely resembled *C. ponderosana*.

Collections treated as *C. l. subretiniana* were those with 'red-brown forewings and gray and orange hind wings' (Stehr 1967) and originated from Benton Meadows, OR; Washoe City, NV; and North Truckee, CA. The first two locations fall well within the *C. subretiniana* range (Powell and De Benedictis 1995*b*). The collections from North Truckee, CA (AOL from *Abies grandis* and PUR from *Pinus contorta*) were from the area now known to contain the nominate species *C. lambertiana*; however, only two matings are included and they have been left with the *C. subretiniana* group. Two collections of less certain identity from Yellowstone Nat. Pk., WY, and Burmis, AB (Table 1) have been left separate and were not used in mating experiments. They may belong to the 'near *C. subretiniana*' group of Powell and De Benedictis (1995*b*).

Immature larvae were allowed to complete their development in the laboratory on shoots of the host from which they were collected (Stehr 1954) or, when necessary, on artificial diet (Grisdale 1970). Throughout the experiments laboratory conditions were controlled at 21°C, 70% RH, and a 17-h photophase centred at 1200 hours local time.

Chromosome Preparation. Chromosomes were studied using progenies from mating success and egg weight experiments (see Tables 5 and 9) . All members of the group except *C. ponderosana* and many of the hybrids were examined (see Table 3). Meiotic metaphase figures were obtained from third- to fifth-instar male larvae and from female pupae (Ennis 1976). They were injected with 0.02% aqueous colchicine 3 h before sacrifice. Testes and ovaries were dissected out in 1% fresh sodium citrate and fixed by the method of Crozier (1968), modified by using acetic-ethanol for fixation. They were processed immediately or transferred to 70% ethanol for storage at 4°C.

Material fixed as above was transferred to a drop of 60% acetic acid on a slide and teased apart with minuten pins until a dispersed cell suspension was obtained. The slide was placed on a slide warmer at 40°C, a drop of acetic-methanol added, and the slide tilted in all directions to assist spreading. When dry, slides were stained with 2% aceto-carmine and made permanent.

Mating Experiments. Moths utilized for matings and crosses (Table 1) were selected to represent the collection norm and their appearance recorded; identifications of moths used in inter se matings were confirmed by the appearance of the progeny. Each pair of moths was placed in an individual ventilated jar containing a piece of moistened foliage (Stehr 1954; Harvey 1977). Mature foliage of balsam fir was provided for oviposition by females

of all spruce–fir-feeding species; foliage of jack pine was provided for females of the pine-feeding species. For matings between hybrids both foliage types were provided. Moths were generally used within 48 h of eclosion, although in some cases one sex was held at 2°C for a day or 2 until mates were available. Only apparently healthy, normal adults were used; deformed or low vigour adults were avoided. Matings were checked and provided with moisture daily over the next 10 days. Seven days after set up of the mating, the foliage was carefully examined and all egg masses were removed. Eggs in individual egg masses were counted and weighed (Harvey 1977) and 5 weeks later were stored at 0°C to satisfy diapause (Harvey 1961). Following diapause, progenies were reared on previously frozen balsam fir foliage or artificial diet (Harvey 1977).

For clarity of terminology the following conventions have been used thoughout. In all designations of mating combinations the female is listed first. Matings rated as successful (i.e. 'fertile') were those producing five or more eggs that hatched; normally the number of fertile eggs was much greater. Matings within the same species are designated as 'inter se', between different species as 'crosses'. All progenies of these matings are the F_1 generation. Progenies of matings between species (crosses) are F_1 hybrids. Matings between F_1 moths can be inter se or, if between species or hybrids, are called F_1 crosses; such matings produce the F_2 generation and F_2 progeny. Matings between F_2 individuals are called F_2 crosses and can be of several types. Back-crosses are matings between an F_1 hybrid moth and a moth of one of the parental species; there are two kinds of F_1 back-crosses, depending on whether the male or female is the parental species.

Egg Weights. Average weights of eggs in the initial two clusters (E_i) produced by successful matings were determined as described by Harvey (1977). Only weights of fertile eggs have been used to characterize species and hybrids (Harvey 1977). For each successful mating this single average value (E_i) was used in statistical analyses. Although egg weight data were collected over several years, treatment of the insects and other techniques remained constant over that period.

Mean E_i values by species were compared by a series of paired 't' tests at $P \leq 0.01$ using table values for mean E_i and SEM (see Table 4). To assist in the interpretation of E_i values among hybrids, and because of the role of the X-chromosome in determining egg size (Campbell 1958), E_i ratios were calculated for each of the F_1 hybrid values using data reported in Table 9, as follows:

E_i ratio = E_i of F_1 progeny / mean E_i male parental type.

Some of the statistical methods used to analyse the data may not satisfy the requirement for independence among samples. However, because not all the basic data are still accessible, it is no longer possible to do a more adequate analysis. The use of an alpha level of 0.01 should help maintain the experiment-wide error at a low level. Thus, in spite of these caveats, the results should be of value.

