

Effects of photoperiod, temperature, and host plant age on induction of reproductive diapause and development time in *Danaus plexippus*

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Abstract. 1. Diapause induction in monarch butterflies *Danaus plexippus* was studied using adults captured from the wild in Minnesota and Wisconsin, and individuals reared under outdoor and controlled conditions.

2. Oocyte presence in females and ejaculatory duct mass in males were used to indicate reproductive status.

3. Some wild individuals were in diapause in mid-August, and all males and females were in diapause by late August and early September respectively.

4. Individuals reared under decreasing day lengths and fluctuating temperatures were more likely to be in diapause than were individuals reared under long or short day lengths or constant temperatures.

5. Individuals fed potted old *Asclepias curassavica* plants were more likely to be in diapause than were those fed potted young host plants; when cuttings of *Asclepias syriaca* plants from the field or greenhouse pots were used, there was no effect of host plant age.

6. Extremely high temperatures increased the number of day-degrees required for development from egg to adult, while decreasing day lengths and older host plants tended to decrease the number of day-degrees required for development.

7. There appears to be a continuum of reproductive development in monarchs, with gradual declines in mean ejaculatory duct mass and oocyte production during the late summer.

8. None of the experimental treatments led to 100% diapause, and diapause was more likely to occur in monarchs subjected to more than one diapause-inducing cue.

Key words. *Danaus plexippus*, day-degrees, development time, diapause induction, host plant characteristics, monarch butterfly, photoperiod, reproductive diapause.

Introduction

Organisms living in temperate regions face seasonal challenges, such as absence of food, harsh winter conditions, and the need to synchronise reproduction with suitable environmental conditions. Insect adaptations to these challenges include migration, dormancy, and seasonal polyphenism; various combinations of these traits constitute

a diapause syndrome (Andrewartha, 1952; Tauber *et al.*, 1986; Danks, 1987; Leather *et al.*, 1993). Diapause is a state of arrested development, characterised by low metabolic activity, reduced motor activity, and increased resistance to environmental extremes, and is controlled neurohormonally in insects (Nijhout, 1994). Diapause induction occurs along a spectrum ranging from complete control by external factors to complete control by genetic factors (Tauber *et al.*, 1986). External stimuli that induce diapause almost always precede adverse conditions, and include both abiotic and biotic factors (reviews by Tauber *et al.*, 1986; Leather *et al.*, 1993).

The monarch butterfly *Danaus plexippus* (L.) (Nymphalidae) is a tropical species that has expanded its range into temperate

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regions, taking advantage of the distribution of its host plant genus, *Asclepias* (Brower, 1985; Masters *et al.*, 1988). Not adapted to survive temperature extremes of their expanded North American range, monarchs undergo long-distance migration each autumn (Urquhart & Urquhart, 1978) and delay reproduction until the following spring, entering a state of reproductive diapause with minimal reproductive tract development (Herman, 1973; Brower *et al.*, 1977; Herman *et al.*, 1989). Eastern and western North American monarchs migrate to overwintering sites in central Mexico and California respectively, where they remain until adults migrate north. The eastern population exhibits a refractory period during which butterflies do not undergo gonadal development in response to 10 days of summer-like conditions (25 °C and 16 h photophase), while this refractory period is either shorter or non-existent in the western population (Herman *et al.*, 1989). Monarchs in Australia also diapause to a lesser degree (James, 1993). Monarch ovarian dormancy in Australia (and possibly California) is classified more accurately as oligopause, an intermediate condition that lacks the refractory period associated with diapause (James, 1982). This dormancy can be induced by exposure to cool temperatures after adult eclosion, and is associated with longer flight distances than seen in adults exposed to warm post-eclosion temperatures (James, 1983).

The neurohormonal control of eastern North American monarch diapause has been elucidated (Barker & Herman, 1973; Herman, 1975, 1981, 1985), and photoperiod and temperature have been shown to influence reproductive development in post-eclosion monarchs (Barker & Herman, 1976). Barker and Herman identified only optimal and inhibitory conditions, however, and their results did not allow distinction between diapause *per se* and the slow development of reproductive organs under cool conditions.

In the work reported here, several environmental cues involved in diapause induction in eastern North American monarchs were studied. The natural incidence of male and female diapause in Minnesota and Wisconsin (north-central U.S.A.) populations was documented and a series of controlled induction experiments was carried out to test the effects of photoperiod, host plant characteristics, and temperature. The effects of rearing conditions on development rate were also assessed.

Methods

Natural incidence

Weekly samples of adults in south-central Minnesota and south-western Wisconsin ($\approx 45^\circ\text{N}$, 90°W) were collected from mid-summer to early autumn 1995 to assess monarch reproductive status. Adults were held in outdoor cages ($2 \times 2 \times 2\text{ m}$) on the University of Minnesota St Paul campus, fed a 25% (by volume) honey solution daily, and their mating and oviposition behaviour was observed. In addition, weekly cohorts of 15–20 eggs from wild-captured

females were reared outdoors (the initial cohort was collected as eggs and early-instar larvae from the wild on 19 July). Cohorts were kept outdoors in $56 \times 40 \times 31\text{ cm}$ screen cages until adults were dissected. Every day, larvae were fed fresh, wild-collected milkweed *Asclepias syriaca* cuttings that were held in bottles of water to maintain hydration. Adult mass and wing length were measured 24 h after eclosion. Adults were fed once each day and dissected at age 9 days as described below. Temperature data were obtained from National Oceanic and Atmospheric Administration climatological records.

Diapause induction experiments

Three experiments in which developing monarchs experienced varying conditions were conducted in order to explore the effects of specific environmental factors on diapause induction. These experiments tested the effects of photoperiod, host plant characteristics, and temperature on the proportion of adults in diapause (details below). Values for each treatment variable were chosen to mimic natural environmental conditions in the northern part of the monarch breeding range. In each experiment, adults were kept in glassine envelopes in treatment chambers, and fed a 25% honey water solution *ad libitum* every other day until dissection at age 9 days. All animals were first- or second-generation offspring of adults captured in east-central Minnesota and west-central Wisconsin. Only females were assessed in the first two experiments.

