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REPRODUCTIVE TRACT DEVELOPMENT IN MONARCH BUTTERFLIES OVERWINTERING IN CALIFORNIA AND MEXICO

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ABSTRACT. Reproductive organs of male and female monarch butterflies captured in December, February, and March in overwintering colonies in both Mexico and California were examined as soon as possible after capture. In addition, response of such organs to incubation in summer-like conditions was determined for animals from both locations in all three months. Results demonstrated numerous similarities between the two populations, indicating comparable stages of reproductive tract development in the two locations. However, a higher percentage of mating was recorded in Californian females, and data obtained after incubation indicated that diapause might last longer in both sexes of Mexican monarchs.

Additional key words: Nymphalidae, *Danaus plexippus*, diapause, overwintering.

North American monarch butterflies (*Danaus plexippus* L.) aggregate in two major overwintering locations. One is California, where several colonies form each winter (Lane 1984). The other is in the mountains of the states of Michoacan and Mexico in Mexico, where multiple overwintering colonies have now been located (Calvert & Brower 1986). The Mexican colonies are principally aggregations of monarchs that emerge in the eastern United States and Canada, while the Californian colonies are the major overwintering sites for monarchs originating west of the Rocky Mountains. Although it might be reasonably assumed that overwintering monarchs from both locations were in similar reproductive states, direct comparative evidence concerning the reproductive status of such animals is not available. To obtain such evidence, we weighed reproductive organs in both sexes collected in December, February and March from colonies in both locations. Additionally, we compared such weights to those found at eclosion, and to those found when animals from both locations were exposed to summer-like environments. Our data demonstrate striking similarities, and some differences, between the two populations.

MATERIALS AND METHODS

Butterflies came from three localities. Those providing data on eclosion values and prediapause (Herman 1981) response to summer-like conditions were obtained from larvae reared outdoors on *Asclepias syriaca* L. in June and July in Minnesota. Larvae were collected as first

instars immediately after hatching from eggs laid by wild-caught females. Eclosion data came from adults dissected on the day of emergence. Prediapause response to summer-like conditions was measured by holding newly emerged adults, fed daily with 30% honey, in incubators at 25°C on a 16-h photophase for 10 days before dissection. Californian monarchs were airmailed to Minnesota from the Natural Bridges colony near Santa Cruz. These insects were either dissected immediately upon arrival or incubated as above before dissection. Animals from California were examined in three separate years (1977–79), and all results were pooled. Diapause values (Herman 1981) were obtained by holding animals captured during the principal portion of the diapause period (September–November) in summer-like conditions for 10 days before dissection. Diapause data were also obtained over a 3-yr period from animals captured in both Minnesota (September) and California (October–November), and the results were pooled. Mexican animals were collected during 1983–84 at the Chincua and Herrada colonies in Michoacan and Mexico, respectively, and airmailed (in three separate shipments) to Minnesota as soon as possible (within eight days) after capture. These adults were either dissected immediately or incubated as above before dissection.

Anatomy of the reproductive tracts of both monarch sexes, and terminology applied to the tracts, is discussed elsewhere (Urquhart 1960, Herman 1975). Reproductive organs were dissected, cleaned of fat body, blotted to remove excess saline, and weighed to the nearest 0.01 mg. Mature oocytes, defined as oocytes with chorionic ridges, were counted in both ovaries in all females. Mated females were those with sperm in the spermatheca, which in monarchs is that portion of the receptable gland proximal to the common oviduct. Rear-wing maximal length was measured to the nearest 0.5 mm with a ruler. Data were analyzed with Student's *t*-test. In this report "significant" refers to statistical significance at the $P \leq 0.05$ level. All data are presented as mean ± SE. Some of the reproductive tract weight data obtained at eclosion, in prediapause, and in diapause have been reported earlier (Herman 1981, 1985, Herman et al. 1981) but this report includes new data from additional animals.

RESULTS

Wing lengths. Females had rear wing lengths of 37.7 ± 0.1 mm (n = 145) and 37.8 ± 0.1 mm (n = 201) in the Minnesotan and Mexican populations, while males had significantly larger wings (38.1 ± 0.1, n = 145 and 38.2 ± 0.1, n = 188) in the two populations, respectively. Monarch wings examined at eclosion were not significantly different from those of the Minnesotan and Mexican populations. Animals ob-

tained from California in October–November had significantly smaller wings than those from Mexico or Minnesota: 37.5 ± 0.1 mm (n = 161) and 37.9 ± 0.1 mm (n = 141) for females and males, respectively. In addition, Californian adults of both sexes exhibited significant declines of wing length in February–March, to 37.0 ± 0.2 mm (n = 114) and 37.6 ± 0.1 mm (n = 201) for females and males, respectively. These latter values were the lowest recorded during this study.

