

Diapause and voltinism in western and 2-year-cycle spruce budworms (Lepidoptera: Tortricidae) and their hybrid progeny

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Abstract—Breeding experiments and rearing under variable controlled conditions have revealed that western and 2-year-cycle spruce budworms (*Choristoneura occidentalis* Freeman and *C. biennis* Freeman, respectively) from British Columbia, Canada, and their hybrid progeny have the inherent capacity for a variable number of diapause events and hence voltinism. While all crosses have at least one diapause, variability in the relative frequency of a second diapause is determined by genetic traits modified by the photoperiod and, to a lesser extent, temperature experienced during the larval stages. Second diapause appears fixed in *C. biennis* but is facultative and most frequent at short photophases (12L:12D) in *C. occidentalis*. Hybrids and backcrosses had responses intermediate to the parental responses under all environmental conditions. The occurrence of a facultative third diapause in all crosses underlines the inherent capacity for flexibility in voltinism in these species. These results are discussed in the context of past, present, and future distributions of alternative life cycles in closely related species.

Résumé—Des expériences de reproduction et d'élevage dans diverses conditions contrôlées révèlent que les tordeuses occidentale et bisannuelle de l'épinette (respectivement *Choristoneura occidentalis* Freeman et *C. biennis* Freeman) de la Colombie-Britannique et leurs hybrides ont la capacité inhérente d'entrer un nombre variable de fois en diapause et donc de produire un nombre variable de générations dans une période donnée. Chaque hybride entre au moins une fois en diapause, et la variabilité sur le plan de la fréquence relative d'une deuxième diapause dépend de caractères génétiques modifiés par la photopériode et, dans une moindre mesure, par les conditions de température qui ont prévalu au cours des stades larvaires. Il semble que *C. biennis* entre toujours au moins deux fois en diapause, tandis que chez *C. occidentalis*, la deuxième diapause est facultative et se produit plus fréquemment lorsque les photopériodes sont courtes. Les hybrides et les produits de rétrocroisements ont donné des résultats intermédiaires par rapport à ceux de leurs parents et ce, dans toutes les conditions environnementales. La possibilité d'une troisième diapause facultative chez tous les hybrides met en valeur la capacité inhérente de flexibilité des deux espèces sur le plan du voltinisme. Ces résultats font l'objet d'une discussion dans le contexte des répartitions passées, présentes et future d'autres cycles de vie chez des espèces étroitement apparentées.

Introduction

Spruce budworms in the genus *Choristoneura* (Lepidoptera: Tortricidae) are common defoliators in the coniferous forests of North America. Populations periodically outbreak to cause severe defoliation of their host trees over extensive areas. During the first half of the 20th century, forest entomologists recognized that different "forms" of the spruce budworm were associated with different tree species in different forest ecozones. However, the absence of definitive, conventional taxonomic characters to separate these forms resulted in the view of a

single transcontinental species referred to as *Choristoneura fumiferana* (Clemens) (Freeman 1958).

Outbreaks in the 1940s and 1950s focussed attention on the taxonomic ambiguity of the group, eventually resulting in the designation of several new species to create a species complex (Freeman 1967). In the northern coniferous forests of Canada, these are the spruce budworm, *C. fumiferana*, and the jack pine budworm, *C. pinus pinus* Freeman, east of the continental divide, and the western spruce budworm, *C. occidentalis* Freeman, the 2-year-cycle spruce budworm, *C. biennis* Freeman, and the coastal budworm, *C. orae* Freeman, west of the continental divide. Despite the intense study of

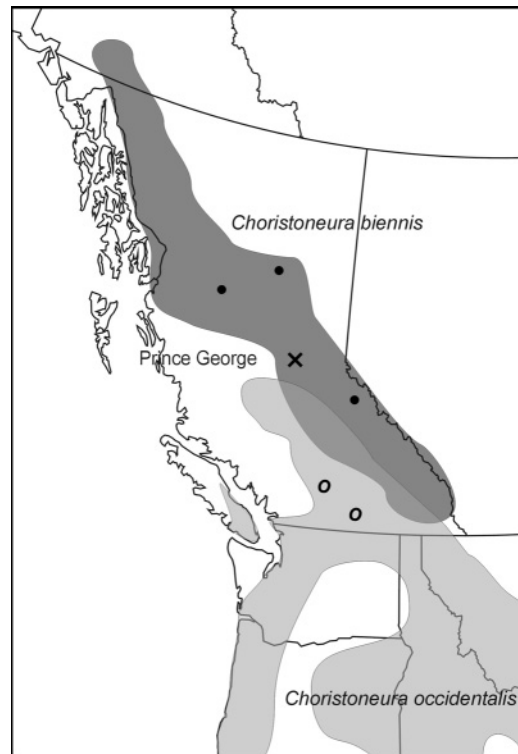
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comparative morphology, genetics, and behaviour that originally supported separation of these species, subsequent studies have revealed broad overlap in biological characteristics, especially among the closely related western species (Sanders 1971; Stock and Castrovillo 1981; Dang 1985, 1992; Sperling and Hickey 1995; Harvey 1996). Indeed, Stehr's (1967) recognition of the close ecological association between each species and its preferred host tree in a particular forest zone remains a more reliable and practical means of separating species than most characters associated with captured specimens.