Experiment 1: photoperiod. The effect of photoperiod was tested using three treatments: long day length (LD 16:8 h), decreasing day length (starting with LD 15:9 h and decreasing by 3 min day^{-1} until adults were dissected), and short day length (LD 13:11 h). The long day length treatment mimicked natural conditions in Minnesota during early summer, when all monarchs are reproductive; the decreasing day length treatment mimicked conditions in late July–late August, when diapause individuals are developing.

Each treatment chamber contained a standard fixture with one 40 W and one 30 W fluorescent bulb (Sylvania Cool White Deluxe brand) suspended 1 m above a tabletop on which larva cages were kept. Programmable appliance timers were used to control photoperiod. The chambers were three adjoining rooms ($3 \times 3 \times 2.75\text{ m}$) on one heating and ventilation system. Temperature was measured every time the monarchs were fed or checked (at least once each day) and did not vary significantly among chambers or over time (mean = 23.2°C , $\text{SE} = 0.4^\circ\text{C}$). This temperature is similar to average summer temperatures in Minnesota, but field temperatures are more variable. Pans of water in the rooms maintained $\approx 25\%$ RH (checked every other day using a sling psychrometer).

At the start of the experiment, 50 newly hatched larvae per treatment were transferred to potted, greenhouse-grown *Asclepias curassavica* plants in screen cages ($56 \times 40 \times 31\text{ cm}$ with 25 larvae per cage). Additional plants were provided as

needed to maintain a constant food supply. The greenhouse in which milkweed was grown was maintained at LD 14:10 h. Plants were watered approximately every 3 days and the pots were fertilised weekly with a 20:20:20 NPK mixture. Larvae pupated in the cages and adult mass and forewing length were measured 24 h post-eclosion.

Experiment 2: photoperiod and host plant. The effects of day length and host plant age were examined in a 3×2 factorial design, with three photoperiod treatments and two host plant treatments. Photoperiod treatments were the same as above, except that the short day length treatment was shortened from LD 13:11 h to LD 10:14 h, and the decreasing day length began at LD 14:10 h instead of LD 15:9 h. Other conditions remained the same as in expt 1. Larvae were reared on greenhouse *A. curassavica* plants. Young plants were ≈ 1 month old; old plants were 8–9-month-old flowering plants that had been cut back and allowed to leaf out for 8–12 weeks. Old plants were watered half as frequently. There were 33–40 larvae per treatment, reared as described above.

Experiment 3: photoperiod, temperature, and host plant. The effects of photoperiod, temperature, and host plant quality were examined in a $2 \times 2 \times 2$ factorial design, with two blocks, using temperature- and photoperiod-controlled Percival growth chambers. Long and decreasing day length treatments were the same as in expt 2; the short day length treatment was omitted. Temperature regimes included a constant (27°C) and a fluctuating temperature (27°C thermophase to 21°C cryophase, with temperature exposures coinciding with light and dark periods).

This experiment was conducted when *A. syriaca* is widely available in Minnesota. To obtain young and old plants simultaneously, a combination of wild and greenhouse-grown *A. syriaca* was used. In the first block, early in the summer, young plant treatments utilised cuttings from wild milkweed. Cuttings from 4- to 5-month-old greenhouse plants were used for old host plant treatments. In the second block, later in the summer, cuttings from old ramets (with seed pods and yellowing leaves) and new growth from plants that had been mowed within the last 1–2 months and had unblemished, green leaves were used for the old and young plant treatments. In both blocks, cuttings were kept in floral tubes and changed daily. Larvae were reared in plastic cages with screened lids (30 × 17 × 11 cm).

Assessing diapause

Females. Nine-day-old females were dissected under 6× magnification; an absence of mature oocytes was used as the criterion for diapause. Females kept outdoors in the summer have mature oocytes 6 days after eclosion (Oberhauser & Hampton, 1995), while females in reproductive diapause have small, undeveloped ovarioles at the same age (Herman, 1973). The degree of ovarian development in females not in diapause was assessed by tallying the number of mature oocytes.

Males. The wet mass of the ejaculatory duct was used to assess male diapause; diapause males have smaller reproductive organs than do reproductively active males (Herman, 1985). Males were dissected under 12× magnification in insect saline, fat bodies and tracheae were cleared from the lower portion of the reproductive tract complex, and the ejaculatory duct was removed. This portion of the tract is the lowest section from the aedeagus to the tubular gland and is separated from the tubular gland by a narrow region. Once removed and cleared of remaining fat bodies, the ejaculatory duct was blotted on absorbent tissue to uniform dryness and weighed to 0.01 mg on a Mettler AE 240 balance (Mettler Instruments, Greifensee, Switzerland).

Wild-caught individuals. Because adults collected from the wild are of unknown ages and mating histories, diapause cannot be assessed in the same manner as in laboratory-reared animals. Mating by males and oviposition by females, however, indicate non-diapause status. Wild-captured males were placed in a large, outdoor mating cage with reproductive laboratory females, and a 1:1 sex ratio was maintained. Males that mated within 5 days were considered reproductive and were subsequently released. The status of wild-captured females was assessed both behaviourally and through dissection. After capture, they were held in individual mesh cages (66 × 66 × 66 cm) with fresh milkweed for 2 days. Females that oviposited were considered reproductive and released. To allow for the possibility that the non-ovipositing females were not mated, they were transferred to a cage with reproductive males for up to 5 days. If they mated, they were transferred to oviposition cages with fresh milkweed. Non-mating females and females that did not oviposit within 5 days of mating were dissected as described above. Because monarchs continue to produce oocytes throughout their lives (Oberhauser, 1997), old reproductive females do not run out of eggs and will thus not be mistaken for diapause females. This variability in treatment minimised the number of wild adults that was killed; females were only dissected if other methods of assessing reproductive status were inconclusive.

