Reference: *Biol. Bull.*, **160:** 89–106. (February, 1981)

STUDIES ON THE ADULT REPRODUCTIVE DIAPAUSE OF THE MONARCH BUTTERFLY, *DANAUS PLEXIPPUS*[1]

WILLIAM S. HERMAN

Department of Genetics and Cell Biology, University of Minnesota, St. Paul, MN 55108

Abstract

Monarch butterflies obtained at monthly intervals during the annual adult cycle were held in summer conditions for 5, 10, and 20 days. Examination of these revealed in both sexes an adult reproductive diapause characterized by depressed growth of those reproductive organs sensitive to juvenile hormone. Diapause began for both sexes in late August or early September; female diapause ended in December while male diapause ended in November. Diapause intensity, maximal in September–October, appeared to be greatest in females. In summer conditions, reproductive tract growth in postdiapause females, but not males, was comparable to that observed in prediapause animals. Previously mated postdiapause, but not diapause, females showed a greater response to summer conditions than did virgins. Storing prediapause monarchs in winter conditions for up to 5 months did not induce diapause, indicating that diapause may be induced in a preadult stage. Diapause monarchs of both sexes responded to juvenile hormone with pronounced growth of the pertinent reproductive organs, suggesting that decreased production of juvenile hormone may be a characteristic of adult diapause. The possible advantages of diapause to the survival of the adult population are discussed.

Introduction

Most monarch butterflies (*Danaus plexippus plexippus*) in the northern United States are reproductively active from May until August, but the monarchs usually begin a period of reproductive inactivity before migrating southward during September and October (Urquhart, 1960; Brower, 1977). With apparently minor exceptions (Brower, 1961; Funk, 1968; Urquhart *et al.*, 1968), female reproductive tracts normally remain undeveloped until the following February or March, when the large overwintering colonies disperse and female reproductive tract development accelerates (Williams *et al.*, 1942; Urquhart, 1960). Similarly, monarch males have periods of depressed reproductive tract development during the overwintering period (Herman, 1975a). The existence of reproductive and non-reproductive adult stages in both sexes of this species strongly suggests an adult reproductive diapause during part of the adult cycle. Some direct evidence supports the existence of such a condition in both sexes (Herman, 1973, 1975a). However, previous studies have merely shown that the reproductive tracts of reproductively inactive overwintering monarchs develop in response to summer-like environments. They have not demonstrated a well-defined period of reduced reproductive tract development in favorable environmental conditions, *i.e.*, a reproductive diapause (Wigglesworth,

Received 7 April 1980, accepted 8 November 1980.

Abbreviations: LD25, regimen of 16 h light: 8 h dark at 25 C; SD10, regimen of 8 h light: 16 h dark at 10°C; JH, juvenile hormone; AJH, Ayerst mixture of juvenile hormone; MO, mature oocytes; OV, ovarian; CG, colleterial gland; AG, accessory glands; TG, tubular glands; ED, ejaculatory ducts.

[1] Supported by USPHS grant HD-07336 and funds from the University of Minnesota Graduate School.

1965), sandwiched between reproductively active stages of the adult life cycle. Thus, although a reproductive diapause at a specific stage of adult monarch life seems probable, its existence has not been conclusively demonstrated.

Against the above background, and as part of a continuing study of the neuroendocrinology of this lepidopteran, (Dallmann and Herman, 1978; Dores *et al.*, 1979; Johnson, 1979; Lessman, 1980), I have attempted to demonstrate and partially characterize the adult monarch reproductive diapause. Particular attention was given those organs of the male and female reproductive tracts known to be sensitive to juvenile hormone, *i.e.*, the female ovaries and colleterial glands, and the male accessory glands, tubular glands, and ejaculatory ducts (Pan and Wyatt, 1971; Barker and Herman, 1973; Herman, 1975a, b; Herman and Barker, 1977). These studies show an adult diapause at certain times in both sexes and indicate pronounced sexual differences during diapause. Furthermore, the data provide information dealing with the induction, duration, intensity, and endocrine regulation of this process.

Materials and Methods

The monarchs used from June–August were obtained from larvae reared outdoors on milkweed (*Asclepias syriaca*) and allowed to pupate and emerge as adults in the laboratory. Those examined in September were captured in the Minneapolis, MN, metropolitan area. Butterflies studied from October–March were collected from a single large overwintering colony near Santa Cruz, CA, in at least 3 years. After capture the latter animals were immediately airmailed to MN, usually arriving without fatalities 2–4 days after capture. Most collections from the colony were made in the first week of each month. Major differences in body weight on arrival in laboratory, or in response to various experimental manipulations, were not observed in any single month from June–March. Animals were not examined in April–May, since in those months the butterflies have left the overwintering colonies, but none have emerged in MN. Except where otherwise noted, all monarchs were held individually in glassine envelopes and fed 30% honey daily.

Most investigations were conducted on animals stored in incubators at 25°C with a long day (LD) 16 h photophase provided by 40 W bulbs with average intensities of 435 lumens. Both male and female butterflies were held at this regimen (designated LD25, and considered comparable to summer conditions) for 10 days in most months of a 3-year period, and in at least 1 year for 5 and 20 days in most months. No data was obtained on March females held for 20 days at LD25, since in the year such studies were planned most females left the colony before the collection date. One set of experiments examined the effects of storage at 10°C with a short day 8 h photophase (SD10), considered comparable to winter conditions, while in another monarchs were held outdoors in the ambient September environment. The latter studies were performed over 3 years, principally during the first weeks of September. During that time, mean ambient temperature in the Minneapolis area normally declines from 19°C to 15°C, and the time from sunrise to sunset decreases from 13 hr:13 min to 12 hr:14 min.

Wet body and organ weights were measured to the nearest 0.1 and 0.01 mg, respectively, using a Sartorium Type 24-33 and an Ainsworth 24N balance, respectively. (Dissection and weighing procedures are described in Herman, 1975b.) Means of body or organ weights were rounded to the nearest mg or 0.1 mg, respectively. A direct relationship obtains between wet and dry weights in all pertinent monarch reproductive organs (Herman, 1975b; Johnson, 1979). This relationship

was confirmed during this study. All mature oocytes (*i.e.*, those with ridged chorions) in both ovaries and in the remainder of the reproductive tract were counted in each female examined, and means rounded to the nearest single oocyte. The gross anatomy of male and female monarch reproductive tracts are described elsewhere (Urquhart, 1960; Herman, 1975b); they generally correspond to that found in other lepidopterans.

The Ayerst mixture (AY-22, 342-3) of juvenile hormone (JH) I mixed isomers (*i.e.*, AJH) was used in most studies in which exogenous hormone was applied. This material has high JH activity in both monarch sexes (Herman, 1975a, b; Herman and Barker, 1977; Lessman, 1979a). In some experiments all *trans, trans, cis* JH I, obtained from CalBiochem, was used instead of AJH. Hormone solvent routinely was mineral oil (USP), and some controls for all hormone treatments were injected (100 μl Hamilton microsyringe with a 30-ga needle) only with suitable volumes of that solvent. In most experiments 100 μg AJH in 10 μl mineral oil was injected. Neck ligature has been described (Herman, 1975b); ligated animals were maintained by daily injections of 100 μl sterile 10% glucose.