The distribution of spruce budworms in Canada, their nearly identical life histories, and, in particular, the ease with which species hybridize and produce fertile offspring in captivity (Harvey 1997) suggest recent evolutionary divergence. These patterns are most evident in the comparatively rich biota of western North America (Powell and De Benedictis 1995; Harvey 1996), where the complex topography creates distinct forest ecozones and corresponding distributions of spruce budworms. In British Columbia (BC), Canada, the western spruce budworm and the 2-year-cycle spruce budworm illustrate the situation (Shepherd *et al.* 1995). The western spruce budworm is at the northern limit of its natural range in BC and is associated most commonly with Douglas-fir, *Pseudotsuga menziesii* (Mirb.) Franco (Pinaceae). The 2-year-cycle spruce budworm appears to be restricted to the interior of BC and the southern Yukon between approximately 50°N and 62°N latitude (Fig. 1). It feeds primarily on spruce (*Picea* spp.; Pinaceae) and true fir (*Abies* spp.; Pinaceae) (Shepherd *et al.* 1995). These two budworms overlap broadly in the latitudes of their ranges (Fig. 1) but less so in their forest ecozone associations: the western spruce budworm occurs at lower elevations in drier, warmer biogeoclimatic zones associated with Douglas-fir, while the 2-year-cycle spruce budworm occurs at higher elevations and latitudes where cool, moist conditions favor its spruce and true fir hosts.

The primary biological distinction between these western species is the apparent predominance of the typical 1-year life cycle for *C. occidentalis* (one winter diapause) but a 2-year life cycle for *C. biennis* (two winter diapauses) (Mathers 1932; Harvey 1961, 1967; Freeman 1967). Voltinism, however, can be variable within insect species, and physiological states such as diapause are often facultative

Fig. 1. Geographic ranges of *Choristoneura occidentalis* and *C. biennis* in British Columbia, Canada, and the Pacific Northwest, United States of America. Closed circles are collection areas for *C. biennis* stock and open circles are collection areas for *C. occidentalis*.



and influenced by prevailing environmental conditions (Tauber *et al.* 1986). Shepherd (1961) reviewed observations of widely dispersed populations of spruce budworms with 1- and 2-year cycles and suggested that temperature selected and maintained the different life histories. He did not, however, compare the two budworms under experimental conditions. Harvey (1967) used experimental conditions but had limited access to wild populations of western species and relatively few family matings with which to examine the effects of environmental conditions on the incidence of second diapause in parental groups or their hybrid progeny.

This paper examines environmental (temperature and photoperiod) influences on the frequency of diapause in the life cycles of western and 2-year-cycle spruce budworms in BC under experimental conditions. Results are presented for wild stocks of *C. biennis*, *C. occidentalis*, and hybrids of the two species as well as

selected backcrosses to provide some insight into the inheritance of this trait. The discussion integrates these new results with recent genetic analysis of the group.

Methods

Stock sources

All parental stocks were collected directly from field sites in BC as final-stage larvae or as pupae and identified by the conventional combination of characters: larval and adult morphology, host-plant association, geographic location, and voltinism. Western spruce budworm was collected near Merritt and Peachland, BC. Two-year-cycle spruce budworm currently has two distinct populations in BC in terms of year of maturity. The population north and west of Prince George, BC, matures in odd-numbered years while the population south and east of Prince George matures in even-numbered years (Nealis and Turnquist 2003). Thus, there was an annual source of moths to mate with the western spruce budworm. Stock maturing in odd years was obtained near Takla Lake north of Fort St. James and the Ospika River north of Mackenzie, BC. Stock maturing in even-numbered years was collected in the Robson Valley between McBride and Valemont, BC (Fig. 1). Voucher specimens are retained at the Pacific Forestry Centre, Victoria, BC.

Choristoneura occidentalis and *C. biennis* collected as larvae completed their development on artificial diet (McMorran 1965) at 20 °C and 16L:8D. These conditions are hereafter referred to as “standard conditions”. Pupae were sexed and isolated individually in small plastic cups until eclosion.

Mating, eggs, and first diapause

Moths were mated either in individual pairings to provide information on family effects or en masse. Individual pairings consisted of one male and one female moth placed in a glass Mason® jar containing a fresh shoot of host foliage. Foliage of subalpine fir, *Abies lasiocarpa* (Hook.) Nutt., was used for *C. biennis* females and foliage of Douglas-fir was used for *C. occidentalis* females. For backcrosses, both types of foliage were included. Insect screen, held in place with a metal ring, was used to cover the jar containing the moths and foliage.

Mass pairings used larger screen cages (50 cm × 50 cm × 30 cm) with 10 to 20 pairs of moths.

The following coding system identifies crosses and families. The first two letters of the code indicate the species of the male parent and the last two letters indicate the species of the female parent. Accordingly,

bibi = *C. biennis* ♂ × *C. biennis* ♀;
ococ = *C. occidentalis* ♂ × *C. occidentalis* ♀;
bioc = *C. biennis* ♂ × *C. occidentalis* ♀; and
ocbi = *C. occidentalis* ♂ × *C. biennis* ♀.

Similarly, backcrosses were coded with the male first and the female second. For example, backcross *bioc* × *bibi* had a *bioc* father and a *bibi* (i.e., *C. biennis*) mother. For individual matings, separate families were identified with numbers.

The rearing system followed Gridale (1970). Needles bearing egg masses were placed in a petri dish covered with Parafilm® to which a triple-layer patch of cheesecloth had been attached on the inside surface, facing the egg masses. The dish with the egg masses was placed in a paper envelope with a window cut to expose the cheesecloth patch to light. This setup was placed in standard conditions. As budworms hatched, they moved toward the window, became embedded in the cheesecloth, and spun hibernacula. Mating success in individual pairings was indicated by at least one egg hatch. After 2 weeks, larvae had moulted to a second instar and entered their first diapause. They were then stored at 2 °C for 6–9 months to satisfy their diapause requirements.