Statistical analyses. Logistic regression models, which are appropriate for binomial data (Hardy & Field, 1998), were used in analyses of female diapause. To test the importance of treatment variables, a stepwise analysis of deviance was carried out for each experiment and the log-odds ratios and Wald statistics for all design variables were examined. For the analyses of ejaculatory duct mass and mature oocyte production, a stepwise linear regression tested the effects of treatment variables, interaction terms, and adult mass. The effects of treatment variables on mass and development time were analysed using ANOVA.

Results

Natural incidence of diapause

All wild-caught females collected and held until 23 August were reproductive, although sample sizes during August

Table 1. Reproductive activity in wild-captured butterflies.

Week captured	Females			Males			
	<i>n</i>	Number that laid eggs	Number with mature oocytes	Per cent reproductive	<i>n</i>	Number that mated within 5 days	Per cent reproductive
17–26 July	5	3	2	100	8	8	100
27 July–2 August	4	3	1	100	2	2	100
3–9 August	2	2	0	100	5	5	100
10–16 August	3	3	0	100	4	4	100
17–23 August	1	0	1	100	5	3	60
24–30 August	3	1	1	67	8	2	25
31 August–6 September	16	4	1	31	24	0	0
7–12 September	5	0	0	0	10	0	0

Logistic regression analysis of deviance using week as the predictor for diapause was significant (females: Δ deviance = 28.94, Δ d.f. = 1, $P < 0.001$; males: Δ deviance = 69.1, Δ d.f. = 1, $P < 0.001$).

were small (Table 1). By the second week in September, all females were in diapause. Male mating behaviour began to decline a week earlier, in mid-August, and ceased by the end of August (Table 1). Almost all reproductive behaviour ceased in wild-caught adults in the last 2 weeks of collection, corresponding to the peak of migration in Minnesota (K. S. Oberhauser and L. Goehring, pers. obs.).

In cohorts of monarchs reared outdoors (Table 2), all females that emerged on or before 25 August developed mature oocytes, with the exception of one female in cohort 1. In the weeks of 30 August and 10 September, 46 and 100% of the females were in reproductive diapause. There was a significant, negative relationship between date of emergence and diapause. Among females that did not diapause, there was a significant relationship between the number of mature oocytes and date, with those emerging later producing fewer oocytes.

Male reproductive tract development also changed over time. There was a significant relationship between ejaculatory duct mass and date of emergence, with late season cohorts having smaller ejaculatory ducts (Table 2).

The gradual decrease over time of male reproductive tract mass is illustrated in Fig. 1a; the transition period is characterised by intermediate masses during the last third of August. Only in the final cohort did most males have small ejaculatory ducts.

Temperatures experienced by cohorts affected the length of the development period, although day-degrees were roughly consistent among groups, with the exception of the first cohort (Table 3). This cohort experienced 7 days on which temperatures reached or exceeded 30 °C. Cohort 3 experienced 5 days over 30 °C, while cohorts 2 and 4 experienced 4 days over 30 °C. Mean daily temperature fluctuation (maximum – minimum) did not vary among cohorts. Neither female nor male body mass varied among cohorts.

Diapause induction

Photoperiod. Diapause incidence varied significantly between decreasing and other day length treatments

Table 2. Reproductive development in cohorts of monarchs reared outdoors.

Date eggs laid	Date adults emerged	Females				Males		
		<i>n</i>	Per cent reproductive†	Mean mature oocytes‡ (SD)	Mean mass§ (g)	<i>n</i>	Mean ejaculatory duct/mass¶ (SD)	Mean mass§ (g)
NA	9–18 August	7	86	84.2a (67.6)	0.532	2	0.041a (0.002)	0.523
16 July	18–21 August	7	100	46.1ab (43.0)	0.468	8	0.035a (0.006)	0.485
28 July	24–25 August	11	100	43.0ab (31.6)	0.502	9	0.027ab (0.005)	0.502
3 August	30 August–1 September	13	54	15.3b (8.6)	0.485	9	0.021b (0.008)	0.488
12 August	10–13 September	7	0	NA	0.517	8	0.011c (0.008)	0.546

†Per cent containing mature oocytes. Logistic regression analysis of deviance using date of emergence as the predictor for reproductive status was significant (Δ deviance = 10.6, Δ d.f. = 1, $P < 0.001$). ‡Mean excludes females in reproductive diapause. ANOVA $F_{3,27} = 3.01$, $P < 0.05$; means followed by the same letter are not significantly different at the 0.05 level of confidence (Tukey least significant difference comparisons). §No difference in adult mass among cohorts (females: $F_{4,40} = 2.17$, NS; males: $F_{4,36} = 2.37$, NS). ¶Ejaculatory duct mass/adult mass ($F_{4,31} = 16.94$, $P < 0.001$); means followed by the same letter are not significantly different at the 0.05 level of confidence (Tukey least significant difference comparisons).