Results

Species Descriptions. Chromosomes. In males (n) of all species studied there is one large pair of chromosomes, presumably the X-chromosomes. In females ($2n$) of *C. fumiferana* and all other species examined there was one large pair chromosomes (Ennis 1976). Chromosome numbers in all members of the group tested were $n = 30$ (Table 3).

Egg weights. E_i values were relatively uniform within species but showed differences among species, several of which were significant (Table 4). Among the *C. lambertiana* group two exceptional E_i values from Yellowstone National Park (0.142, 0.153 mg) and one from Burmis, AB (0.162) mg) were significantly smaller than any other values for *C. lambertiana*. Both collections came from *Pinus flexilis*, and although the moths were similar in

TABLE 3. *Choristoneura* species and their hybrids[1] examined for chromosome number[2] and morphology

	Male (m)							
Female (f)	*bie*	*ora*	*fum*	*occ*	*ret*	*pin*	*sub*	*pon*
bie	f/m		f/m					
ora	m	m	m					
fum	m	m	f/m		m	f/m		
occ	m			m			m	
ret	m				m		m	
pin	f/m			m		f/m	m	
sub				m			m	
pon			m				m	

[1]Minimum number counted: three females, two males; somewhat larger numbers surveyed for chromosome morphology.
[2]Chromosome numbers in all females: $2n = 60$; in all males: $n = 30$.

appearance to *C. l. ponderosana*, their smaller egg weight raises questions about their identity. These values were therefore listed separately from the other data for *C. lambertiana* in Table 4.

Mating Studies. F_1 matings. Fertile matings were obtained in all combinations among the several North American species where five or more crosses were attempted (Table 5). In contrast, of 21 crosses between the European *C. murinana* and *C. orae*, *C. fumiferana*, or *C. occidentalis* none was successful (*C. murinana* inter se: four out of nine successful). Comparative mating success in F_1 crosses among the North American species (Table 5) can be summarized:

1. Inter se matings (i.e. within species) (diagonal, Table 5) had a significantly higher percentage success than the crosses between species in both fir- and pine-feeding groups (Table 6; $P < 0.01$).

2. All combinations tested involving pine-feeding species (*C. pinus*, *C. subretiniana*, *C. ponderosana*) were successful, although insects were not available for some combinations. Mating success was generally lower for both inter se matings (diagonal, Table 5) and for crosses among the pine-feeding species than among the spruce–fir-feeding species. These differences were significant for both inter se matings (40.4% vs. 71.9%) and for intra-group matings (10.8% vs. 48.4%) (Table 6). There were no significant differences among the spruce–fir-feeding species.

3. Success of matings between the two host groups was lower than both the inter se or intra-group crosses (Table 6; $0.05 > P > 0.01$).

4. Analysis of crosses among the six principal species showed that mating success was greater among species that shared a pheromone type than between species with different pheromone types (Table 7; $P < 0.01$). *Choristoneura subretiniana* and *C. ponderosana* were not included in this analysis because of the paucity of data.

F_2 matings. Survival of insects from egg stage to adult for all the progenies of F_1 matings was close to that of laboratory stocks. For most crosses sufficient moths were obtained to test fertility and mating success among the F_1 adults. Most of the possible F_2 crosses were successful and produced fertile eggs (Table 8). Although comparative data were not available for all combinations, mating success in crosses was generally similar to that of other laboratory matings. Some lower values occurred among pine-feeding species, but appeared less marked than among the F_1 crosses.

Although data were available from only 26 of the 64 possible crosses, fertility of eggs produced by fertile F_2 crosses was, on average, 47.8% (range per hybrid type 17.1–70.7%).

TABLE 4. Summary of egg weights of *Choristoneura* spp. by collection[1]

Species	Population	n[2]	Mean E_i[3] (mg)	SEM[4] (mg)
C. fumiferana	(all across Canada)[5]	852	0.165c	0.001
C. biennis	F'2 LAK	5	0.182	0.0107
	F'4 SKY	22	0.188	0.0038
	F'6 NUK	23	0.179	0.0031
	F'6 RIK	10	0.176	0.0044
	F'0 KAN	18	0.192	0.0040
	F'6 KAR	33	0.168	0.0022
	F'9 SIK	22	0.163	0.0033
	F'9 WAS	9	0.192	0.0055
	All	120	0.180a	0.016
C. orae	F'2 MIT	4	0.150	0.0074
	4'6 TAT	19	0.154	0.0030
	F'0 RAE	14	0.149	0.0026
	F'9 ANA	9	0.161	0.0036
	F'9 ANK	12	0.166	0.0039
	F'4 KEN	1	0.212	
	F'5 COP	10	0.172	0.003
	All	69	0.159b	0.0019
C. occidentalis	F'3 ROK	18	0.171	0.0029
	F'4 WIL	1	0.164	
	H'7 HOP	31	0.161	0.0019
	F'0 VEN	17	0.176	0.0028
	F'6 BOS	16	0.162	0.0031
	F'6 HUP	9	0.168	0.0046
	F'6 SUM	24	0.169	0.0024
	F'5 HAR	1	0.192	
	F'5 COL	7	0.211	0.008
	All	124	0.170c	0.0015
C. carnana californica	F'5 CAR	3	0.173	0.005
C. retiniana	F'0 VID	17	0.148	0.0038
	F'5 VEM	37	0.156	0.0023
	F'6 VIS	3	0.158	0.0114
	All	57	0.154b	0.0028
C. pinus pinus	2'6 JUS	3	0.104	0.0037
	S'65-1954	4	0.124	0.0122
	S'65-2201	1	0.086	
	S'65-1759	1	0.109	
	F'6 FAJ	15	0.097	0.0017
	All	24	0.102d	0.0029
C. l. subretiniana	F'4 AOL, PUR	2	0.195	0.0050
	F'4 LAM	4	0.198	0.0054
	F'5 SUR	6	0.200	0.0051
	All	12	0.199e	0.0030