Student's t test, with statistical significance considered to be the $p = 0.05$ level or better, was used in statistical analysis. Unless otherwise noted, data are presented as mean ± standard error of the mean (SEM) and n indicates the smallest number of individuals examined.

Results

Response of JH-sensitive female organs to the LD25 regimen

Effects of storage at LD25 for 5, 10, and 20 days were evaluated using several hundred newly emerged (June–August) and wild-caught (September–March) female monarchs. Except for the March females, the animals probably contained no mature oocytes (MO) when placed in the regimen (Herman, unpublished), and had ovarian (OV) and colleterial gland (CG) wet weights not significantly different from 2.0 mg and 0.9 mg, respectively, when placed at LD25 (Herman, unpublished). Of 17 females examined on arrival in the laboratory in early March only one contained MO, and mean OV and CG weights were 6.0 mg and 1.3 mg, respectively (Herman, unpublished). Thus in females placed in the LD25 regimen, the responses reported were not normally influenced by significant variations in MO or in OV or CG weights before incubation. All the June–August females were virgins; some captured in September–March had mated.

Figure 1 summarizes the production of MO at LD25 from June–March. For each storage duration the general pattern of change was comparable, *i.e.*, pronounced MO production in June–August, conspicuous depression of such activity from September–November, a rise in December to levels below those of June–August, a return to June–August values by January, and the highest mean responses in February–March. In addition, during June–August and January–March, 97% of females examined at all three storage durations contained MO, while in September–December only 50% of the females dissected had produced MO. The lowest MO production was noted in September–October, when only 23% of the females examined contained MO and no MO were found in animals incubated for 5 days. The only apparently aberrant point in Figure 1 was that obtained at 20 days in November. From the 5- and 10-day data for that month (and the trend for other months) it appears that the November 20-day value was unreasonably low, *i.e.*, not significantly higher than the 10-day value.

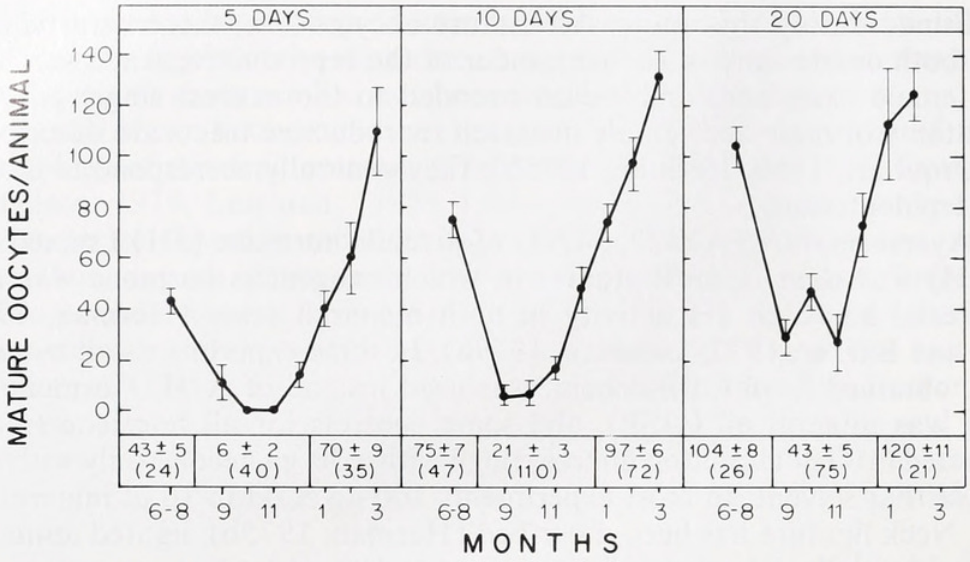

FIGURE 1. Mean mature oocyte production by monarch butterflies held at LD25 for 5–20 days. Combined mean values for June–August, September–December, and January–March, with n in parentheses, are at the bottom of the figure; months indicated by number; vertical bars indicate SEM.

Similar variations at all three storage durations occurred for both OV and CG wet weights (see Table I); *i.e.*, both organs were large in June–August, significantly smaller in September–December, and increased significantly in weight in January–March.

MO production and OV and CG development at LD25 seemed to be correlated with mating status during January–March but not September–December. As shown in Table II, organ weights in mated and virgin females did not differ significantly after storage duration in September–December. Similarly, although MO production was apparently twice as high in mated females at 10 days in September–December, that relationship was not evident at 5 or 20 days. By contrast, in mated females

TABLE I

Female monarch ovarian and colleterial gland development at LD25. Data expressed as mean wet weight in mg ± SEM; n, in parentheses, are the same for both organs; ovary (OV) values are weights of single ovaries; CG = colleterial glands; days indicate duration of storage at LD25.

	Months of arrival in laboratory		
	June–August	September–December	January–March
CG Weight			
5 days	4.9 ± 0.4 (24)	1.6 ± 0.2 (40)	5.0 ± 0.4 (35)
10 days	7.4 ± 0.4 (47)	2.7 ± 0.2 (110)	7.1 ± 0.3 (72)
20 days	8.8 ± 0.7 (26)	4.2 ± 0.3 (75)	7.8 ± 0.7 (21)
OV Weight			
5 days	28.9 ± 2.0	7.2 ± 1.7	34.8 ± 3.4
10 days	38.7 ± 2.5	14.2 ± 1.5	46.8 ± 2.2
20 days	48.5 ± 2.9	25.2 ± 2.0	52.8 ± 3.8

TABLE II

Ovarian and colleterial gland development in virgin and mated monarchs held at LD25. Data presented as mean ± SEM; single ovary (OV) and collaterial gland (CG) data in mg, mature oocyte (MO) data in total number/female; n in parentheses is same for MO, OV, and CG.

	Months of arrival in laboratory			
	September–December		January–March	
	Mated	Virgin	Mated	Virgin
OV Weight				
5 days	4.4 ± 1.0 (10)	8.1 ± 1.6 (30)	34.0 ± 4.0 (11)	21.3 ± 3.8 (12)
10 days	15.4 ± 3.3 (34)	13.8 ± 1.6 (76)	53.1 ± 2.7 (38)	37.9 ± 2.8 (27)
20 days	26.5 ± 3.7 (23)	24.4 ± 2.3 (52)	61.3 ± 5.0 (6)	49.3 ± 4.6 (13)
MO Production				
5 days	0	7 ± 3	89 ± 11	34 ± 10
10 days	32 ± 8	16 ± 3	119 ± 7	69 ± 7
20 days	46 ± 9	42 ± 6	153 ± 14	107 ± 13
CG Weight				
5 days	1.4 ± 0.2	1.7 ± 0.2	4.7 ± 0.5	3.1 ± 0.3
10 days	3.3 ± 0.4	2.5 ± 0.2	7.6 ± 0.4	6.2 ± 0.5
20 days	4.1 ± 0.7	4.3 ± 0.4	8.4 ± 1.4	7.6 ± 0.8

held at LD25 during January–March, OV and CG weights, and MO production, were always significantly higher after 5 or 10 days, and MO production was also clearly elevated after 20 days. However, mated female OV and CG weights at 20 days in January–March were not significantly higher than those of virgins. This may be due to the absence of data on March females held for 20 days. March females (usually all mated; Herman, unpublished), would probably have very high OV and CG weights and contain numerous MO after 20 days at LD25 (Figure 1). Therefore mating was correlated with enhanced JH-sensitive organ development at LD25 in January–March animals, but not in females examined during September–December. In addition, virgin MO production and OV weight in females from CA during January–March were not significantly different from those of June–August MN females, which were virgins (see Figure 1). CG weights from January–March virgins were, however, less than those of June–August at each storage duration.