Reproductive organ weights. Mexican and Californian females showed no significant weight differences in the ovaries (OV) and colleterial glands (CG) on arrival in either December or February, but both organs were slightly and significantly heavier in Californian females in March (Fig. 1). The OV and CG weights of Mexican animals on arrival never exceeded the eclosion values, but those of the Californian adults in March were significantly heavier than at emergence. Mature oocytes (MO) were never observed on arrival in females from either location.

Response of the OV and CG to summer-like conditions was qualitatively similar, but quantitatively different, in the two groups of females (Fig. 1). Incubated Californian females in all three months had final organ weights significantly above the diapause value, generally comparable to or above those in prediapause females, and larger than those of incubated Mexican females. Mexican females exhibited OV and CG weights after incubation that were close to the diapause values in December, but well above those values in February and March. Mating was more often observed in Californian animals, with 29%, 37%, and 96% mated in December, February and March, respectively, while Mexican females exhibited only 17%, 8%, and 15% mating, respectively, in the same months.

Accessory glands (AG), tubular glands (TG), and ejaculatory ducts (ED) were typically heavier than at eclosion in males examined on arrival (Fig. 2). There were no significant differences in gland weights on arrival in December or March between the Californian and Mexican males, but all three glands of Californian males were significantly heavier in February. After incubation, all three glands of Californian males exhibited responses significantly above those of diapause males, and similar to prediapause animals, in all three months (Fig. 2). Mexican male glands showed lesser responses only in December and February, and the December response of TG and ED approached the diapause value. All three male glands exhibited the same level of development in both groups of incubated males in March.

On arrival, testes (TE) in both populations were always smaller than at eclosion, while seminal vesicles–vas deferens (SV) complexes were always larger (Fig. 3). In addition, Californian males had comparable TE, but significantly smaller SV, in all three months. Incubated Cali-