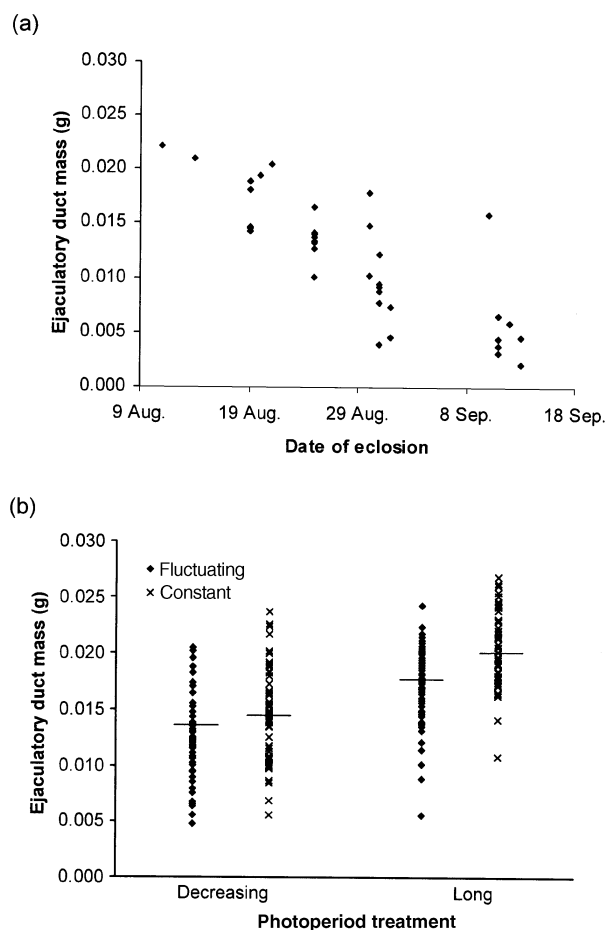


Fig. 1. Male ejaculatory duct mass. (a) Weekly cohorts reared during August and September 1995. (b) Males in photoperiod, host plant quality, and temperature induction experiment. All individuals are included as marks to show the range of values, and treatment means are indicated by bars. Only temperature and day length treatments are illustrated because host plant did not have a significant effect on ejaculatory duct mass.

(Table 4, Fig. 2a) (significant contrasts are evident when the log-odds ratio is different from 1). The log-odds ratio of 8.14 indicates that the odds of inducing diapause in the

decreasing day length treatment are about eight times those of other photoperiod treatments. Adult size did not vary among treatments, and the size of reproductive and diapause females did not differ. While the difference in the number of mature oocytes produced in reproductive females (Fig. 3a) was not significant at the 0.05 level ($F_{2,44} = 2.84$, $P = 0.069$), there was a trend towards more oocytes produced by females in the long day length treatment.

Development time in day-degrees, from hatching to eclosion, was significantly longer in the long day treatment (Fig. 4a). Mean development times for diapause and reproductive females did not differ significantly.

Photoperiod and host plant. Females were most likely to be in diapause when reared under decreasing day length and fed old milkweed (Fig. 2b). To assess the importance of each treatment variable, four models were tested in a stepwise analysis of deviance: null, a photoperiod effect, a plant-quality effect, and a combination of both cues. The most parsimonious model for predicting diapause included only host plant and decreasing day length variables (Table 4). The odds of diapause for females reared on old plants were 12 times those for females reared on young plants. The odds of diapause in decreasing day length treatments were five times the odds for other day lengths. Both factors contributed to the likelihood of diapause but the lack of a significant interaction implies that their functions are additive.

To rule out an effect of nutrition on reproductive development, mass at emergence was examined. There was a relationship between mass and host plant age, however females fed old plants were significantly larger (young plant mean = 0.473 g, old plant mean = 0.537 g; $F_{1,113} = 60.16$, $P < 0.001$). The sizes of diapause and reproductive females did not differ significantly.

Stepwise linear regression of mass, host plant, and photoperiod (the latter two were included as indicator variables in the model) on oocyte production in reproductive females revealed that only the long day length treatment had a significant effect, with females reared in this treatment producing more mature oocytes (Table 5, Fig. 3b).

Monarchs reared under decreasing day lengths and fed old plants developed more quickly (in day-degrees)

Table 3. Summary of cohort development.

Cohort (n)	Development period	Mean temperature†		Mean daily fluctuation‡		Mean development time§		Mean development time (day-degree)¶	
		°C	SD	°C	SD	Days	SD	DD	SD
1 (21)	20 July–20 August	23.6ab	2.0	10.8	2.7	30.3a	0.8	363.3a	7.8
2 (26)	1–25 August	23.7ab	2.0	10.4	2.7	22.8d	0.5	282.4c	5.7
3 (26)	7 August–1 September	24.0a	1.8	9.7	3.0	23.6c	0.6	294.4b	6.1
4 (16)	15 August–13 September	22.0b	4.0	11.1	3.2	26.9b	1.0	279.8c	7.3

†Mean temperature experienced during development period, hatching to eclosion. Numbers followed by the same letter were not significantly different at the 0.05 confidence level ($F_{3,103} = 3.33$, $P < 0.05$, Tukey least significant difference comparisons). ‡Means did not differ among cohorts ($F_{3,103} = 1.12$, NS). §Time from hatching to adult. Means differed among cohorts ($F_{3,85} = 509.3$, $P < 0.001$). ¶Degree days = Σ (daily mean temperature – 12°C threshold temperature) from hatching to adult. Means differed among cohorts ($F_{3,85} = 740.1$, $P < 0.001$).

Table 4. Summary of final binomial regression models testing factors affecting female diapause probabilities.

Predictor	Coefficient	SE	P	Log-odds	95% CI
Experiment 1: photoperiod (effects of long and short day treatments also tested)					
Constant	-2.00	0.47	< 0.0001		
Decreasing day	2.10	0.64	< 0.01	8.14	2.30–28.14
Deviance	59.73				
d.f.	61				
Experiment 2: photoperiod and host plant (effects of long and short day length treatments and interactions also tested)					
Constant	-4.54	1.08	< 0.0001		
Old host plant	2.51	1.06	< 0.05	12.26	1.53–98.44
Decreasing day	1.62	0.60	< 0.01	5.04	1.54–16.5
Deviance	72.28				
d.f.	112				
Experiment 3: photoperiod, host plant, and temperature (effects of long and short day treatments, host plant and block, and interactions also tested)					
Constant	-4.66	0.76	< 0.0001		
Decreasing day	3.65	0.74	< 0.0001	38.5	9.1–163.0
Fluctuating temperature	1.17	0.41	< 0.01	3.2	1.5–7.2
Deviance	150.68				
d.f.	214				

(Table 6, Fig. 4b). There was no significant difference in development time between diapause and reproductive females, and no relationship between development time and size, after controlling for host plant.