From the above, it appears that adult reproductive diapause, defined as a period of significant reduction of the response of the JH-sensitive reproductive organs to LD25, lasts from September–December in the female monarch.

The response of JH-sensitive male organs to LD25

Experiments conducted simultaneously with those reported above for females, examined the responses of over 400 males stored at LD25 for 5, 10, and 20 days. Again, both newly emerged (June–August) and wild-caught (September–March) animals were used. The pattern of development of the male accessory gland (AG), tubular gland (TG), and ejaculatory ducts (ED) were all similar.

Figure 2 presents the summarized data obtained for the ED. ED weights were

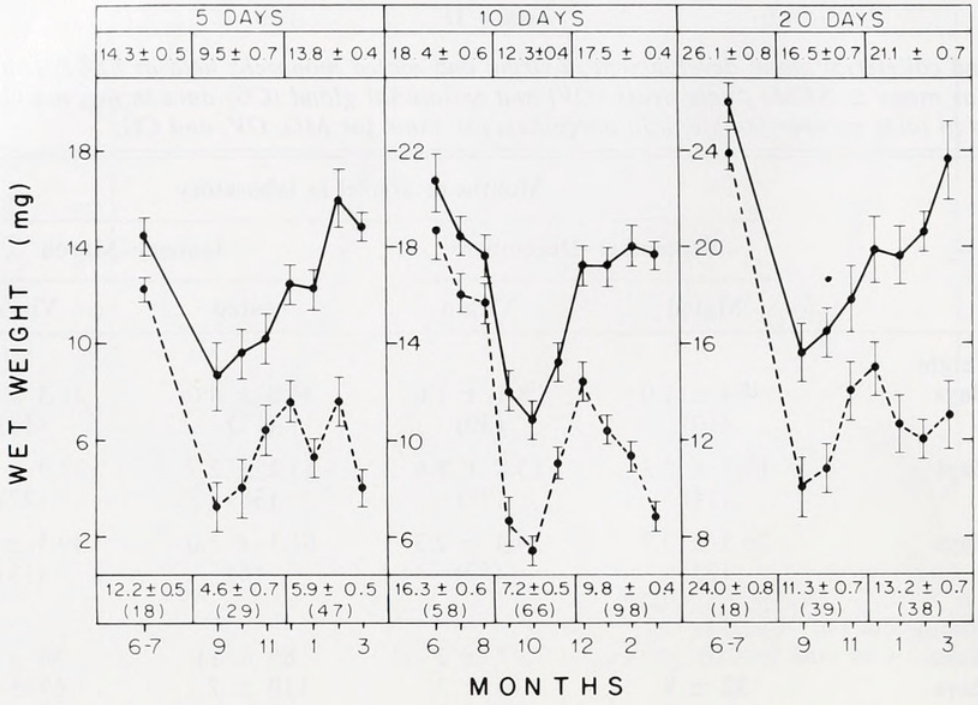

FIGURE 2. Solid and dashed lines, respectively, indicate mean gross and net changes in ejaculatory-duct wet weights in monarch butterflies held at LD25 for 5–20 days. Combined mean values for June–August, September–November, and December–March are at the top (gross changes) and bottom (net changes) of the figure; n in parentheses are the same for both gross and net responses; months indicated by number; vertical bars indicate SEM.

high in June–August. These glands' 5-day values, and those for 20-days, were nearly identical in June–July, and are combined in Figure 2. August data at 5 and 20 days were not obtained. The August mean at 10 days was significantly lower than that of June, but not July, and that difference is indicated in Figure 2. ED development declined at all three storage durations, to an apparent nadir in September–October, and was followed by a slight increase in November. The December ED mean at 10 days was not significantly different from that in July–August, and the December ED means for all three storage durations were significantly higher than those determined from the combined September–November data. ED development was erratic after December: The 5-day response was significantly elevated in February–March, the 10-day response remained unchanged, and the 20-day response increased significantly only in March.

Consideration of the response of the three male glands to LD25 was complicated by changes in the initial weights of all three glands during the annual adult cycle. Thus, at eclosion the mean wet weights of the AG, TG, and ED were, respectively, 0.9 mg, 1.6 mg, and 2.0 mg, and all June–August data were obtained from males placed at LD25 on the day of eclosion. By contrast, mean AG, TG, and ED wet weights upon butterflies' arrival in the laboratory in November were 1.3 mg, 2.0 mg, and 3.8 mg, respectively, while in March the corresponding mean values were 3.6 mg, 6.5 mg, and 10.7 mg. Therefore, the mean *net* response was examined (*i.e.*, weights after LD25 storage minus mean weight on arrival in the laboratory) for all three glands in each month. The calculations yielded qualitatively comparable response patterns for all three male glands. They are illustrated in Figure 2 by the dashed-line curves obtained for the ED net response.

Net ED responses were highest in June–August, fell to low levels in September–

November, and seemed to rise to a post-November peak in December. Net ED means in January–March, with the single exception of the 5-day February values, were consistently lower than those seen in December; *i.e.*, net response apparently declined after December. In addition, although the December–March net weight means at all storage durations were higher than those of September–November, they were only significantly higher in males stored at LD25 for 10 days. It therefore appears that while gross ED response in December–March approached that found in June–August, net ED response did not increase substantially after September–November and never reached a level comparable to that observed in June–August.

The gross and net responses of the AG and TG are summarized in Table III. The general pattern of mean gross and net development for both glands was the same as that illustrated more fully for the ED in Figure 2. However, the AG were the only male glands in which the gross and net increases in December–March were all significantly higher in September–November, and in which the 5- and 10-day gross values in December–March were significantly higher than those observed in June–August.

The above data indicate that an adult reproductive diapause occurs from September–November in male Monarch butterflies.

Comparison of the male and female responses to LD25

To compare the responses to LD25 of the JH-sensitive male and female organs during diapause and postdiapause periods, mean values for MO production and

TABLE III

Effect of LD25 environment on development of male monarch accessory and tubular glands. Data presented as mean mg wet weight ± SEM; n, in parentheses, is same for both glands; days refer to time held at LD25; net = weight after LD25 storage (i.e., gross weight) minus mean weight of same glands upon arrival in the laboratory.