Second diapause

Following cold storage and completion of the first diapause, patches of cheesecloth containing the second instars were placed in glass tubes (3.5 cm diameter × 15 cm long) with moist filter paper and incubated under standard conditions to promote emergence. A creamer cup containing diet was inverted over the open mouth of the tube. Emerging budworms crawled up the tube, entered the creamer cup, and established feeding sites. A cardboard lid was placed on the creamer cup and the rearing unit was transferred to the rearing conditions specified by the experimental treatments (see below).

As the diet began to discolor, larvae were transferred to fresh diet in a second rearing cup and thinned to 10 larvae per cup. At this time, a strip of Parafilm® approximately 1 cm wide and

5 cm long with a double layer of cheesecloth embedded on each side was folded upon itself to form an accordion shape and added to the rearing cup to provide a substrate for budworms to form a hibernaculum for a second diapause. Thus, all budworm larvae had the choice of either entering a second diapause or continuing to feed on fresh diet within the same rearing chamber. Larvae that continued to feed and develop to final-instar larvae were scored as single-diapause larvae and removed from the cup. They were either used for subsequent controlled matings or discarded. Larvae that established hibernacula were scored as second-diapause larvae. My unpublished measurements confirm Shepherd's (1961) observation that larvae entering a second diapause construct a hibernaculum as a third instar and then moult to the fourth instar. These larvae were treated as described above for first-diapause individuals and stored for several months at 2 °C. As with the first diapause, more than 95% of the larvae survived a second diapause under these conditions in all cases.

Limited availability of controlled environment chambers necessitated carrying out a series of experiments. At the beginning of each experiment, the required treatment conditions were assigned randomly to available chambers to guard against spurious procedural effects. In each experiment, all of the different crosses were assigned to each chamber to avoid confounding cross and rearing conditions within an experiment.

Experiment 1: Effect of temperature

The stock material for this experiment was collected in 1999 and the experiments were conducted in 2000. Following the first diapause, each large family (>50 second instars) was divided into three equal portions. Each portion was randomly assigned to one of three constant temperatures — 16, 20, or 25 °C — at one photoperiod (16L:8D). All progeny from smaller families were assigned to 20 °C only.

In addition to the family matings, there were mass matings in which parental stock but not the specific parents were known. These offspring were distributed randomly to one of the three treatment temperatures. In all cases, individual larvae were scored as either entering or not entering a second diapause.

Larvae that did not enter a second diapause were reared to adults and used to form

backcrosses. Because most *bibi* individuals entered second diapause (see Results), backcrosses could be produced only between the hybrids and the 1-year-cycle parental group, *ococ*. Crosses within hybrid lines were also made. Progeny were reared under standard conditions and the incidence of second diapause was recorded.

Larvae that entered second diapause were stored in cold conditions as described above and subsequently used to produce backcrosses between *bibi* and each hybrid. Some of these progeny were reared under standard conditions in 2000, and the frequency of second diapause was recorded. Other progeny were used to examine the interaction between temperature and photoperiod on the frequency of second diapause (see experiment 3).

Experiment 2: Effect of photoperiod

Stock material for experiment 2 was collected in 2000 and the experiments were carried out in 2001. The design of experiment 2 was identical to that of experiment 1 except that progeny of families were distributed among three different photoperiods (12L:12D, 16L:8D, and 20L:4D) at a single temperature (20 °C). Larvae entering second diapause were stored at 2 °C for 6 months and then incubated at 16, 20, or 25 °C, 16L:8D, to rear them to adults. Some of these individuals entered a third diapause. The frequency of third diapause was scored by cross and temperature (see below).

Experiment 3: Interaction of temperature and photoperiod

Stock material for experiment 3 was collected in 2002 and the experiments were conducted in 2003. Pure and hybrid offspring were formed as described above except that moths were mated en masse, *i.e.*, there was no discrimination of individual families. Following cold storage, groups of larvae that had completed first diapause were assigned randomly to a particular temperature (16 or 26 °C) and photoperiod (12L:12D, 16L:8D, or 20L:4D) combination in a fully factorial design.

Experiment 3 produced insects that entered a second diapause under different combinations of temperature and photoperiod. This experiment therefore provided material with which to examine the influence of previous environmental conditions on the third diapause response under three different photoperiods (12L:12D,

Table 1. Mating success (percentage of pairings with at least one egg hatch), sample size (n), and number of families utilized in experiments 1 and 2 for pure and hybrid crosses of *Choristoneura biennis* and *C. occidentalis* (see Methods).

Cross	Success (%)	Sample size (n)	No. of families in experiment 1	No. of families in experiment 2
<i>bibi</i>	59.6	89	3	37
<i>ococ</i>	58.2	79	9	9
<i>bioc</i>	52.1	94	27	19
<i>ocbi</i>	53.2	77	32	43

16L:8D, and 20L:4D) and a single temperature (20 °C) in 2004.

Analysis

Results are summarized in tables and figures as the proportion of individuals entering second or third diapause. In all cases, these proportions were estimated from sample sizes greater than 50 individuals and in most cases greater than 100 individuals (see Tables 2 and 3).