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C. l. ponderosana	F′3 LAR	1	0.218	
	F′4 CLP, RFL	3	0.206	0.0028
	F′4 PON	3	0.202	0.0058
	F′5 SEV	6	0.208	0.0050
	F′5 AVE	2	0.212	0.0045
	F′7 PAN	12	0.201	0.0059
	F′5 POB	1	0.203	
	All	28	0.205e	0.0029
C. l. uncertain (see text)	F′4 MAM	2	0.147	0.0055
	4′8 BAL	1	0.162	
	All	3	0.152	0.0058

[1] Collection locations in Table 1. 2′6 JUS and 4′6 TAT are F_1 values.
[2] Number of matings represented by E_i values.
[3] Mean E_i (mean weight of initial eggs, Harvey 1977); letters show significant differences at $P \leq 0.01$, based on paired '*t*' tests between species and subspecies.
[4] SEM = standard error of the mean.
[5] Data from Harvey (1983*a*).

TABLE 5. Percentage success[1] in single pair matings [2] in *Choristoneura*

	Mating success (%)							
	Male							
	Spruce–fir-feeding species					Pine-feeding species		
Female	*occ*	*bie*	*fum*	*ora*	*ret*	*pin*	*sub*	*pon*
occ	72.2[3,4]	55.6	58.3[3]	38.6	51.7	34.0[3]	30.5	41.2
bie	55.8	67.6	52.5	52.9	46.2	34.5	—	70.0
fum	43.2	51.7	68.2	55.8	64.1	40.7	23.3	33.8
ora	38.9	21.4	40.0	73.5	(60.0)	48.3	—	—
ret	21.4	(50.0)	34.6	(75.0)	77.9	33.3	39.4	—
pin	11.1[3]	42.9	32.2	37.5	(25.0)	39.1	6.3	—
sub	30.8	—	18.9	—	12.5	11.8	46.2	—
pon	—	(20.0)	21.7	(50.0)	—	14.3	—	36.0

[1] Percentage of matings to produce fertile eggs. Values in parentheses based on less than 10 attempted matings.
[2] *n* values: inter se, exclusive of *fum* × *fum*, mean = 205; *fum* = 920; crosses (46 combinations), mean = 43.7.
[3] Marked *occ* matings may have included moths from the 'area of hybridization' (see text).
[4] Underlined values show inter se matings.

TABLE 6. Analysis of success in single-pair matings of *Choristoneura* species, grouped by host

	Spruce–fir-feeding species			Pine-feeding species			
Type of mating	*n*	Mean %	SEM	*n*	Mean %	SEM	'*t*'[1]
Inter se	5	71.9	1.9	3	40.4	3.4	8.7**
Intra group	20	48.4	3.0	3	10.8	2.4	8.7**
Between groups (by female host)	11	39.9	3.8	13	23.2	3.4	2.3*
'*t*' inter se vs. intra group		6.56**			5.67**		

[1] '*t*' values after arcsine square-root transformation of percentages: ns, $P > 0.05$; *, $0.05 > P > 0.01$; **, $P < 0.01$

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TABLE 7. Analysis of success in single-pair matings[1] of *Choristoneura* species, grouped by pheromone type

Type of mating	Female releases: Aldehyde *n*	Aldehyde Mean %	Aldehyde SEM	Acetate *n*	Acetate Mean %	Acetate SEM	'*t*'[1]
Inter se	3	69.3	1.44	5	54.5	8.8	1.41ns
Intra group	6	52.9	2.16	12	34.7	6.22	2.77*
Inter group	14	44.1	3.57	13	29.5	3.17	3.37**

[1]See previous table.

No trends or relationships to direction of the cross, host, or origin were detected. Survival and rearing performance of the F_2 progenies were similar to those of F_1 and wild progenies.

Back-crosses. All combinations tested produced some successful matings, although only a few of the possible back-crosses were attempted. [Data not presented.]

Characteristics of Hybrids. Fertile progenies were produced by all combinations tested and all F_1 and F_2 insects showed good levels of survival under laboratory conditions so there is no evidence of hybrid inviability. Nevertheless, some hybrids showed characteristics such as small egg weights, which could reduce their viability under extreme natural conditions.