	Months of arrival in laboratory		
	June–August	September–November	December–March
Accessory glands			
5 days—gross	4.4 ± 0.2	3.3 ± 0.2	5.2 ± 0.2
net	3.5 ± 0.2	1.8 ± 0.3	2.6 ± 0.2
	(18)	(29)	(47)
10 days—gross	6.3 ± 0.2	4.7 ± 0.2	7.2 ± 0.2
net	5.4 ± 0.2	3.2 ± 0.2	4.6 ± 0.2
	(59)	(66)	(98)
20 days—gross	10.9 ± 0.5	7.3 ± 0.3	9.2 ± 0.3
net	10.0 ± 0.5	5.7 ± 0.3	6.6 ± 0.3
	(20)	(37)	(38)
Tubular glands			
5 days—gross	12.0 ± 0.5	5.5 ± 0.4	9.0 ± 0.4
net	10.4 ± 0.5	2.8 ± 0.5	4.2 ± 0.3
10 days—gross	12.7 ± 0.6	7.5 ± 0.3	10.9 ± 0.3
net	11.0 ± 0.4	4.7 ± 0.3	6.2 ± 0.3
20 days—gross	17.2 ± 0.6	10.2 ± 0.4	13.3 ± 0.5
net	15.6 ± 0.6	7.4 ± 0.4	8.5 ± 0.5

male and female organ wet weights obtained at 5, 10, and 20 days were converted to percentages of the pertinent mean values at the same storage durations in June–July. Means of these individual percentages were used to estimate the changes in the responses of the pertinent organs (Table IV).

The diapause (September–December) female response was collectively about one-third of that in prediapause (June–August), while the diapause (September–November) male response was about two-thirds of that seen in prediapause. In addition, the postdiapause (January–March) female response was collectively 83% higher than that observed in diapause, but the postdiapause (December–March) male response was only 23% higher than that seen in diapause. The above differences would be even more pronounced if net, rather than gross, male response had been used for comparison. Similarly, 20-day March female data would presumably accentuate these sexual differences. Thus, development of the JH-sensitive female organs is more depressed during diapause, and more enhanced during postdiapause, than is that of the JH-sensitive male organs.

When individual male and female organs were considered, it appeared that MO production was the single event most profoundly influenced during diapause and postdiapause; it exhibited the lowest percentage response to each storage duration during diapause and the highest percentage response at each storage duration during postdiapause. The means obtained for MO percent response, however, were not significantly different from those obtained for the OV and CG responses during either diapause or postdiapause. In males, the AG and ED had statistically equivalent responses in both periods, and the responses of these two organs were significantly different from those of the TG. Postdiapause AG and ED development, based upon gross response percentages, was not significantly different from that of the female CG, but this apparent similarity was eliminated by consideration of net responses.

Figure 3 shows that after 5 days at LD25, MO production in diapause females was only about 14% and 9% of that found in pre- and postdiapause females, respectively. By contrast, in females held at LD25 from 5 to 20 days, mean MO production increased 720% (6–43 MO) in diapause monarchs, but only 240% and

TABLE IV

Comparison of responses of JH-sensitive male and female monarch reproductive organs during diapause and postdiapause. Data expressed as mean ± SEM; raw data used to determine percentages presented in Figs. 1 and 2 and Tables 1 and 3; numbers in parentheses refer to number of percentages available for determining each mean value; abbreviations presented in earlier figures and tables.

	Percent of June–July mean response	
	Diapause	Postdiapause
Female		
MO production (3)	28 ± 7	136 ± 7
OV weight (3)	38 ± 6	116 ± 4
CG weight (3)	39 ± 4	96 ± 3
All above (9)	35 ± 4	118 ± 7
Male		
AG weight (3)	70 ± 2	102 ± 8
TG weight (3)	53 ± 3	77 ± 1
ED weight (3)	66 ± 1	90 ± 4
All above (9)	63 ± 3	86 ± 5

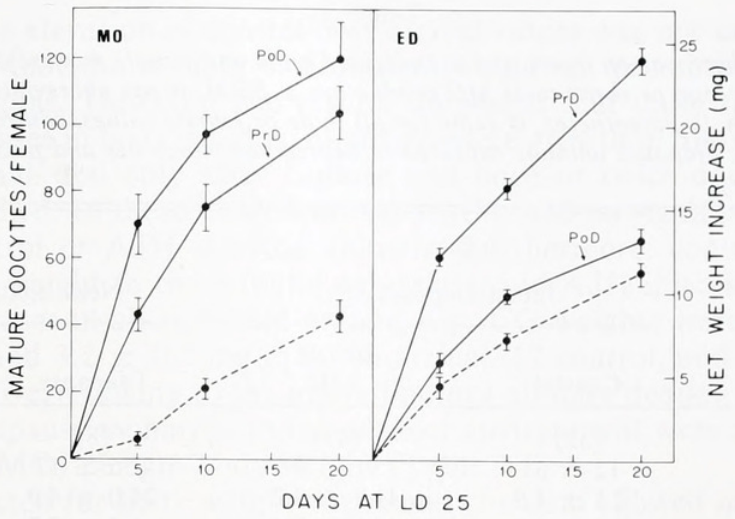

FIGURE 3. Mean mature oocyte production (MO) and ejaculatory duct (ED) net wet weight changes in monarch butterflies held at LD25 for various intervals. Dashed line indicates diapause animals, and solid lines indicate prediapause (PrD) and postdiapause (PoD); vertical bars indicate SEM. Numbers of animals tested same as in Figures 1 and 2.

170% in pre- and postdiapause animals, respectively. Comparable, but quantitatively less striking changes occurred in the male ED (Figure 3). After 5 days at LD25 the mean net wet weight increase of these organs in diapause males was 37% and 78% that observed in pre- and postdiapause males, respectively. By contrast, the mean net wet weight increase of the ED in diapause males held at LD25 for 5 to 20 days was 246% (4.6–11.3 mg), while the comparable values for pre- and postdiapause males were 107% and 224%, respectively. (Males differed from females in that weight changes in the postdiapause ED more closely resembled those of diapause animals.) Comparable plots of the OV and CG data of Table I, and the AG and TG data of Table III, produced similar results. Thus, monarch reproductive diapause was characterized by a severe depression of JH-sensitive organ development during the first 5 days at LD25. Storage of monarchs at LD25 from 5 to 20 days, however, results in a developmental rate of the pertinent organs during diapause that apparently exceeds that observed in prediapause and postdiapause.

Effect of juvenile hormone on diapause and postdiapause monarchs

Diapause males and females were fed immediately after arrival in the laboratory, injected with 100 μg AJH or used as controls, held at LD25 for 10 days, and then dissected. Comparable experiments were performed in each of 3 diapause months (i.e., September–November) in each of 2 years. The results of individual experiments, and the combined data (see Table V,a), show that diapause monarchs of both sexes responded to AJH.

Less extensive experiments examined the response of neck-ligated diapause (October) and postdiapause (February) monarchs to JH injections. Animals were fed, neck-ligated, and then injected with either mineral oil or 10 μg JH I, immediately after arrival in the laboratory. They were then held at LD25 for 6 days. The results (see Table V,b) indicated significantly greater postdiapause responses in all JH-sensitive organs in both sexes.