Second and third diapause were scored as binomial responses and analyzed using binary logistic regression models with temperature and (or) photoperiod included as continuous variables and cross as a factor (MINITAB® Release 14, Minitab Inc., State College, Pennsylvania). Preliminary analysis including family as a nested factor indicated additional variation for this factor within crosses. A full analysis of quantitative inheritance patterns, however, is beyond the scope of this paper and so the present analysis is restricted to the relationship between cross and the environmental factors. Because of consistently clear differences in the overall responses of pure and hybrid crosses (Figs. 2, 3), the two hybrid crosses (*bioc* and *ocbi*) were analyzed as a separate partial table, treating cross as an additional factor. The pure crosses (*bibi* and *ococ*) were each analyzed as separate partial tables.

Inferences for simple regressions were based on a z -test of coefficients, given an adequate fit. Inferences for multiple models were guided by a backward elimination procedure that compares the likelihood ratio statistics (G) and corresponding degrees of freedom for models with and without the terms of interest (Agresti 1996). I began with the most complex model and successively removed terms, beginning with higher level terms. At each step, the fit of the simpler model was compared with that of the more complex model by examining the

difference in deviances (G) with the appropriate degrees of freedom under the null hypothesis of no difference between the two models. The final model was the one for which further removal of terms resulted in a significant ($P < 0.05$) change in deviance. All final models with higher level terms retained the main effects that constituted the significant interactions. Goodness-of-fit for models was evaluated with the Hosmer–Lemeshow test (designated hereafter as $H-L$). For this test, the greater the P value, the less evidence of lack of fit (Agresti 1996).

Results

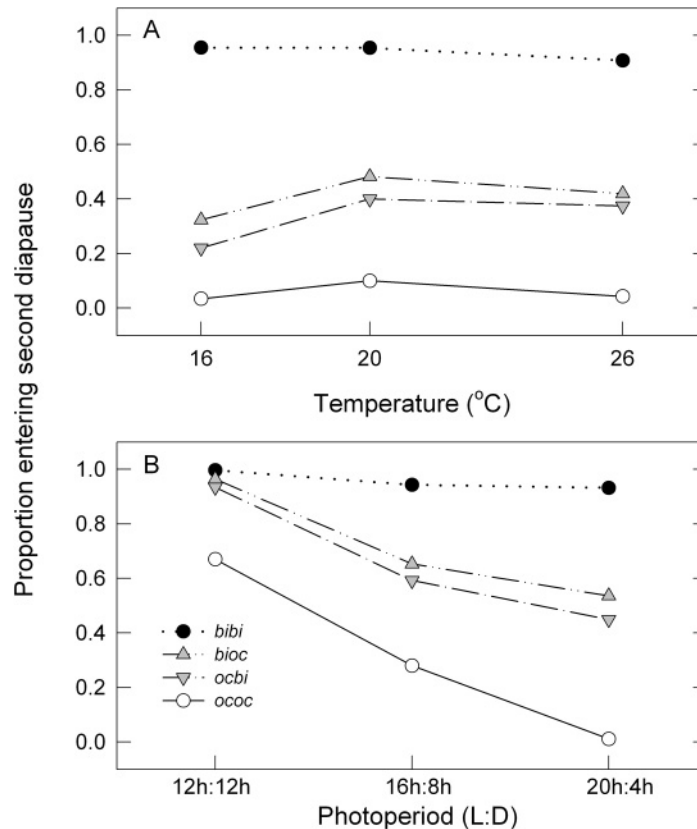
Male and female *C. occidentalis* and *C. biennis* interbreed readily and produce viable hybrid progeny. The mating success of individual pairings of the two species was only slightly less than that within pure lines (Table 1) and similar to reports by Harvey (1997) under laboratory conditions.

Experiment 1: Effect of temperature

There were clear differences among crosses in the overall proportion of individuals entering second diapause at all temperatures (Fig. 2A). The proportion entering second diapause was uniformly high among *bibi* and low among *ococ*. There was a slight but significant effect of temperature on the likelihood of second diapause in both pure lines (*bibi*: $G = 5.73$, $df = 1$, $P = 0.017$; $H-L = 0.588$, $df = 1$, $P = 0.44$; *ococ*: $G = 5.56$, $df = 1$, $P = 0.018$; $H-L = 3.55$, $df = 1$, $P = 0.06$), although the two lines exhibited opposing trends.

The hybrid crosses, *bioc* and *ocbi*, were intermediate to the pure lines in their response to temperature, with the proportion of individuals entering second diapause increasing slightly with temperature (Fig. 2A). The final model required a quadratic term in temperature to

Fig. 2. Proportions of pure (*bibi* and *ococ*) and hybrid crosses (*bioc* and *ocbi*) entering second diapause: (A) effects of temperature (experiment 1) and (B) effects of photoperiod (experiment 2).



provide an adequate fit ($H-L = 6.57$, $df = 4$, $P = 0.16$). Significantly more *bioc* than *ocbi* entered second diapause at all temperatures ($G = 64.3$, $df = 1$, $P < 0.001$). Analysis of the marginal tables summed over all families and temperatures for each hybrid line indicated no association between the likelihood of entering second diapause and the sex of the individual (*ocbi*: $\chi^2 = 1.57$, $df = 1$, $P = 0.21$; *bioc*: $\chi^2 = 1.15$, $df = 1$, $P = 0.28$).

Experiment 2: Effect of photoperiod

There were clear differences among crosses in the overall proportion of individuals entering second diapause at all photoperiods (Fig. 2B). As in experiment 1, the proportion of the *bibi* cohort entering second diapause was uniformly high at all photoperiods. For the hybrid crosses, however, the proportion of individuals entering second diapause was greater in experiment 2 than in experiment 1, and the response to photoperiod was more marked than the response to temperature (compare Figs. 2A, 2B). Unlike the

response to temperature, there was no significant difference in the response to photoperiod between the two hybrid crosses ($G = 0.51$, $df = 1$, $P > 0.10$; $H-L = 0.55$, $df = 3$, $P = 0.91$). Almost all *ocbi* and *bioc* individuals entered second diapause at the shortest photophase (12L:12D), and approximately half entered second diapause at the longest photophase (20L:4D).