Chromosomes. Chromosome numbers and morphology appeared to be normal in all F_1 hybrids tested (Table 3) so defects in hybrids from this source are not expected. In all males, pure and hybrid, the first meiotic metaphases were normal (i.e. no univalents, multivalents). There were some indications of possible heteromorphisms in a small number of individuals. All males had one large pair of chromosomes, presumably the XX, thus confirming the sex mechanism as XY♀ : XX♂. Most of the female hybrids were normal but in a few the pachytene chromosome patterns suggested heterozygosity (Ennis 1976). Here again numbers of individuals examined were too few to quantify.

Egg weights. Weights of eggs laid by hybrid females were dependent on the egg weights of the parents. For most crosses, the weights of the eggs laid by the hybrid females from the two reciprocal F_1 crosses are similar and closely reflect the mean parental values (diagonal, Table 9). Thus, for eggs produced by *C. occidentalis* × *C. retiniana* hybrids (parental E_i

TABLE 8. Percentage success[1] in matings of F_1 moths of eight *Choristoneura* species and hybrids among them

Female grandparent	Mean mating success (%): Male grandparent *occ*	*bie*	*fum*	*ora*	*ret*	*pin*	*sub*	*pon*
occ	70.2	61.9	63.7	32.0	78.8	80.6	83.9	27.3
bie	72.7	62.5	77.8	75.0	74.4	68.4	(75.0)	56.0
fum	75.2	65.4	67.8	53.3	66.3	49.6	20.7	40.0
ora	50.0	+[2]	(75.0)	78.6	84.2	48.9	—	+
ret	(32.3)	81.8	72.7	90.0	45.0	+	57.4	+
pin	(42.9)	+	(28.6)	+	—	39.3	72.2	—
sub	82.6	38.1	27.8	—	18.5	—	32.3	—
pon	—	+	20.0	—	—	—	—	50.0

[1]Percentage of matings to produce fertile eggs. Number of matings attempted: *fum* = 900; other species mean = 179; hybrids mean = 38.
[2]Plus (+) indicates that two or more matings were obtained, but data unavailable for analysis.

TABLE 9. Mean weights of initial eggs (E_i) laid by F_1 females produced by *Choristoneura* matings

Female parent	Mean weights (mg)[2]							
	Male parent							
	bie	*ora*	*fum*[3]	*occ*	*ret*	*pin*	*sub*	*pon*
bie	0.179	—	**0.256**	0.186	0.174	0.121	0.231[1]	0.220
ora	0.184	0.159	**0.229**	0.178	—	0.124	—	
fum[3]	**0.124**[1]	**0.122**	**0.165**[3]	**0.137**	**0.132**	**0.096**	**0.153**	**0.167**
occ	0.196	0.176	**0.259**	0.170	0.177	0.160	0.216	0.232
ret	0.188	—	**0.226**	0.167	0.154	0.122	0.213	
pin	0.183[1]	(0.155)[1]	**0.236**	0.168	0.169	0.102	0.187	
sub	0.187	—	**0.261**	0.193	0.184[1]	—	0.203	
pon	0.180		**0.292**[1]					0.203

[1]Number of crosses: inter se matings, 160.9; remainder, 23.0, except marked values[1] where $n \leq 6$.
[2]Standard errors for mean E_i (SEM): inter se, Table 4; remainder of crosses: SEM: 0.001–0.010, mean 0.0038; except marked values[1] where SEM: 0.009–0.014, mean 0.010.
[3]E_i values for crosses where one parent was *C. fumiferana* are bold.

values: 0.170 and 0.154 mg, respectively), E_i values were *ret* × *occ* = 0.167 mg, *occ* × *ret* = 0.177 mg. Similarly, progeny of the crosses between *C. retiniana* and *C. pinus* (parental E_i values: 0.154 and 0.102 mg, respectively) produced egg weights of 0.122 mg (*ret* × *pin*) and 0.169 mg (*pin* × *ret*). In both crosses of *C. occidentalis* with either *C. orae* or *C. retiniana*, egg weights of the hybrids were closer to those of *C. occidentalis* than to the other species. In all crosses of *C. pinus* with *C. occidentalis* and of *C. retiniana* with *C. pinus*, the hybrid egg weights were closer to those of the male parent. These and other comparisons suggest that E_i values in F_1 hybrids tend to be, on average, slightly higher than the values in the parental species.

The most striking effects in hybrids were the E_i values produced by all F_1 hybrids where one parent was *C. fumiferana* (Table 9, bold values). The parental *C. fumiferana* used in these crosses were mostly from eastern Canada and their mean egg weight was close to the overall mean for *C. fumiferana* (0.165 ± 0.001 mg). When *C. fumiferana* was the male parent all E_i values produced by the hybrids were much greater (0.226–0.292 mg) than either parental species, by more than 50% in some cases. When *C. fumiferana* was the female parent, E_i values for all the hybrids but one were much below (0.096–0.167 mg) those of either parent species, even in the case of the very small-egged *C. pinus*. The crosses of *C. ponderosana* × *C. fumiferana* were an exception in that the hybrid E_i values (0.167), although well below the female parental value, were about equal to the value for *C. fumiferana* (0.165).