Photoperiod, host plant, and temperature. Female diapause in this experiment was most likely to occur under decreasing day length and fluctuating temperature conditions (Fig. 2c). A summary of the resulting binomial regression model from the analysis of deviance is shown in Table 4. The odds of diapause under decreasing day length conditions were 38 times those under long day lengths. The odds of diapause under the fluctuating temperatures were three times those of the constant temperature regime. The effects of photoperiod and temperature were similar in the two blocks, and host plant had no effect even when the blocks were analysed separately. There were no interaction effects.

Stepwise linear regression on the number of mature oocytes in reproductive females revealed several significant relationships (Table 5, Fig. 3). Heavier females contained more eggs, while those reared in decreasing day length treatments and fluctuating temperature regimes produced fewer eggs. Females in the second block produced fewer mature oocytes than females in the first block, although the pattern of relative mature oocyte production in each treatment was consistent between blocks. There was no effect of plant quality on oocyte production.

Males from decreasing day length treatments had smaller reproductive tracts, as did males reared under fluctuating temperature treatments (Table 5, Fig. 1b). There was no effect of the interaction of day length and temperature, indicating that the effects of these two factors are additive; there was also no effect of block or plant quality on male reproductive tract mass.

Mean development time in day-degrees varied among treatments. In block 1, where old greenhouse plants and

young plants from the wild were used, individuals fed old plants and kept in fluctuating temperatures developed more quickly (Table 6, Fig. 4c). In block 2, where both old and new plants were from the wild, monarchs raised in fluctuating temperatures developed more quickly, as did those fed young plants and kept in long day lengths (Table 6, Fig. 4d). There was no difference in mean development time between diapause and reproductive females, and no correlation between ejaculatory duct mass and development time.

Discussion

Assessing the timing and progression of diapause

There was a pronounced change in female reproductive behaviour and physiology at the end of the summer (Table 1). By late August, a third of females did not oviposit in captivity and by the second week of September all were in diapause. There was a similar progression in cohorts reared outdoors; half of the females that emerged during the last week of August and all females that emerged after 1 September were in diapause (Table 2). The onset of male diapause followed a similar pattern. Mating behaviour in wild-caught males tapered off beginning in mid-August, and reproductive tract mass decreased steadily over 5 weeks in the cohorts (Table 1). By the end of August, mean ejaculatory duct mass was roughly half that of the earliest cohort, indicating diapause. While sample sizes of wild-captured adults were low, the clear pattern and correspondence with cohorts reared outdoors suggest that the observed patterns are real.

The time during which these changes occur in the northern part of the monarch breeding range is characterised by decreasing day lengths (2 min day⁻¹ in July to 3 min day⁻¹ in August) and generally decreasing temperatures,

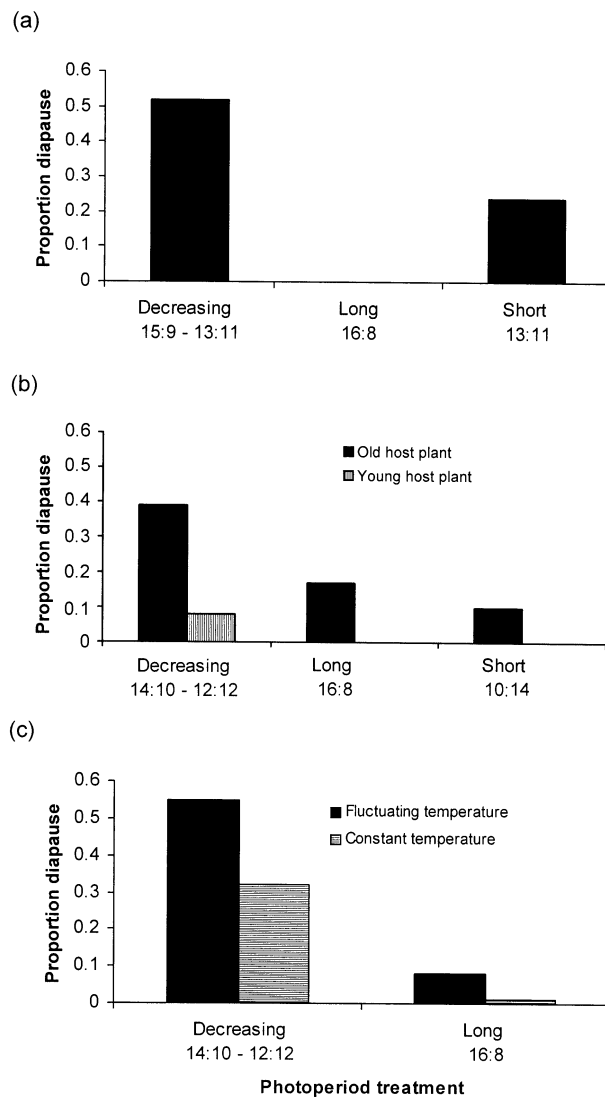


Fig. 2. Proportion of females in diapause from experiments: (a) photoperiod, (b) photoperiod and host plant quality, and (c) photoperiod, temperature, and host plant quality. The effect of host plant quality is not illustrated in (c) because host plant did not have a significant effect on the likelihood of diapause in expt 3.

especially during cryophase. The duration of thermophase also decreases. The influence of these factors and host plant characteristics on monarch diapause induction is discussed below.