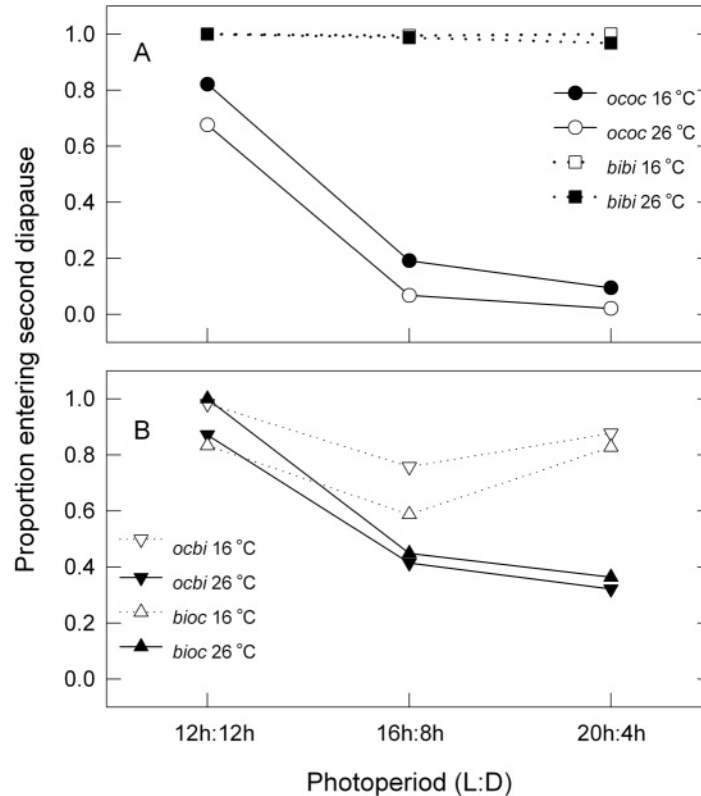
There was also a strong effect of photoperiod on the second diapause response of *ococ*, with more than half of the individuals entering second diapause at the shortest photophase (12L:12D) and almost none at the longest photophase (20L:4D). To check this unexpected result, experiment 2 was repeated in 2002 using wild parental *ococ* and *bibi* stocks only. Although the overall proportion of *ococ* individuals entering second diapause in this repeated trial was slightly lower than in the first trial (see Fig. 2B), the invariance of the *bibi* response and the significant effect of photoperiod on the frequency of second diapause in *ococ* were confirmed (Table 2).

Table 2. Frequency of second diapause and proportion of individuals entering second diapause in pure *C. biennis* (*bibi*) and *C. occidentalis* (*ococ*) crosses and backcrosses of hybrids (*bioc* and *ocbi*) with *bibi* at combinations of long (20L:4D) and short (12L:12D) photophases and two temperatures (16 °C or 26 °C).

Cross	Photophase	Temperature = 16 °C				Temperature = 26 °C				
		Second diapause	No second diapause	Proportion entering second diapause	Second diapause	No second diapause	Proportion entering second diapause	Second diapause	No second diapause	Proportion entering second diapause
<i>bibi</i> *	Short	171	0	1.000	298	1	0.997			
	Long	268	3	0.989	229	44	0.839			
<i>ococ</i> *	Short	50	11	0.820	158	129	0.550			
	Long	21	275	0.0709	5	115	0.0417			
<i>bibi</i> ♂ × <i>bioc</i> ♀	Short	104	0	1.000	80	2	0.976			
	Long	135	1	0.993	54	3	0.947			
<i>bioc</i> ♂ × <i>bibi</i> ♀	Short	108	3	0.973	52	0	1.000			
	Long	83	0	1.000	113	21	0.843			
<i>bibi</i> ♂ × <i>ocbi</i> ♀	Short	267	1	0.996	127	6	0.955			
	Long	154	3	0.981	137	56	0.710			
<i>ocbi</i> ♂ × <i>bibi</i> ♀	Short	152	2	0.987	95	11	0.896			
	Long	100	4	0.961	53	28	0.654			

*Additional controls of pure lines were reared at 16L:8D and 20 °C; the proportion of individuals entering second diapause was 1.0 in *bibi* and 0.26 in *ococ*.

Fig. 3. Proportions of pure (*bibi* and *ococ*) and hybrid crosses (*bioc* and *ocbi*) entering second diapause under combinations of varying temperature and photoperiod (experiment 3): (A) pure crosses and (B) hybrid crosses.



Experiment 3: Interaction of temperature and photoperiod

As in the other experiments, most *bibi* individuals entered a second diapause irrespective of environmental conditions, with only a slight decrease in the proportion of insects entering a second diapause at the longest photophase and highest temperature (Fig. 3A). The response of *ococ* also was similar to that in earlier experiments, with the effect of photoperiod slightly modified by temperature (Fig. 3A). The final model for each of the pure lines contained the two main effects, temperature and photoperiod, but not their interaction (*bibi*: $G = 17.6$, $df = 2$, $P < 0.01$; $H-L = 6.23$, $df = 4$, $P = 0.18$; *ococ*: $G = 475.7$, $df = 3$, $P < 0.01$; $H-L = 1.78$, $df = 4$, $P = 0.78$). A quadratic term in photoperiod was included for *ococ* to account for the curvilinear relationship (Fig. 3A).