Discussion

Taxonomic problems among these *Choristoneura* species stem from the difficulty in assigning individual insects to species and from the absence or low level of genetic incompatability among them. Most of the identifications were done at the time of collection and without the benefit the understandings of this group of species gained since that time. It is important, therefore, to try to put the identity of the western collections in context with the new understandings gained by the detailed studies of Powell and associates (Powell 1995*a*).

All moths utilized for matings and crosses were carefully selected and the identifications confirmed by the appearance of the progeny. However, the newer techniques such as pheromones, DNA, and isozyme analysis were not available at the time and the degree of

certainty about identifications is lower than would be considered acceptable today. Comparisons of the identifications of the western species with distributions shown by Powell and De Benedictis (1995*b*) indicate that most of the collections of all entities originated from areas where there is little uncertainty about their identities. Although the possible participation of incorrectly identified moths in matings to produce E_i values and mating data cannot be categorically ruled out, origins and identifications of most of the insects are generally accurate and the extent of errors in the results is considered to be small.

The data presented here were assembled from experiments conducted by different people at different times and the results must be interpreted with care. These conditions have also placed some limitations on the statistical procedures which could be applied. However, the experiments were all performed by the methods described and both treatment of the insects and laboratory conditions were kept as uniform as possible. Consequently, the conclusions from the summarized results are considered to be valid.

Chromosomes in *Choristoneura* are very small and their study is complicated by their large number (Ennis 1976). Smith (1944) reported that both *C. fumiferana* and *C. pinus* had $2n = 60$ chromosomes and demonstrated the presence of a sex chromosome in Lepidoptera (Smith 1945). Smith also showed that in *C. fumiferana* the female is the heterogametic sex and contains one relatively larger pair of chromosomes. Although a few females of *C. biennis* had only a single large chromosome (Ennis 1976), subsequent studies showed this result to be exceptional. Ennis (1976) reported haploid numbers of 30 chromosomes for *C. biennis* and *C. occidentalis*. This study confirms these results and that 30 is the haploid chromosome number for all members of the group tested to date and appears to be common to all Tortricinae (Ennis 1976).

Mating Success. Determination of mating success was simply a test of the ability of *Choristoneura* species to mate and produce viable progeny under a single set of conditions in the laboratory. Although environmental and other factors may influence the probability of mating under natural conditions, no attempt was made to reproduce those conditions to improve the likelihood of matings. The appreciable levels of mating success in all combinations tested suggest a rather low level of influence of such factors on mating probability. The apparently normal levels of fertility of all successful crosses also argue against mechanical or gametic factors affecting matings among these species.

Differences in mating success related to pheromones were not unexpected. The principal pheromone component of *C. occidentalis*, *C. biennis*, and *C. fumiferana* is an aldehyde (tetradecenal), whereas that of *C. orae, C. retiniana*, and the pine-feeders is the related acetate (Sanders et al. 1977; Harvey 1985*a*; Silk and Kuenen 1988; Daterman et al. 1995). Mating success was lower among acetate-pheromone species than among the aldehyde-pheromone species, both in inter se matings (54.5% vs. 69.3%, $P > 0.05$) and in matings among members of the group (34.7% vs. 52.9%, $0.05 > P > 0.01$) (Table 7). The lower percentage success in the acetate group may be attributable to dissimilarities among the pheromones in this group (Daterman et al. 1995; De Benedictis et al. 1995) combined with the generally lower success in all the pine-feeding species under laboratory mating conditions. The failure to obtain any successful matings with the European *C. murinana* may be explained by the difference in its principal pheromone (Priesner et al. 1980; Harvey 1985*a*) but may also involve other genetic dissimilarities.

Sex pheromones function principally in long distance communication but they also act as primers for copulation release stimuli for spruce budworm (Grant 1987) and could influence behaviour in the confines of a small jar. In addition, short distance effects such as female forewing scales may release copulatory behaviour (Grant 1987). Nevertheless, in crosses between species with different pheromones, mating success was significantly greater in crosses in which the female released aldehyde and the male responded to acetate (44.1%),

than in the reciprocal crosses (29.5%: $P \leq 0.01$) (Table 7). These results suggest that behavioural cues that act at short distances also differ between these two groups.

Only a small number of the 224 possible back-crosses were attempted. Sanders et al. (1977) reported viable progeny in 16 combinations. Percentage mating success in back-crosses among *C. fumiferana*, *C. retiniana,* and *C. pinus* was as great or greater than in inter se and cross matings in their study. Liebhold et al. (1984) and Liebhold (1986) reported successful matings of two maternal back-crosses of *C. occidentalis* × *C. retiniana* hybrids, but they did not report comparative mating success. Successful back-crosses of *C. retiniana* × *C. occidentalis* were also obtained by De Benedictis et al. (1995). Successful back-crosses of an additional 10 combinations were obtained, bringing to 28 the total recorded to date. By combining the data of Sanders et al. (1977) with the assembled data I can report that for 425 attempted back-cross matings representing 26 combinations, the mating success was 58%. Progenies of these back-crosses were generally fertile.