Environmental cues and diapause induction

Decreasing day length. In each experiment, there was a significant effect of decreasing day length on diapause induction in females (Fig. 2). Likewise, ejaculatory duct mass was smaller in males reared in decreasing day length treatments in expt 3 (Fig. 1b). It is unlikely that the salient feature of the photoperiod treatments was the absolute

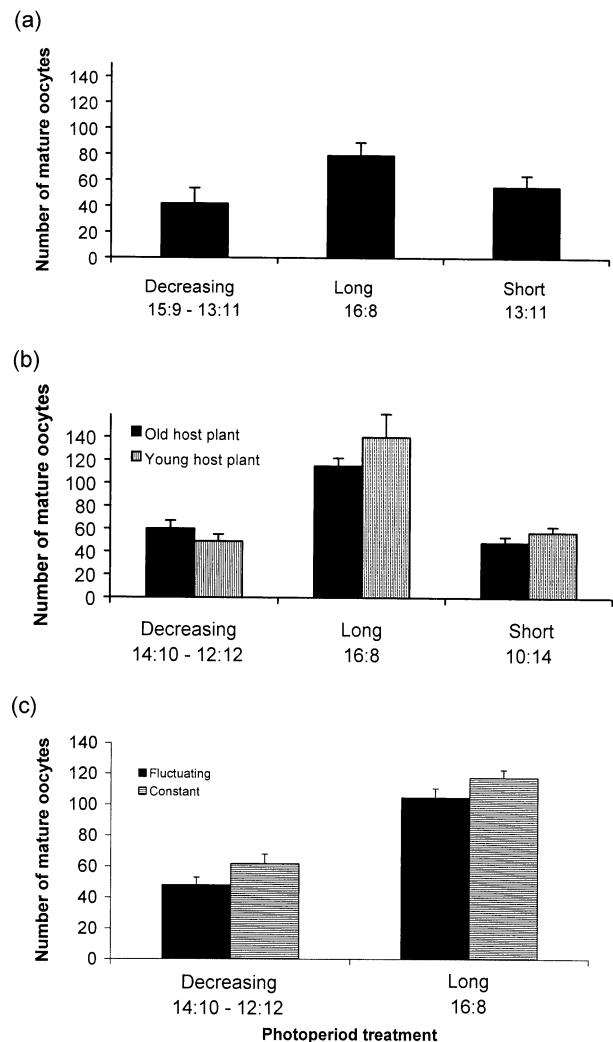


Fig. 3. Mean mature oocyte production in reproductive females for each treatment in experiments: (a) photoperiod, (b) photoperiod and host plant quality, and (c) photoperiod, temperature, and host plant quality. Error bars represent SE. The effect of host plant quality is not illustrated in (c) because host plant did not have a significant effect on oocyte production in expt 3.

length of photophase rather than the rate of change over time; if monarchs respond to an absolute critical day length, the short day length treatment should have induced diapause as effectively as the decreasing day treatment. These results do not, however, rule out the possibility that an intermediate average day length induces diapause in monarchs. The number of available incubators did not allow simultaneous testing of a constant, intermediate photophase.

There is increasing evidence that changes in photoperiod induce diapause (Solbreck, 1979; Tauber *et al.*, 1986; Nylin, 1989; Han & Gatehouse, 1991; Blanckenhorn, 1998). Decreasing photoperiod is likely to have a more pronounced effect in higher latitudes where the change is more perceptible (Taylor & Spalding, 1986; Han & Gatehouse, 1991; Gatehouse

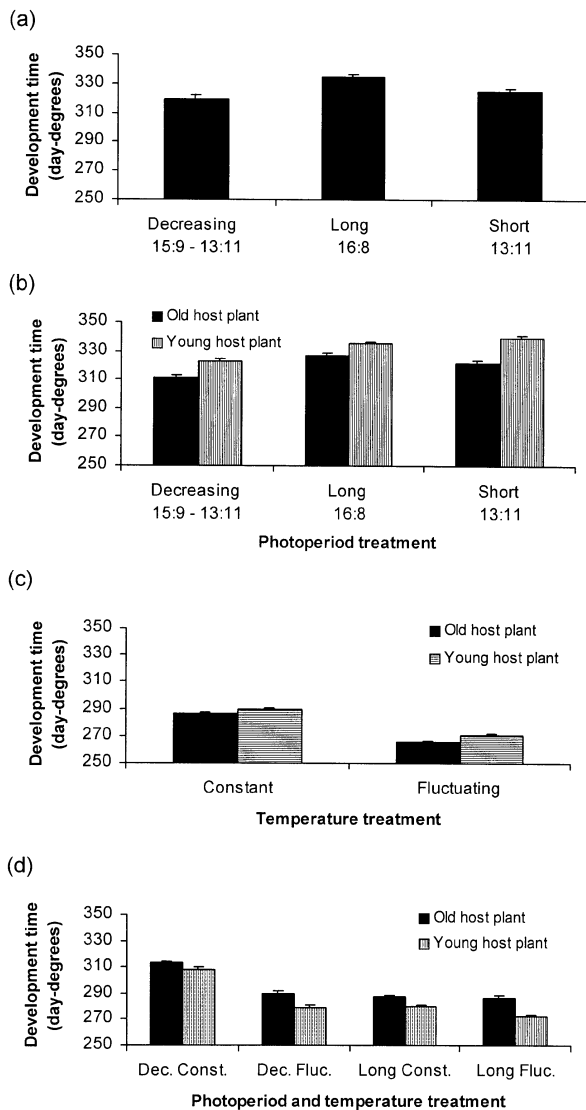


Fig. 4. Development times (egg to adult in day-degrees) in experiments: (a) photoperiod, (b) photoperiod and host plant quality (*Asclepias curassavica*), (c) photoperiod, temperature, and host plant quality, block 1 (old *Asclepias syriaca* from greenhouse, young *A. syriaca* from wild), and (d) photoperiod, temperature, and host plant quality, block 2 (old and young *A. syriaca* from the wild). The effect of photoperiod in expt 3, block 1 is not illustrated in (c) because it did not have a significant effect on development time in this block.

& Zhang, 1995) and also in insects (like monarchs) in which the offspring of different generations are exposed to different photoperiods (Solbreck, 1979). The results reported here support Solbreck's suggestion that response to decreasing day length enables synchronisation with habitat at different latitudes; however it will be important to test monarch responses to less pronounced changes in photoperiod, such as those experienced in the central and southern U.S.A. in late summer and early autumn.