Overall, hybrid crosses were again intermediate to the pure lines but, unlike the results of experiments 1 and 2, differences between the two crosses were more obvious (Fig. 3B).

There was a high proportion of individuals entering second diapause at the shortest photophase in both crosses (Fig. 3B), with the same modifying effect of temperature that was obvious in *ococ* (Fig. 3A). The trend at longer photophases was more complex. At the highest temperature (26 °C), the proportion of individuals entering second diapause continued to decrease with longer photophases at the same rate for both hybrid crosses. At the lowest temperature (16 °C), however, the proportion of individuals entering second diapause decreased between the shortest and intermediate photophases and then increased again at the longest photophase (Fig. 3B). All interactions were required in the final model for the hybrid crosses, making further interpretation problematic ($G = 527.3$, $df = 8$, $P < 0.01$; $H-L = 8.93$, $df = 5$, $P < 0.11$).

Additional information on the interaction of photoperiod and temperature is available for backcrosses between hybrids (*ocbi* and *bioc*) and the parental group *bibi* from experiment 1.

Table 3. Frequency of second diapause and no second diapause and proportion of individuals entering second diapause in primary crosses (pure lines and hybrids), backcrosses, and crosses within hybrid lines of *C. occidentalis* and *C. biennis* reared at 20 °C, 16L:8D.

Cross	Second diapause	No second diapause	Proportion entering second diapause
Primary crosses			
<i>ococ</i>	12	90	0.118
<i>bibi</i>	549	28	0.951
<i>ocbi</i>	1090	1489	0.423
<i>bioc</i>	307	337	0.477
Backcrosses			
<i>bioc</i> ♂ × <i>ococ</i> ♀	622	1373	0.312
<i>ococ</i> ♂ × <i>bioc</i> ♀	712	1629	0.304
<i>ocbi</i> ♂ × <i>ococ</i> ♀	334	1047	0.242
<i>ococ</i> ♂ × <i>ocbi</i> ♀	633	1494	0.298
<i>bibi</i> ♂ × <i>bioc</i> ♀	110	4	0.965
<i>ocbi</i> ♂ × <i>bibi</i> ♀	55	1	0.982
Crosses within hybrid lines			
<i>ocbi</i> ♂ × <i>ocbi</i> ♀	652	546	0.544
<i>bioc</i> ♂ × <i>bioc</i> ♀	571	545	0.512

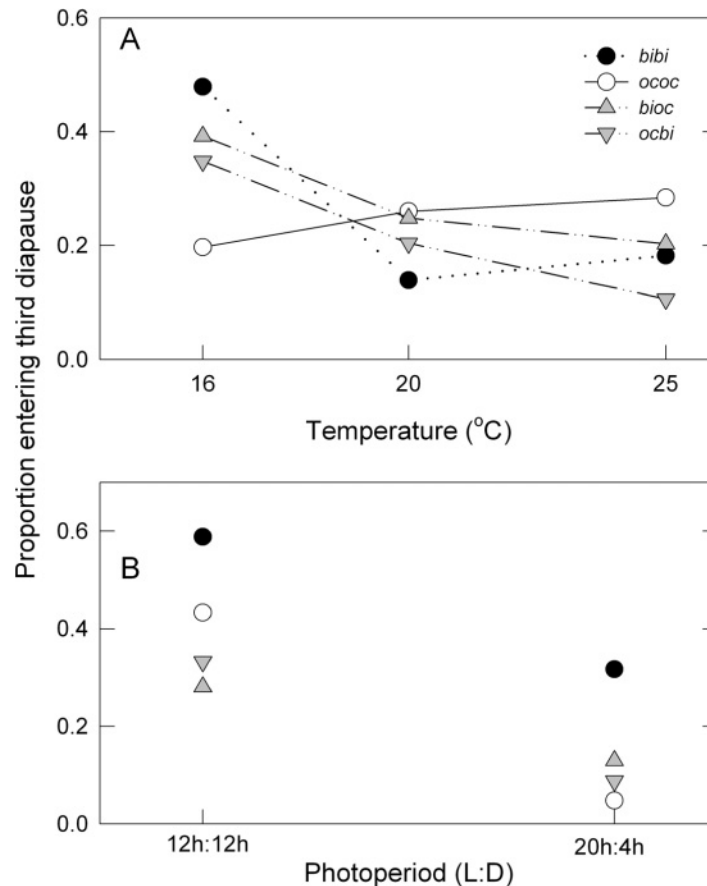
Wild parental stocks were added as controls. These results confirm that the relative frequency of second diapause is greater at shorter photophases and lower temperatures than at longer photophases and (or) higher temperatures (Table 2). As in experiment 3, the effect was less pronounced in *bibi* than in *ococ*. Most *bibi* individuals entered a second diapause irrespective of the environmental conditions, whereas the response in *ococ* was highly dependent on photoperiod and, to a lesser extent, temperature (Table 2). Backcrosses between *bioc* and *bibi* also showed a high frequency of second diapause at all combinations of environmental conditions, although the frequency for the long photophase – high temperature combination was considerably lower than that of pure *bibi* for the same photoperiod–temperature combination, showing the influence of the hybrid parent. This influence of the hybrid parent was also evident in backcrosses between *ocbi* and *bibi*. As the overall frequency of second diapause was lower in these backcrosses, the influence of environmental conditions was more marked but consistent with the conclusion that second diapause, where it varies at all, will be most frequent at short photophases and lower temperatures.