It must be concluded, therefore, that when pre-mating factors such as allopatry, differences in timing of mating behaviour, and pheromone differences are eliminated, as in the laboratory environment, there appear to be no remaining barriers to mating. All the combinations I attempted, including back-crosses and F_2 crosses, were successful and fertile and produced fertile offspring. However, some post-mating factors such as egg weights and developmental differences of the hybrids (Harvey 1985*a*; 1985*b*) and differences in host specificity may help to maintain the separation of the species if pre-mating factors break down.

Egg Weights in Species. Weights of eggs decrease progressively in successive clusters so the overall egg weight means vary with weight of the female. However, the mean weights of initial eggs (E_i), determined as specified, are relatively independent of moth size and of environmental conditions during development but are determined by the female's genetic constitution (Harvey 1983*b*). E_i is therefore suitable for comparison of egg weights in different species of *Choristoneura*.

Campbell (1958) reported differences among egg weights of entities now identified as *C. fumiferana, C. biennis*, and *C. pinus*, those of *C. pinus* being the smallest. These differences are confirmed herein. Similar differences also occur among the other *Choristoneura* species included in this study. Variations within species were generally small (Table 4).

The first six species listed in Table 4 are all spruce–fir-feeding *Choristoneura* (Stehr 1967; Powell and De Benedictis 1995*a*). They fall into two groups, based on E_i values. The larger group with E_i values, on average, from 0.165 mg for *C. fumiferana* to 0.180 mg for *C. biennis* also includes *C. occidentalis* and *C. carnana californica*. E_i values in *C. fumiferana* range from 0.219 mg in the northwest to 0.157 mg in the east (Harvey 1983*a*) so weights in western collections are similar to *C. biennis* and *C. occidentalis* whereas those in the east are similar to *C. orae*. The second and smaller group with significantly smaller E_i values [0.154–0.159 mg ($P < 0.01$)] includes *C. orae* and *C. retiniana*.

All the pine-feeding species (*pin, pon, sur*) differ morphologically from the spruce–fir-feeding forms in size, pupal shape, and adult colour (Stehr 1967; Powell 1980). However, E_i values show much greater variation among species in this group. E_i values in *C. pinus* are the smallest of all the species studied (0.104 mg), and show relatively little variation, as reported by Campbell (1958). Egg weights for the closely related *C. lambertiana subretiniana* and *C. lambertiana ponderosana* are identical (0.203) and almost double those of *C. pinus* (Table 4). As the chromosome numbers are identical (Table 3) this doubling cannot be explained as a simple genetic factor. Both 'morphs' (Stehr 1967) occurred in most collections of *C. ponderosana* and there were no egg weight differences between them.

Two collections from *Pinus flexilis* were among several collections described as related to *C. lambertiana* but of 'uncertain status' (Harvey 1985*a*). Both these collections included moths with both distinctive and washed out patterns (Stehr 1967; Powell and De Benedictis

1995*b*). E_i values from three matings were appreciably smaller (0.152, Table 4) than any from the *C. ponderosana* or *C. subretiniana* collections, but still larger than any *C. pinus*. Although Powell and De Benedictis (1995*b*) include *P. flexilis* as a host of *C. ponderosana* in Wyoming, they do not list this host for *C. subretiniana*. The differences in appearance and egg size of these moths suggest they may not have been either subspecies, but they could have been included in the 'near *C. subretiniana*' of Powell and De Benedictis (1995*b*). They also appear morphologically different from the 'coastal lodgepole pine-feeding populations' of Powell and De Benedictis (1995*b*) and the other entities described by Gray and Slessor (1989) and Gray and Gries (1993). Their status remains uncertain.

E_i values of populations of *C. fumiferana* are correlated to geographical factors, showing a cline in mean egg weights from small eggs in the southeast to larger eggs in the northwest (Harvey 1983*a*). The possibility of a relationship between latitude and altitude was studied in both *C. biennis* and *C. occidentalis* even though the number of collections was small. The species with the widest distribution after *C. fumiferana* is *C. occidentalis*. Collections of this species originated from as far south as New Mexico (lat. 35.8°N, long. 105.7°, 2740 m) north to southern British Columbia (lat. 51.4°N, long. 119.6°, 460 m). Mean E_i of the 10 collections from California (lat. 40.3°N) to Adams Lake, BC (lat 51.4°N) ranged from 0.206 mg in the south to 0.169 mg in the north, which suggests that a weak cline may be present. However, collections of both *C. biennis* and *C. occidentalis* from higher latitudes came from lower altitudes and vice versa, consequently collection locations spanned only a narrow bioclimatic range. Selection pressures for egg size may not have been as great as for *C. fumiferana* so that these two species have not adapted to as wide a range of conditions as *C. fumiferana*. Unfortunately, the data do not span the entire range of either species and were insufficient to separate latitudinal from altitudinal effects.