The above figures reflect net changes for females, since there were no major differences in the OV and CG weights obtained from diapause and postdiapause female controls and no female control produced any MO. However, the gross data

TABLE V

Effects of juvenile hormone on intact and neck-ligated male and female monarchs. Data presented as mean wet weight (mg) or mean total MO production ± SEM; organ abbreviations as in previous tables and figures; n, in parentheses, is same for all male or female values; controls uninjected and mineral oil injected; diapause animals captured in September–November and postdiapause animals captured in February.

	(a) Intact diapausers		(b) Neck-ligated + 10 μg JH I	
	Control	100 μg AJH	Diapause	Postdiapause
Female	(32)	(45)	(8)	(9)
MO production	12 ± 3	91 ± 9	56 ± 17	143 ± 15
OV weight	12.5 ± 1.9	41.1 ± 3.2	24.0 ± 4.9	49.7 ± 4.4
CG weight	2.3 ± 0.3	5.6 ± 0.4	5.3 ± 0.7	7.5 ± 0.8
Male	(31)	(34)	(5)	(9)
AG weight	5.7 ± 0.2	7.3 ± 0.4	3.6 ± 0.5	5.3 ± 0.3
TG weight	8.8 ± 0.4	11.2 ± 0.5	6.2 ± 0.7	8.8 ± 0.6
ED weight	14.1 ± 0.5	18.1 ± 0.7	11.2 ± 1.3	14.8 ± 0.8

are misleading with regard to males. As mentioned earlier, postdiapause male glands on arrival in the laboratory were significantly heavier than those of diapause males, and the postdiapause male controls in this experiment were significantly larger than those of diapause controls. Thus control diapause males (n = 17) had weights of 1.3 ± 0.1 mg, 2.4 ± 0.3 mg, and 4.0 ± 0.4 mg for the AG, TG, and ED, respectively, while the corresponding values for eight postdiapause male controls were 3.1 ± 0.3 mg, 7.0 ± 1.0 mg, and 10.7 ± 1.2 mg, respectively. The mean net response to JH I showed the AG, TG, and ED were 2.3 ± 0.5 mg, 3.8 ± 0.7 mg, and 7.1 ± 1.3 mg, respectively, in diapause males, but only 2.2 ± 0.3 mg, 1.8 ± 0.6 mg, and 4.1 ± 0.8 mg, respectively, in postdiapause males. Therefore, it appears that the gross response of the postdiapause male glands to JH I is significantly higher than that of diapause males, but the net response is either comparable (AG) or significantly less (TG and ED).

Effect of natural environment on diapause monarchs

Other experiments examined the response of wild-caught September monarchs to storage in natural (*i.e.*, outdoors) conditions. In these studies, monarchs captured during early September were either dissected immediately or held outdoors on a porch adjacent to the laboratory for 5–7 days. Some animals of each sex were uninjected, while others were injected with hormone solvent or 100 μg AJH.

In initial experiments all control and AJH injected animals were fed both after capture and daily during the storage period. All of these animals exhibited significant increases in body weight; *e.g.*, 26 males on arrival in the laboratory weighed 465 ± 16 mg, while 35 control males (fed daily) weighed 689 ± 18 mg after 5–7 days outdoors. In addition, even though AJH injections significantly elevated all pertinent organ weights of both sexes over control values, all JH-sensitive organs of both male and female controls were significantly heavier than those of animals dissected immediately after capture; *e.g.*, ED values were 5.3 ± 1.0 mg, 8.2 ± 0.5 mg, and 11.7 ± 0.4 mg for 26 on arrival, 35 control, and 24 AJH-injected males,

respectively. The elevation of control over arrival values was not anticipated, since it was expected that the outdoor environment would not promote development of JH-sensitive organs. However, the results suggested that daily feeding, and the associated increases in body weights, might promote growth of JH-sensitive organs. Therefore, animals fed only after capture and once or twice during the storage period were studied. In these, there were no deaths, and no significant body weight changes in control or AJH injected animals. Furthermore, control organs were identical in wet weight to those found on arrival, and AJH injections again led to significant increases in all pertinent organs; *e.g.*, CG weights were 0.8 ± 0.1 mg, 0.8 ± 0.1 mg, and 3.2 ± 0.5 mg in 32 on-arrival, 17 control, and 7 AJH-injected females, respectively. Owing to the above findings all data dealing with the effects of storage of diapause monarchs in the outdoor environment were converted to mg wet weight (or MO) per gm body weight (Table VI).

When corrected for body weight variations, the data showed no change in any JH-sensitive male or female organ of control animals during 5–7 days storage in natural conditions. (By contrast, both male and female organs developed markedly when newly emerged July monarchs were held outdoors for similar periods, *e.g.*, male ED weighed 23.5 ± 1.3 mg and female OV weight was 33.5 ± 4.2 mg.) Both monarch sexes exhibited significant weight increases in all JH-sensitive organs after AJH injections. MO were found in only one of 33 females on arrival, and only two of 39 control females, while 23 of 33 females treated with AJH contained MO. Thus, diapause male and female monarchs held in their natural environment responded to AJH, but did not exhibit significant development of JH-sensitive organs in the absence of exogenous hormone.

Alterations in organs lacking sensitivity to juvenile hormone

JH regulation of the male testes and seminal vesicle-vas deferans complex, or the female bursa copulatrix and receptacle gland has not been demonstrated (Herman, 1975a, b; Johnson, 1979). Observations on these four organs during the investigations described above revealed that patterns of development were not comparable to those noted for JH-sensitive male and female reproductive organs. None

TABLE VI

Effects of outdoors environment, with or without juvenile hormone injections, on wild-caught diapause monarchs in September. Data presented as mean mg wet weight (or MO)/gm body weight ± SEM; abbreviations as in previous tables and figures; n in parentheses; experiments conducted in September; all animals held in outdoors September environment for 5–7 days; controls either uninjected or injected with mineral oil.

	AG	TG	ED
Males			
On arrival (26)	4.3 ± 0.8	7.4 ± 1.5	12.3 ± 2.4
Controls (50)	4.4 ± 0.3	7.3 ± 0.7	12.5 ± 1.1
100 μg AJH (31)	6.2 ± 0.4	11.6 ± 0.8	18.1 ± 1.1
	MO	OV	CG
Females			
On arrival (33)	5 ± 5	8.8 ± 4.0	2.2 ± 0.5
Controls (39)	2 ± 2	6.8 ± 1.4	2.0 ± 0.2
100 μg AJH (33)	42 ± 9	31.3 ± 3.7	6.6 ± 0.6

of these four organs exhibited clearly depressed responses during diapause coupled with significantly elevated weights during postdiapause when intact animals were held for 5, 10 (see Table VII), or 20 days at LD25. In addition, when diapause monarchs held outdoors were injected with AJH, only the receptacle gland showed significant enlargements. In similarly treated diapause animals held at LD25, only the testes apparently responded to AJH (Table VII). Since these four organs showed no selective depression of response to LD25 during diapause, and no consistent responses to AJH injections, it appears that they are all controlled by regulatory mechanisms different from those influencing the JH-sensitive male and female reproductive organs.