Backcrosses and crosses within hybrid lines

Table 3 summarizes the observed frequency of second diapause among progeny of a second set of primary crosses (both pure and hybrid), backcrosses, and crosses within hybrid lines reared under standard conditions. Note that the proportion of progeny entering second diapause for both the pure lines and the hybrids provides independent support for the results for standard conditions presented in Figure 2. The proportion of individuals entering second diapause was similar among backcrosses between either hybrid and *ococ* (min. = 0.242, max. = 0.312). The overall proportion of these backcrosses entering second diapause was intermediate to that of the parental groups (Table 3), suggesting further regression toward the parental mean. Information on only two backcrosses with *bibi* (*bibi* × *bioc* and *ocbi* × *bibi*) is available from these trials. In both cases, the frequency of second diapause was as high as in the pure wild *bibi* (Table 3).

Interestingly, the proportion of individuals entering second diapause was slightly higher among the crosses within hybrid lines than in the corresponding parental groups. But, as in the first-generation crosses, the frequency of

Fig. 4. Proportions of pure (*bibi* and *ococ*) and hybrid crosses (*bioc* and *ocbi*) entering third diapause: (A) effects of temperature and (B) effects of photoperiod.



second diapause was intermediate to that of the respective backcrosses (Table 3).

Additional information is available for backcrosses with *bibi* (see Table 2). Note that different temperatures and photoperiods were used in these tests. Nonetheless, it is clear that the proportion of second-diapause individuals resulting from backcrosses between hybrids and *bibi* was uniformly high (>0.96) at low temperatures, irrespective of photoperiod. Second diapause was less frequent at higher temperatures and longer photophases, especially in backcrosses between *ocbi* and *bibi* (Table 2). The effects of photoperiod and the interaction between temperature and photoperiod are discussed more fully below. At this point, it is sufficient to note that as in backcrosses involving *ococ* as the parental group (Table 3), backcrosses between hybrids and *bibi* resulted in observed frequencies of second diapause intermediate to the parental responses.

Third diapause

The observation of frequent third diapause in all crosses was unexpected. Also unexpected, given the results of experiment 1, was the influence of temperature on the frequency of third diapause (Fig. 4A). The effect of photoperiod on third diapause was similar to its effect on the second diapause response, with third diapause occurring relatively more frequently at short (12L:12D) than at long (20L:4D) photophases (Fig. 4B). Regression analysis showed that previous temperature did not significantly influence the proportion entering third diapause but previous photoperiod did, at least in *bibi* ($G = 39.6$, $df = 1$, $P < 0.01$) and *ocbi* ($G = 16.0$, $df = 1$, $P < 0.01$). Insufficient or missing data precluded statistical analysis for *ococ* and *bioc*.

Discussion

The high success rate of mating between the

two species confirms the capability of western species of *Choristoneura* to interbreed and produce viable offspring (Harvey 1997). Given that *C. biennis* and *C. occidentalis* respond to the same pheromone (Sanders 1971), mate indiscriminately, and produce viable offspring, the results reported here reinforce Campbell's (1967) speculation that there are few biological factors isolating these species and genetic introgression is likely to occur in areas of sympatry (Fig. 1). There is ample evidence that such introgression between *C. occidentalis* and other western species of conifer-feeding *Choristoneura* already occurs in mixed forests in Oregon (Volney *et al.* 1984; Powell and De Benedictis 1995).

The experimental results revealed a strong genetic effect on the second diapause response at the population level, modified by photoperiod and, to a much weaker extent, temperature. The importance of the environmental variables depended on the nature of the cross. A consistently high proportion of 2-year-cycle budworms entered second diapause irrespective of photoperiod or temperature, adding weight to Harvey's (1967) speculation that second diapause in *C. biennis* is genetically fixed. In contrast, the frequency of second diapause in western spruce budworm varied from over three quarters of the generation at short photophases and low temperatures to none at long photophases, irrespective of temperature, suggesting considerable polymorphism in the diapause response of this species.

The intermediate response of hybrid lines under all combinations of environmental conditions suggests polygenic inheritance of voltinism in *Choristoneura*. The second diapause response of offspring of backcrosses and crosses within hybrid lines regressed toward the parental mean in the classical pattern of quantitative inheritance (Tauber *et al.* 1986), although other genetic models cannot be dismissed. There was evidence that at least a portion of the inheritance is sex-linked: hybrid responses most closely resembled that of the paternal contribution to the cross, although the effect was not as strong as suggested by Harvey's (1967) limited data set. Several other species-specific characters have been associated with sex-linked genes (males are homogametic in Lepidoptera) in *Choristoneura* (Sperling 1994). Variation in the second diapause response was also influenced by family, but in a

complex way, and associated with the subtle and nonlinear effects of temperature. These genetic patterns will be investigated in a second publication.

The induction of a third diapause in both pure and hybrid lines, although qualified because of observation under a sequence of artificial conditions, reveals the inherent capacity for a range of voltinism in the genus. The frequency of third diapause was greatest in 2-year-cycle spruce budworm that had been invariant with respect to second diapause. Short photophases induced the highest frequency of third diapause in the normally 2-year-cycle spruce budworm, just as short photophases had induced the greatest frequency of second diapause in the normally 1-year-cycle western spruce budworm. Temperature, however, also had an influence on third diapause in the 2-year-cycle spruce budworm, raising the possibility that in very cool years some individuals could take 3 years to complete a generation.