Mean egg weights of *C. biennis*, which appears to replace *C. occidentalis* in the higher altitudes and latitudes, are significantly greater. The most southerly collection of *C. biennis*, from near Canal Flats, BC (lat. 50.5°N, 1435 m), had a mean E_i of 0.192 mg. The most northerly collection was from mile 1061 of the Alaska Hwy, near Koidern, YT (lat. 62°N, 760 m) with a mean E_i of 0.188 mg. Collection means for the group ranged from 0.163 to 0.192 and showed no evidence of a relationship between E_i and latitude, longitude, or altitude. Campbell (1962) reported a higher mean egg size for *C. biennis* from near Lake Louise, AB. E_i values calculated from his data gave a mean value of 0.232 ± 0.002 for 18 females, with the smallest being 0.222. These values are greater than any we encountered and are not readily explained.

Among the other species, data were too limited for tests for latitudinal effects. The E_i values for *C. orae* and *C. retiniana* showed very little variation and data for both species represented limited latitudinal and altitudinal ranges: *C. orae* at 50°N and 30 m near Kitimat, BC; *C. retiniana* at about 42°N and 1770 m in the Warner mountains of California. Ranges of both species are now known to be much wider and further studies would be useful. Although *C. pinus* occupies a wide range, E_i data are limited and inadequate to test for clines in E_i values. Both *C. ponderosana* and *C. subretiniana* also appear to occupy limited ranges so adaptations of egg size are not expected.

Effects in Hybrids. Mating success between hybrid individuals (Table 8) was generally equal to or better than those of the initial F_1 crosses (Table 5) and comparable to conspecific matings of wild moths. Egg weights are determined by the genetic constitution of the female producing the eggs, regardless of the male with whom she has mated. However, hybrid effects on egg weights can be measured only after rearing the hybrids to maturity and obtaining their eggs. Consequently egg weights of hybrids always represent at least the second laboratory generation. Fortunately for study of this group of species, survival of hybrids during rearing was equal to that of other stocks and adequate numbers of moths were obtained to determine egg weights of hybrids.

TABLE 10. E_i ratios in eggs produced by F_1 hybrids

Female parent	E_i ratios [1,2] Male parent [3]							
	bie	*ora*	*fum*	*occ*	*ret*	*pin*	*sub*	*pon*
bie	1.00	—	**1.55**	1.09	1.13	1.22	1.14	1.08
ora	1.02	1.00	**1.39**	1.06	—	1.22	—	—
fum	0.69	0.77	**1.00**	0.81	0.85	0.86	0.79	0.83
occ	1.09	1.11	**1.57**	1.00	1.15	1.50	1.06	1.14
ret	1.04	—	**1.37**	0.98	1.00	1.20	1.05	—
pin	1.02	(0.98)	**1.43**	0.99	1.10	1.00	0.92	—
sub	1.04	—	**1.58**	1.14	1.19	—	1.00	—
pon	1.00	—	**1.77**	—	—	—	—	1.00
Mean[3]	0.99	0.95	**1.52**	1.01	1.08	1.20	0.99	1.02

[1] E_i ratio = E_i progeny/E_i mean, species of male; data of Table 9.
[2] Species inter se ratios underlined = 1.00; *C. fumiferana* crosses bolded.
[3] E_i values in hybrids grouped by male parent.

Campbell (1958) reported that egg weights of some F_1 hybrids were much larger and others smaller than those of either parent. Consequently some differences in E_i values of hybrids were expected. Most of these effects were small, except for the crosses involving a parent of *C. fumiferana* (Table 9). These relationships are more readily seen from the E_i ratios which compare the E_i value of the hybrids with those of the male parental species represented by the species mean (Table 10). Except for crosses involving *C. fumiferana* as a parent, E_i ratios in all crosses were, on average, 1.10 ± 0.025 (range 0.90–1.57). There was no indication of differences related to the direction of the cross. The excess by which the mean value exceeds 1.00 is a measure of the difference between egg weights in hybrids and those in the species, and may be a measure of 'hybrid vigour'. Further, in spite of marked differences in E_i values of the pine-feeding species, crosses between them and with spruce–fir-feeding species did not produce divergent E_i ratios, which suggests that their egg-weight determining mechanisms are closer to *C. biennis, C. occidentalis, C. orae,* and *C. retiniana* than to *C. fumiferana*. The closeness of this ratio to 1.00 is further evidence of the close relationships among members of this group. These results are in agreement with those of Campbell (1958) but extend the series beyond what he reported.