Effects of SD10 storage on June–July animals

Effects of SD10 storage on monarch reproductive tract development at SD10 and to LD25 after various periods of SD10 storage were studied. Monarchs emerging in the laboratory during June–July were placed at SD10 on the day of eclosion and held there for up to 5 months, with weekly feeding. Several animals of each sex were removed from SD10 at 30-day intervals and either dissected immediately or fed and placed at LD25 for 10 days, with daily feeding, prior to dissection.

The male reproductive glands enlarged continuously during the SD10 storage period. Thus, eight males held for 30 days at SD10 possessed AG, TG, and ED with wet weights of, respectively, 1.2 ± 0.1 mg, 2.6 ± 0.4 mg, and 5.2 ± 0.8 mg, while four males held for 150 days had AG, TG, and ED weighing 2.8 ± 0.4 mg, 6.9 ± 1.5 mg, and 18.0 ± 4.5 mg, respectively. The 150-day value for the ED was not significantly different from that of June–July animals held for 10 days at LD25 immediately after eclosion. The data from all males held at SD10 for 1–5 months is summarized in Table VIII. Slight but significant enlargements were noted at 30 days for the female OV and CG, but further changes were not observed in females stored at SD10 for 60–150 days, and no female held at SD10 produced any MO.

TABLE VII

Effects of various treatments on monarch reproductive organs lacking sensitivity to juvenile hormone. Data presented as mean mg wet weight ± SEM; LD25 animals held for 10 days; SV-VD, BC, and RG are seminal vesicles-vas deferans, bursa copulatrix, and receptacle gland, respectively; reproductives from June–August, diapause-LD25 animals from September–November (males), or September–December (females); diapause-outdoors from September; and postdiapause animals from December–March (males) or January–March (females); about half controls given 10 µl mineral oil.

	Males			Females		
	Testes	n	SV-VD	BC	n	RG
LD25						
Reproductive	12.2 ± 0.3	59	12.0 ± 0.3	13.6 ± 0.5	47	2.1 ± 0.1
Diapause	8.7 ± 0.2	79	14.4 ± 0.4	13.2 ± 0.7	112	1.9 ± 0.0
Postdiapause	8.9 ± 0.2	98	14.6 ± 0.2	15.9 ± 2.2	72	1.5 ± 0.0
Diapause-outdoors						
Controls	10.4 ± 0.3	35	10.6 ± 0.4	11.5 ± 0.5	43	2.6 ± 0.1
100 µg AJH	11.0 ± 0.4	24	10.8 ± 0.6	11.3 ± 0.5	26	3.0 ± 0.1
Diapause-LD25						
Controls	9.0 ± 0.2	31	14.4 ± 0.5	11.9 ± 0.3	31	1.9 ± 0.1
100 µg AJH	9.8 ± 0.3	33	14.3 ± 0.6	12.6 ± 0.4	27	1.6 ± 0.1

TABLE VIII

Effect of SD10 storage on development of monarch reproductive organs and their subsequent response to LD25. Data presented as mean mg wet weight (or MO) ± SEM; SD10 only = held at SD10 for 30–150 days; LD25 only = held at LD25 for 10 days after eclosion; SD10 + LD25 = SD10 for 30–150 days + 10 days at LD25; see text for other abbreviations; n in parentheses.

	AG	TG	ED
Males			
At eclosion (35)	0.9 ± 0.0	1.6 ± 0.1	2.1 ± 0.1
SD10 only (37)	2.0 ± 0.1	5.2 ± 0.4	12.2 ± 1.0
LD25 only (28)	7.0 ± 0.3	13.8 ± 0.5	19.2 ± 0.7
SD10 + LD25 (33)	7.1 ± 0.2	13.4 ± 0.9	21.8 ± 1.0
	MO	OV	CG
Females			
At eclosion (16)	0	1.9 ± 0.1	0.8 ± 0.0
SD10 only (34)	0	3.3 ± 0.2	1.1 ± 0.1
LD25 only (29)	72 ± 9	38.3 ± 3.3	7.4 ± 0.5
SD10 + LD25 (36)	93 ± 9	41.2 ± 6.6	7.8 ± 0.4

These studies also showed that storage at SD10 for any duration was without apparent effect on subsequent response to LD25. There was no indication of a diapause-like response in any male or female monarch held at SD10 for any length of time, and all but one female placed at LD25 after SD10 storage produced MO. Thus, the mean values from combined results (see Table VIII) from all males and females held at LD25 after storage at SD10 for 30–150 days did not differ significantly from results from June–July monarchs placed at LD25 on the day of emergence.

DISCUSSION

The above experiments demonstrate an adult reproductive diapause, defined as a period of significant reduction in the response of certain reproductive organs to summer-like (LD25) conditions, in both male and female monarch butterflies. This period of reduced response lasts from September–November in males, and from September–December in females. It can be easily demonstrated, in all male and female organs known to be sensitive to JH, by holding monarchs collected in the wild at monthly intervals at LD25 for 5, 10, or 20 days. As in many other insects (Tauber and Tauber, 1976), the adult monarch reproductive diapause is characterized by: (1) beginning when environmental conditions might still permit some successful reproduction (*i.e.*, late August to early September in MN); (2) a period of diminishing diapause intensity (*i.e.*, after October in the overwintering colonies); and (3) ending before the return of environmental conditions that promote full reproductive activity in both sexes (*i.e.*, in November or December in the overwintering colonies).

The above experiments seem to document a monarch butterfly adult diapause and its duration. However, information is lacking on its induction, maintenance, and termination, and the relative roles of photophase and temperature. The available data indicate that storing newly emerged animals in constant, suboptimal environments does not induce diapause in adult monarchs. In this report, holding new adults at SD10 for up to 5 months did not result in a diapause-like response

to LD25. Similarly, earlier studies (Barker and Herman, 1976) have shown that monarch females emerging in June–July, and held on short photophases and relatively low temperatures, still produce more MO than are found in diapause females on arrival in the laboratory. Therefore, although adult monarchs are sensitive to both photoperiod and temperature, adult reproductive diapause may be programmed in some earlier developmental stage.

The data of Tables V and VI, and earlier studies (Pan and Wyatt, 1971; Barker and Herman, 1973, 1976; Herman, 1973, 1975a, b; Herman and Barker, 1977), strongly suggest that effective JH hemolymph titers are depressed during monarch reproductive diapause. Thus, animals of both sexes held at LD25 exhibit low levels of JH-sensitive organ development, but such development is enhanced by injections of AJH. Similarly, JH-sensitive organs did not grow in diapause animals held in the natural environment in September, but such growth is induced in both males and females by JH treatment. Furthermore, hemolymph JH titers in wild-caught monarchs of both sexes are lower in diapause than in the reproductively active months of June–July (Lessman, 1980). Therefore, it is likely that effective JH hemolymph titers during monarch reproductive diapause are significantly less than those during periods of reproductive activity. Whether this reduction of effective JH titer is due to decreased JH biosynthesis, enhanced JH inactivation, both processes, or some other factor(s) remains to be determined. Reduction of JH-sensitive male and female organ response to JH I during diapause (see Table V,b), suggests enhanced JH inactivation, or decreased target tissue sensitivity, during the diapause.