Temperature. Diapause was twice as likely to occur in females reared under a conservative fluctuating temperature treatment where night temperatures were lower than day temperatures (Fig. 2c), and males reared under fluctuating temperatures developed smaller ejaculatory duct tracts (Fig. 1b). While it is possible that the monarchs responded to cool average temperatures *per se*, as opposed to temperature fluctuation, adults kept in an incubator at constant 21 °C under summer photoperiods do not diapause (K. S. Oberhauser, pers. obs.).

Although not as consistent a cue as photoperiod, temperature is seasonably variable. James (1983) showed that cool temperatures induce reproductive dormancy in post-eclosion monarchs, regardless of photoperiod during the immature states. Most investigations of temperature have focused on the modulating effect of a particular critical temperature on photoperiod cues, with few studies focusing on the primary effect of temperature, in particular thermoperiod, on diapause induction (Beck, 1982; van Houten *et al.*, 1987). Response to a fluctuating temperature regime may be a function of reaching a threshold temperature in cryophase, of the duration of each phase of the cycle, or of the differences between phases (Beck, 1983). While the precise mode of action is uncertain, the results suggest that temperature intensifies the effect of photoperiod on diapause induction and that monarchs respond to some aspect of thermoperiod with amplitude as little as 6 °C and thermophase/cryophase duration of 14 h. Thermophase/cryophase amplitudes typical of late summer in the north central U.S.A. are closer to 10 °C (Watson *et al.*, 1999; Table 2). The duration of thermophase, which decreases as the season progresses, may also be an important cue.

Host plant characteristics. Response to host plant characteristics was mixed. In expt 2, in which potted, greenhouse-grown *A. curassavica* were used, monarchs fed old plants were more likely to be in diapause (Fig. 2b). In expt 3, in which limits imposed by plant rearing necessitated comparing cuttings from greenhouse and wild *A. syriaca*, plant characteristics had no effect. It is possible that monarchs respond differently to *A. curassavica* and *A. syriaca*. This difference may also have resulted from incomplete control of factors affecting plant characteristics. Greenhouse plants were manipulated consistently, whereas controlling for changes in wild milkweed was difficult. All greenhouse plants were kept on the same photoperiod (LD 14:10 h), whereas wild plants experienced natural conditions. Thus, the experimental design would not have detected insect response to plant cues affected by the light:dark regime experienced by the plants. In addition, plant cuttings may not convey accurate age cues. Latex flow depends on a pressure delivery system destroyed in cuttings, and it is possible that latex quality provides a cue to plant age. Results with *A. curassavica* suggest a plant function in diapause induction in monarchs, and the effects of plant age warrant further study.

Several studies (Sims, 1980; Hare, 1983; Koveos & Tzanakakis, 1989; Hunter & McNeil, 1997) have demonstrated differential diapause response in animals reared on

Table 5. Stepwise linear regression of reproductive development in expts 2 and 3.

Predictor	Coefficient	SE	P
Oocyte production in photoperiod and host plant experiment			
Adj. $R^2 = 0.38$, $n = 100$, overall $P < 0.001$ (no effect of host plant or mass)			
Constant	52.2	5.2	< 0.001
Long day length	74.6	9.5	< 0.001
Oocyte production in photoperiod, host plant, and temperature induction experiment			
Adj. $R^2 = 0.525$, $n = 173$, overall $P < 0.0001$			
Constant	20.5	30.4	0.501
Mass	281.2	50.3	< 0.001
Block	-38.1	5.7	< 0.001
Decreasing day length	-42.2	5.9	< 0.001
Fluctuating temperature	-17.0	5.6	0.003
Ejaculatory duct mass in photoperiod, host plant, and temperature induction experiment			
Adj. $R^2 = 0.413$, $n = 212$, overall $P < 0.0001$			
Constant	0.0135	0.0028	< 0.001
Decreasing day length	-0.0053	0.0005	< 0.001
Fluctuating temperature	-0.0027	0.0047	< 0.001
Mass	0.0117	0.0005	0.014

different plant species, but the mechanisms by which plant cues within a species affect diapause are largely uninvestigated. Any cue from the plant must be a consistent response to late season growing conditions (e.g. withdrawal of protein from leaf tissue, changes in phytochemical concentrations, toughening of leaves, presence of flower and seed pod). Rankin (1985) demonstrated delayed reproduction in female *Oncopeltus fasciatus* when fed sub-optimal milkweed (green pods and flowers), suggesting an effect of starvation on diapause induction. Hunter and McNeil (1997) proposed a nutritional mechanism for diapause induction in

Choristoneura rosaceana, suggesting that plant protein levels affect insect development rate in relation to a photoperiod-sensitive stage for diapause induction. In the mite *Petrobia harti*, more females lay diapause eggs when they are fed leaves from flowering plants vs. non-flowering plants under diapause-inducing photoperiods (Koveos & Tzanakakis, 1989).

Response to multiple cues. When the effects of multiple cues were tested, a second cue resulted in an increase in the percentage of animals in diapause. In expt 2, feeding on old plants increased the percentage of females in diapause

Table 6. Analysis of factors affecting development time.