E_i ratios from crosses where one parent was *C. fumiferana* were markedly different from all others. In hybrids where *C. fumiferana* was the male, E_i ratios ranged from 1.37 to 1.77 times the value for *C. fumiferana* (mean = 1.53 ± 0.053) (Table 10). In the reciprocal crosses ratios ranged from 0.69 to 0.86 (mean 0.80 ± 0.022) times the inter se value of the male parent. These results tend to support the involvement of the X-chromosome in determination of E_i (Campbell 1958). They also indicate that the separation of *C. fumiferana* from the other members of the group is greater than that existing among the other species. Evidence from isozyme and DNA studies also indicates that *C. fumiferana* is more widely separated from the other members of the group (Harvey 1996; Sperling and Hickey 1994). Perhaps it is significant that the strong effect in hybrids is produced by the only species in which there is any evidence of adaptability of egg weight to climatic conditions; this may imply some differences in egg size determination in *C. fumiferana*.

Fecundity in *C. fumiferana* is determined by a genetic system having two components, one determining the proportion of body mass allocated to eggs, the other determining egg weight (Campbell 1958, 1962). It is therefore not surprising that there were dramatic differences in fecundities of crosses where E_i differences occurred, particularly those

involving a parent of *C. fumiferana*. For example, in the *occ* × *fum* hybrids which produced large eggs ($E_i = 0.277$ mg) mean fecundity was only 126 eggs (Campbell 1958; Harvey 1985*a*). In the opposite cross which produced small eggs ($E_i = 0.133$ mg), twice as many were produced (256). Similar trends in both E_i and fecundity were observed in the *fum* × *pin* hybrids. Neither mean pupal weights of F_1 females nor mean weights of total egg mass differed between reciprocal crosses in either combination. Thus the changes in numbers of eggs are largely dependent on their individual weights (Campbell 1962).

Wide differences in egg weights, such as shown among some of the hybrids, will have consequences for survival under natural conditions. Harvey (1985*b*) found evidence that heavier eggs in *C. fumiferana* yield overwintering larvae that are better able to survive harsh or prolonged winter conditions than are lighter eggs. If such a generalization also holds for small and large eggs produced by reciprocal hybrids between *C. fumiferana* and *C. occidentalis*, then the smaller-egged *fum* × *occ* hybrids could be strongly selected against under harsh conditions, whereas the large eggs from *occ* × *fum* hybrids, although fewer in number, should have higher overwintering survival. Crosses between *C. fumiferana* and *C. occidentalis* probably do not occur naturally but similar directional differences occur in crosses of *C. fumiferana* with *C. biennis* and *C. orae* which are sympatric in places (Harvey 1985*a*; Shepherd et al. 1995). Thus progenies of the cross where *C. fumiferana* was the male would be most likely to survive, although numbers would be low (Harvey 1985*a*, 1985*b*). These effects on egg weight and the number of surviving hybrids may reduce the likelihood of appreciable hybridization between some of these species.

Other Differences in Hybrids. Chromosome morphology revealed little evidence of structural differences in chromosomes of the hybrids examined (Table 3). There were, however, signs of abnormality in some hybrids that may warrant further study. Ennis (personal communication) suggests that, although technically difficult, the most fruitful area of study would be the search for XY chromosome differences in females, as differences in the size of the Y chromosome may be diagnostic.

Where natural hybridization is possible several additional mechanisms may act to reduce or eliminate any significant gene flow. Although eggs produced by such crosses have a normal level of fertility, and larval development to maturity approaches normal levels under laboratory conditions, some developmental effects have been documented as reviewed by Harvey (1985*a*). These include differences between hybrids in development rates and, in some crosses, in incidence of second diapause (Harvey 1967), both of which may reduce the normal synchrony of adult emergence times. Other differences may include the pheromone produced by the hybrid (Sanders et al. 1977; De Benedictis et al. 1995).

The apparent lack of barriers to copulation and the apparently normal fertility in all combinations of North American coniferophagous *Choristoneura* confirms the close relationship among members of this group of species. However, fertile matings between species under laboratory conditions are known in other groups and do not, in themselves, invalidate their taxonomic separations (Oliver 1979). These results support the conclusions of Smith (1953), Sanders et al. (1977), Liebhold et al. (1984), Harvey (1985*a*), and Powell and De Benedictis (1995*a*) that pre-mating factors must constitute the main barriers to reproduction among sympatric taxa of this genus. Further studies of post-mating factors are needed, of course, but to understand reproductive isolation among these *Choristoneura*, the main emphasis should be on pre-mating factors.

Several authors have commented on the closeness among the North American coniferophagous members of this group of taxa and the inadequacy of classical methods of separation (Powell 1980; Harvey 1985*a*; Volney 1989; Powell 1995*b*). The group appears to be evolving rapidly and has not yet completed the development of morphological differences on which taxonomic separations are usually made. Powell and his associates (1995) have utilized pheromone specificity, distribution, and morphology to separate some

of the taxonomic entities. For further progress in unravelling the problems of taxonomic separation in this group, additional characters, such as isozymes (Harvey 1996), DNA (Sperling and Hickey 1994), haemolymph polymorphism (Stehr 1964; Harvey and Stehr 1967), and others must also be used (De Benedictis 1995). This important group still offers a formidable challenge to entomologists.

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