Comparison of results from male and female monarchs demonstrates differences in diapause and postdiapause development of JH-sensitive organs. The prediapause response level was first reached in December in males but not until January in females held at LD25. Similarly, the response of female JH-sensitive organs to LD25 storage during diapause was only 35 ± 4% of that observed in June-July while the comparable percentage for the male diapause response was 63 ± 3%. Therefore, female diapause is both longer and more intense than that of males. Postdiapause sexual differences were also observed. After arrival in the laboratory, female JH-sensitive organs remained substantially unaltered for most of the postdiapause period (Herman, unpublished), and the response of these organs to LD25 storage typically met or exceeded the prediapause values. By contrast, JH-sensitive male organs exhibited progressive enlargements (similar to those noted at SD10) on arrival in the laboratory. Furthermore, the gross responses of the pertinent male organs to LD25 storage rarely equalled those of prediapause animals, and the net responses were typically not significantly higher than those observed during diapause. Apparently, postdiapause female (but not male) development in the natural environment is repressed, and the postdiapause female (but not the male) retains the ability to respond to LD25 in a manner comparable to that of the prediapause animal.

Studies on neck-ligated diapause monarchs show that males are more sensitive to JH I, JH II, and AJH than are females, *e.g.*, the average effective dose (ED_{50}) for AJH effects on the pertinent organs was 49 μg in males and 253 μg in females (Lessman, 1980). Similarly, posteclosion female monarchs routinely reach a peak hemolymph JH titer of about 10^6 *Galleria* units/ml before the onset of rapid oogenesis, while male values during rapid reproductive gland growth average about 10^4 *Galleria* units/ml (Lessman, 1980). Unpublished experiments in my laboratory show an absence of female tract development, but significant male reproductive gland growth, in allatectomized animals held at LD25 for several weeks. Appar-

ently, male reproductive glands can exhibit substantial growth when hemolymph JH is low or absent, and they can also be stimulated by JH levels ineffective in females. Thus the shorter and less intense male diapause, and the development of the male tract in normal environmental conditions during postdiapause, may be due to either male (but not female) sensitivity to low hemolymph JH titers, or to male (but not female) tract development in the absence of JH.

Mating stimulates both oogenesis and oviposition in reproductively active summer monarchs, and the available evidence suggests that enhanced oocyte production in mated females is associated with elevated JH titers (Herman and Barker, 1977). A comparable situation does not exist in monarch females captured in the wild from September–February, since about 50% of the females examined during that period were mated but practically none contained mature oocytes on arrival (Herman, unpublished). Similarly, prior mating did not stimulate the female reproductive tract in monarch females held at LD25 during diapause. By contrast, mated postdiapause females had significantly larger OV and CG, and produced more MO, than did virgins after LD25 storage. Apparently, mating elevates effective JH titers (as measured by the growth of JH-sensitive female organs) in both reproductive and postdiapause, but not diapause, females held at LD25. It would be interesting to know more about the mechanism(s) by which mating influences effective JH titers, and the alterations in this mechanism during overwintering.

Adult diapause might facilitate survival of the overwintering monarch population. Existing data indicate that monarch diapause only occurs in adults emerging in late August or September; there is no reason to believe that individual adults that have previously been reproductively active can subsequently become diapause monarchs (Barker and Herman, 1976; Herman, unpublished). When the diapause generation begins to emerge there are still many flowering plants blooming, and thus many sources of nectar. Field observations document that feeding is a major activity of premigratory diapause adults. Adult diapause also begins in the Minneapolis area about 1 month before the first frost. Field observations show that milkweed plants are rapidly deteriorating during this period. Since rearing data (Urquhart, 1960; Herman, unpublished) indicate that oviposition to adult emergence usually takes 3–4 weeks outdoors in June–August, it appears that the cessation of reproduction associated with diapause prevents a wasteful expenditure of reserves that would probably yield few if any viable adults. Conserving protein, especially, might be important to monarchs, since their reproduction entails substantial loss of fat body protein (Herman, unpublished). Since mating is rare in the premigratory population (Herman, unpublished), bursa copulatrix weights are low in the absence of spermatophores and male reserves are not depleted by production and export of spermatophores. Similarly, the possibility of mating-induced oogenesis and oviposition, like that observed in summer reproductives (Herman and Barker, 1977), is reduced. Thus, diapause initiation apparently results in a young monarch population with high levels of nutrient reserves, unencumbered by well-developed reproductive tracts, and hence highly suited for the long migratory flights characteristic of the species (Brower, 1977).

A "diapause break" during migration is unlikely, since migratory activity (Urquhart, 1960; Brower, 1977) and diapause both reach peaks in September–October. Furthermore, migratory flight may speed up JH degradation (Lessman, 1979) and effectively decrease hemolymph JH levels. Thus, both reproductive diapause intensity and flight-enhanced JH hydrolysis would inhibit futile reproductive activity and the resultant loss of stored reserves. In addition, the lipid accumulated

during the premigratory period of reproductive diapause (Brown and Chippendale, 1974) and presumably mobilized by the adipokinetic hormone for flight fuel (Dallmann and Herman, 1978), could be replenished indirectly by nectar feeding during migration. Thus, most monarchs probably would arrive at the overwintering colonies in excellent condition to withstand the coming winter. The data available supports this expectation (Tuskes and Brower, 1977).

After the overwintering colonies form, female reproductive diapause would last into December, when suboptimal environments could ensure suppression of female reproductive activity (Barker and Herman, 1976). Similarly, females' longer diapause might enhance their ability to withstand the rigors of winter, since diapause is frequently characterized by reduced metabolic activity (Tauber and Tauber, 1976). The earlier end of diapause in males, and the ensuing increase in male gland development and mating behavior in environments that completely suppress female tract development (Herman, unpublished), could transfer nutrients (especially protein?) from males to females, as it appears to do in reproductively active summer monarchs (Boggs and Gilbert, 1979). If so, such a mechanism could partly explain the ability of postdiapause females, even though they are already 5–6 months old, to produce oocytes at a rate at or above that of summer reproductives. Transfer of nutrients and sperm simultaneously also could help males retain their genes in the monarch population. This line of reasoning implies a decline in male, but not female, condition during the postdiapause period. Evidence for such a decline comes from the developmental pattern of male glands at LD25 during the postdiapause period. In addition, a decline in male vigor during overwintering is indicated by data on aging showing that at LD25 postdiapause males do not live as long as postdiapause females (Herman, unpublished). Thus, the available evidence, although inconclusive, implies that postdiapause male mating activity in the overwintering colony may significantly enhance female's survival and fecundity. It further suggests that many males may normally be sacrificed to insure survival of the monarch population. The result of all diapause and postdiapause activities in both sexes would be a population of previously mated, but nutrient poor (or dead), males and a population of relatively well-nourished, and mated, females with no substantial decrease in life expectancy. Such a population would seem to be admirably suited to re-establish the reproductive generations. It might also account for the fact that most females, but far fewer males, emigrate from the overwintering colonies (Williams *et al.*, 1942; Herman, unpublished). Since many of the above speculations can be tested experimentally, it will be of interest to see if future research confirms or refutes some or all of these ideas.