This inherent capacity for variable voltinism in *Choristoneura* can be extended to the case of diapause-free development. Harvey (1957) was able to produce non-diapause stock of *C. fumiferana* by rearing at long (24-h) photophases, although this stock retained its ability to enter diapause when photophase was reduced. Similarly, non-diapause lines of *C. occidentalis* are readily obtained by altering light conditions (Lyon *et al.* 1972). Such a relationship between photoperiod and facultative diapause in insects is well known. In these cases, the diapause response is often associated with the sensitivity of a particular stage of the insect to the environmental stimulus (Tauber *et al.* 1986). Harvey (1957) proposed that it was the first instar of spruce budworm that was sensitive to the photoperiodic influence on non-diapause, and Lyon *et al.* (1972) cited similar evidence in their development of non-diapause strains of *C. occidentalis*. In the experiments reported here, all first instars were treated identically, so sensitivity must extend to later stages. The significant effects of photoperiod experienced by *bibi* and *ocbi* between their first and second diapause and following their second diapause on the likelihood of these crosses entering a third diapause suggests that sensitivity to photoperiod and the capacity to enter diapause may persist as late as the fifth instar.

Comparative studies of conifer-feeding *Choristoneura* species reveal the dynamic interactions

between biological characters and ecological relationships that have shaped evolutionary radiation in the past and continue to influence present populations. The few distinctive morphological characters and extensive polymorphisms in the group suggest recent divergence of species. The genetic evidence supports this (Stock and Castroville 1981; Powell and De Benedictis 1995; Sperling and Hickey 1995; Harvey 1997). The apparent lack of either pre- or post-mating reproductive isolating mechanisms in *C. biennis* and *C. occidentalis* means that the accepted separation of these species depends to a great extent on their voltinism and the related association with forest types, which are, in turn, closely associated with climatic zones.

The western spruce budworm exploits several host tree species over its extensive latitudinal range (Furniss and Carolin 1977), but the host range appears more restricted in northern regions. Harvey (1985), for example, noted that *C. occidentalis* is commonly found on subalpine fir in the southern portions of its range but does not penetrate these high-elevation forest zones in BC. Larval collections by me and R. Turnquist in BC since 1997 confirm that *C. occidentalis* is found almost exclusively on Douglas-fir, while *C. biennis* is found feeding on subalpine fir and white spruce, *Picea glauca* (Moench) Voss.

These distinct forest types occupy different biogeoclimatic zones in BC: Douglas-fir occurs in drier, warmer zones in the south, whereas *Abies* and *Picea* species occur in cooler, wetter areas at higher elevations or latitudes. The lower temperatures and shorter growing seasons typical of these spruce-fir forests undoubtedly have favored fixation of the extended life cycle characteristic of *C. biennis*. All conifer-feeding *Choristoneura* species are constrained by their specialization to feed principally on current-year growth, and their survival is influenced by the limited seasonal window during which these buds are suitable (Shepherd 1992; Lawrence *et al.* 1997). When this seasonal window is further constricted by inclement conditions associated with higher elevations and latitudes, extending the feeding stages over two seasons reduces risk considerably and may have other survival benefits (Mathers 1932).

Both western and 2-year-cycle spruce budworms have geographic ranges extending hundreds of kilometres along a north-south gradient, which means that both species experience

a range of photoperiods. In the case of 2-year-cycle spruce budworm, the invariant second diapause response with respect to photoperiod and temperature ensures that this species will express at least a 2-year life cycle throughout its range. However, in western spruce budworm, second diapause is more frequent in northern populations (Harvey 1985). This facultative diapause induced by photoperiod is a common pattern in species occupying a broad latitudinal range (Tauber *et al.* 1986). Such variation facilitates adaptive responses of populations to environmental variability while maintaining genetic polymorphism within the species.

All of the present range of *C. biennis* and most of the Canadian range of *C. occidentalis* were glaciated until the beginning of the Holocene. Although it is possible that these species were already differentiated in the fragmented forests south of the glaciers during the Pleistocene, the evidence suggests that their separation is more recent and associated with the reestablishment of northern coniferous forests over the past 7000 years (Volney 1985; Powell and De Benedictis 1995). The diverse forests south of the glacial margin may have been occupied by a complex of interbreeding populations of *Choristoneura* species rich in life-history polymorphisms and flexible in their host associations. Genetic communication among these populations would have been enhanced by periodic outbreaks and mass migration of moths, which are characteristic of the genus. Remnants of this hypothetical situation can still be found in the Pacific Northwest (Volney *et al.* 1984; Powell and De Benedictis 1995) and may be specifically epitomized by the ecological variability and reproductive promiscuity of the western spruce budworm. Such elasticity suits that species' broad ecological circumstances. As populations moved northward with their hosts with the retreating glaciers, however, constraints associated with reduced host species diversity and inclement weather would have favored life-history traits more suited to these environments. A 2-year life cycle, still inherent in the genetic heritage of *C. occidentalis*, is one such trait. Selection for this life-history trait would favor continued northward expansion of the range of *Choristoneura*. It would also reinforce distinct host associations, as the ranges of the hosts themselves are determined primarily by similar climatic variables. The result is the

current distribution and ecology of *C. occidentalis* and *C. biennis* in BC.

The recent divergence of these two species and its dependence on prevailing bioclimatic conditions implies that these systems remain dynamic. Changes in host species ranges or mixing of forest types associated with changes in either climate or forest land-use patterns or even the direct effects of climate change could result in populations of budworms that have new combinations of characters and are heterogeneous in voltinism. Such biological signals of environmental change provide fascinating evidence of evolution in action.

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