Experiment 2: photoperiod and host plant† ($n = 227$, comparisons of means showed that decreasing day and old host plants resulted in shorter development times)				
Source	d.f.	SS	F	P
Photoperiod	2	10051	35.5	< 0.001
Plant	1	9752	68.9	< 0.001
Photoperiod \times plant	2	836	2.96	0.05
Residual	221	31285		
Experiment 3: photoperiod, host plant, and temperature				
Predictor	Coefficient	SE	Student's <i>t</i>	<i>p</i>
Experiment 3: block 1† , $n = 244$, adj. $R^2 = 0.52$				
Constant	290.35	0.97	298.40	< 0.001
Old host plant	-4.42	1.34	-3.22	< 0.01
Fluctuating temperature	-19.99	1.27	-15.69	< 0.001
Experiment 3: block 2† , $n = 243$, $R^2 = 0.59$				
Constant	284.23	1.30	217.86	< 0.001
Old host plant	9.42	1.35	7.00	< 0.001
Fluctuating temperature	-15.34	1.35	-11.37	< 0.001
Decreasing day length	16.75	1.34	12.44	< 0.001

†ANOVA used to analyse expt 2, and stepwise linear regression used to analyse development time in expt 3 due to unbalanced sample sizes in expt 3 treatments. Indicator variables used for treatments. Expt 3 blocks were analysed separately due to different host plant sources in two blocks (see text).

under decreasing day length treatments, as did a fluctuating temperature regime in expt 3. The lack of a significant interaction between the cues suggests that they act additively but not synergistically. Blanckenhorn (1998) reported similar findings with diapause response in dung flies; shorter photoperiod/cooler temperature combinations resulted in increasing proportions of females in reproductive diapause. Using multiple cues to assess current and near future habitat suitability could be an optimal strategy for organisms in unpredictable environments, in which selection should favour individuals best able to exploit habitat while it is available.

Individual variation in response to environmental cues. There is significant within-population variation in response to diapause-inducing stimuli in monarchs. First, while the percentage of diapause in males and females increases with combinations of cues, diapause occurred in response to a single cue. Second, there was a gradual shift to diapause in monarchs reared under natural conditions; increasing numbers of individuals were in diapause as the season progressed. Finally, none of the experiments resulted in 100% diapause; the highest proportion of individuals in diapause in any treatment was 56%. This variation could be due to genetic or environmental effects; the experiments cannot differentiate between these possibilities but this is a promising avenue for further study.

There was also variation in the degree of reproductive development. Reproductive females in outdoor conditions produced fewer mature oocytes as the season progressed (Table 2), and in laboratory experiments, treatments that contained the highest proportions of diapause females also resulted in reproductive females with fewer mature oocytes (Fig. 3), although this effect was not statistically significant in expt 1. Ejaculatory duct masses were not distributed bimodally (Fig. 1), which is expected if there is a clear distinction between diapause and reproductive males. Instead, their mass declined gradually over time in cohorts reared outdoors, and there was a great deal of overlap among the males in the different treatments in expt 3. It is possible that females with fewer mature oocytes and males with ejaculatory ducts of intermediate mass eclosed in a physiological state that could have developed into either diapause or reproductive maturity, depending on environmental conditions.

Variation in response to environmental cues has been described in other insects. Both reproductive and diapause seasonal forms of the leafwing *Anaea andria* eclose from identical larval photoperiods of 13 h day length (Riley, 1988). In *Papilio zelicaon*, Sims (1980) demonstrated shortened critical photoperiod and decreased frequency of diapause after five generations of selection for non-diapause. Variation in diapause response may be expected particularly along geographical gradients (Sims, 1980; Taylor & Spalding, 1986), with variation typically declining at higher latitudes (Vinogradova, 1986). Seasonal habitat variability from year to year could favour this kind of *bet-hedging*.

In addition to within-population variation in monarch diapause, there appears to be a great deal of between-

population variation, with populations in different locations showing a spectrum of dormancy ranging from no dormancy to complete diapause (Tuskes & Brower, 1978; James, 1982, 1983; Herman, 1985; and references therein). The degree to which these differences are environmentally or genetically determined remains to be discovered.

While results reported here are the first to document effects of environmental cues on diapause induction in eastern North American monarchs, important questions about diapause induction in this species remain. The study did not identify conditions leading to 100% diapause. Constant temperatures in expts 1 and 2 may have had an inhibitory effect because constant temperatures are rare under natural conditions. It is possible that the actual temperature, in addition to the degree of fluctuation, is important, and that lower temperatures, or a greater fluctuation, would have resulted in more individuals in diapause. It is also possible that monarchs respond to cues that are difficult to reproduce in a laboratory. In addition, the metamorphic stage at which diapause is induced in monarchs remains to be determined because monarchs were kept under experimental conditions throughout their development.

Development rates

Development times (in day-degrees) for monarchs reared outdoors were approximately equivalent, with the exception of the first cohort that experienced several days above 30 °C (Table 3). Temperatures above 30 °C may retard growth without being lethal (Zalucki, 1982; Baker *et al.*, 1985; Malcolm *et al.*, 1987; H. York and K. S. Oberhauser, in press), and the method used to calculate day-degrees does not correct for the effects of upper threshold temperatures.

The effects of temperature on monarch development rates are well documented, and the results reported here are similar to those reported elsewhere (Rawlins & Lederhouse, 1981; Zalucki, 1982; Malcolm *et al.*, 1987; Masters, 1993). In addition, monarchs reared under conditions most likely to induce diapause tended to require fewer day-degrees to develop from egg to adult (Table 6). Decreasing and short day length treatments in expt 1, decreasing day length and old host plants in expt 2, and fluctuating temperatures in expt 3 all tended to shorten development time; however the effects of host plants differed in the two blocks of expt 3, and decreasing day lengths in block 2 of expt 3 were associated with longer development time. While the experiments were not designed to test factors that may affect development time, these results warrant further investigation. A similar finding was reported by James (1987), who found that two Australian migratory butterflies, *Vanessa kershawi* and *Junonia villida*, developed faster under short day lengths.

These experiments did not compare growth rates on different host plants explicitly, but development time (in day-degrees) was shorter in expt 3, in which *A. syriaca* was used, than in expts 1 and 2, in which *A. curassavica* was used (Table 1, Fig. 4). These two species vary in cardenolide

content, with *A. curassavica* having much higher concentrations of cardenolides (Malcolm & Brower, 1986); the effects of cardenolide concentration on development rates would also be a productive avenue for future study.

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