These studies have dealt with monarchs captured or reared in MN from June–September, and with monarchs captured in CA from October–March. However, monarchs from the eastern United States typically overwinter in Mexico, while CA monarchs rarely migrate into the eastern portion of the country (Urquhart, 1960; Urquhart and Urquhart, 1978; Brower, 1977). This population separation suggests the possibility of physiological differences between the two groups of butterflies. Data indicating geographical differences in diapause of other insect species has been presented (Tauber and Tauber, 1976). However, to my knowledge no data substantiates such differences in monarchs. The findings of Tables I and II indicate no obvious differences in eastern September and western October monarchs. Similarly, the fact that western postdiapause virgins produce oocytes at rates comparable to those found in eastern prediapause virgins argues for comparable activities in the two populations. Thus, the above discussion has assumed that

overwintering monarchs in Mexico and CA, and reproductively active monarchs in both the eastern and western United States, are in similar physiological states. It will be of interest to see if future studies on western reproductively active, and overwintering Mexican, monarchs support this assumption.

ACKNOWLEDGMENTS

I would like to express my sincere gratitude to those who assisted in the capture, rearing, and feeding of the monarchs used in these studies. In this regard, special thanks are due to Mr. Paul Cherubini, Mr. and Mrs. David Preus, and Alex, Max, Carter, and especially Charlotte Herman. I also deeply appreciate the efforts of those residents of Minnesota, particularly Mrs. Dorothy Jackson, who located migratory swarms of butterflies and were kind enough to guide me to them. Dr. Louise Rollins-Smith conducted some of the dissections done in the early stages of this work, and her highly competent assistance was invaluable.

LITERATURE CITED

BARKER, J. F., AND W. S. HERMAN. 1973. On the neuroendocrine control of ovarian development in the Monarch butterfly. *J. Exp. Zool.* **183:** 1–10.

BARKER, J. F., AND W. S. HERMAN. 1976. Effect of photoperiod and temperature on reproduction of the Monarch butterfly, *Danaus plexippus*. *J. Insect Physiol.* **22:** 1565–1568.

BOGGS, C. L., AND L. E. GILBERT. 1979. Male contribution to egg production in butterflies: Evidence for transfer of nutrients at mating. *Science* **206:** 83–84.

BROWER, L. P. 1961. Studies on the migration of the Monarch butterfly. I. breeding populations of *Danaus plexippus* and *D. gilippus berenice* in south central Florida. *Ecology* **42:** 76–83.

BROWER, L. P. 1977. Monarch migration. Natural History **86:** 40–53.

BROWN, J. J., AND G. M. CHIPPENDALE. 1974. Migration of the Monarch butterfly, *Danaus plexippus*: energy sources. *J. Insect Physiol.* **20:** 1117–1130.

DALLMANN, S. H., AND W. S. HERMAN. 1978. Hormonal regulation of hemolymph lipid concentrations in the Monarch butterfly. *Gen. Comp. Endocrinol.* **36:** 142–150.

DORES, R. M., S. H. DALLMANN, AND W. S. HERMAN. 1979. The regulation of post-eclosion and post-feeding diuresis in the Monarch butterfly, *Danaus plexippus*. *J. Insect Physiol.* **25:** 895–901.

FUNK, R. S. 1968. Overwintering of Monarch butterflies as a breeding colony in southwestern Arizona. *J. Lepid. Soc.* **22:** 63–64.

HERMAN, W. S. 1973. The endocrine basis of reproductive inactivity in Monarch butterflies overwintering in central California. *J. Insect Physiol.* **19:** 1883–1887.

HERMAN, W. S. 1975a. Juvenile hormone stimulation of tubular and accessory glands in male Monarch butterflies. *Comp. Biochem. Physiol.* **51A:** 507–510.

HERMAN, W. S. 1975b. Endocrine regulation of posteclosion enlargement of the male and female reproductive glands in Monarch butterflies. *Gen. Comp. Endocrinol.* **26:** 534–540.

HERMAN, W. S., AND J. F. BARKER. 1977. Effect of mating on Monarch butterfly oogenesis. *Experientia* **33:** 688–689.

HILL, H. F., A. M. WENNER, AND P. H. WELLS. 1976. Reproductive behavior in an overwintering aggregation of Monarch butterflies. *Am. Midl. Nat.* **95:** 10–19.

JOHNSON, C. G. D. 1979. *The accessory reproductive glands of the male Monarch butterfly, Danaus plexippus: their development, fine structure, and biochemistry*. Ph.D. Thesis, University of Minnesota, St. Paul, MN. 162 pp.

LESSMAN, C. 1979. Enhancement of juvenile hormone degradation by flight and heat in adult Monarchs. *Am. Zool.* **19:** 915.

LESSMAN, C. 1980. Biology of juvenile hormone in the Monarch butterfly, *Danaus plexippus*. Ph.D. Thesis, University of Minnesota, St. Paul, MN. 156 pp.

PAN, M. L., AND WYATT, G. R. 1971. Juvenile hormone induces vitellogenin synthesis in the Monarch butterfly. *Science* **174:** 503–505.

TAUBER, M. J., AND C. A. TAUBER. 1976. Insect seasonality: diapause maintenance, termination, and postdiapause development. *Ann. Rev. Entomol.* **21:** 81–107.

TUSKES, P. M., AND L. P. BROWER. 1978. Overwintering ecology of the Monarch butterfly, *Danaus plexippus*, in California. *Ecol. Entomol.* **3:** 141–154.

URQUHART, F. A. 1960. *The Monarch Butterfly*. University of Toronto Press, Toronto, Canada. 361 pp.

URQUHART, F. A., and N. R. URQUHART. 1978. Autumnal migration routes of the eastern population of the Monarch butterfly (*Danaus p. plexippus* L.; Danaidae; Lepidoptera) in North America to the overwintering site in the neovolcanic plateau of Mexico. *Can. J. Zool.* **56:** 1759–1764.

URQUHART, F. A., N. R. URQUHART, AND F. MUNGER. 1968. A study of a continuously breeding population of *Danaus plexippus* in southern California compared to a migratory population and its significance in the study of insect movement. *J. Res. Lepid.* **7:** 169–181.

WIGGLESWORTH, V. B. 1965. *The Principles of Insect Physiology*. Methuen and Co., Ltd. London.

WILLIAMS, C. B., G. F. COCKBILL, M. E. GIBBS, AND J. A. DOWNES. 1942. Studies on the migration of Lepidoptera. *Trans. Entomol. Soc. Lond.*, **92:** 101–283.