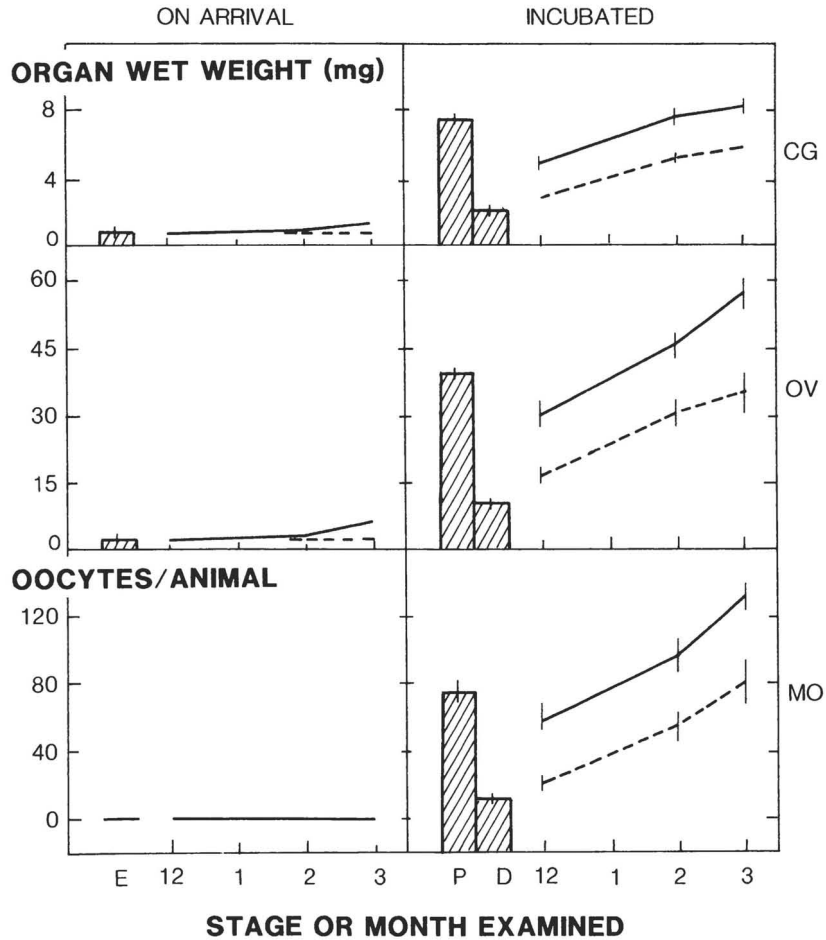

FIG. 1. Wet weights of colleterial glands (CG) and ovaries (OV), and total number of mature oocytes (MO) in females collected in Californian (solid lines) and Mexican (dashed lines) overwintering colonies. On-arrival data obtained from at least 17 animals/data point, and incubated data obtained from at least 18 animals/data point. E = organ weights at eclosion (n = 26), P = organ weights from prediapause animals (n = 47), and D = organ weights from diapause animals (n = 85); 12 = December, 1 = January, 2 = February, and 3 = March. Vertical lines indicate SE; negligible values are omitted.

fornian males consistently had both TE and SV near the diapause level, while Mexican animals exhibited slightly smaller TE and significantly larger SV (Fig. 3).

Receptacle glands (RG) and bursae copulatrix (BC) showed little variation from eclosion level in Mexican females on arrival, as did RG and BC of December females from California (Fig. 3). Both organs

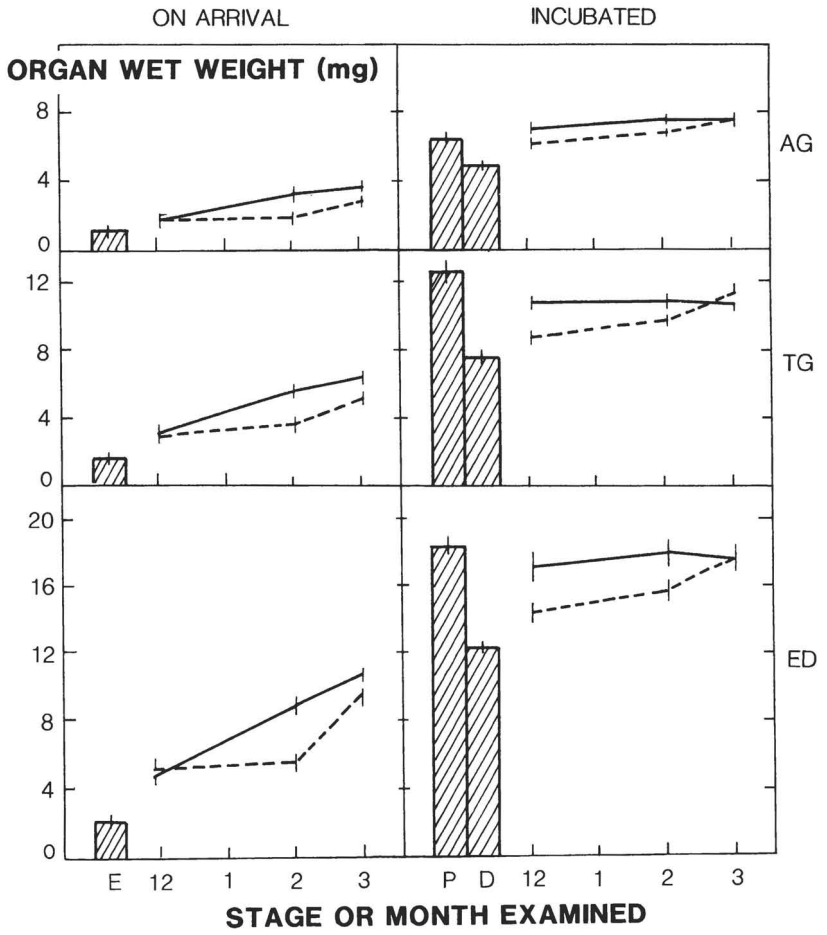

FIG. 2. Wet weights of accessory glands (AG), tubular glands (TG), and ejaculatory ducts (ED) from monarch males collected in Californian and Mexican colonies. On-arrival data obtained by dissection of at least 27 animals/data point, and incubated data obtained from at least 24 animals/data point. Data presentation and other abbreviations as in Fig. 1. Values of n for E, P, and D were 35, 59, and 79, respectively.

exhibited weights significantly above eclosion values in Californian animals examined on arrival in both February and March. Incubation reduced RG size to similar values in both groups of females (Fig. 3), and caused little BC weight change.

DISCUSSION

Our data show major similarities and some differences in the reproductive tracts of monarch butterflies from the Mexican and Californian

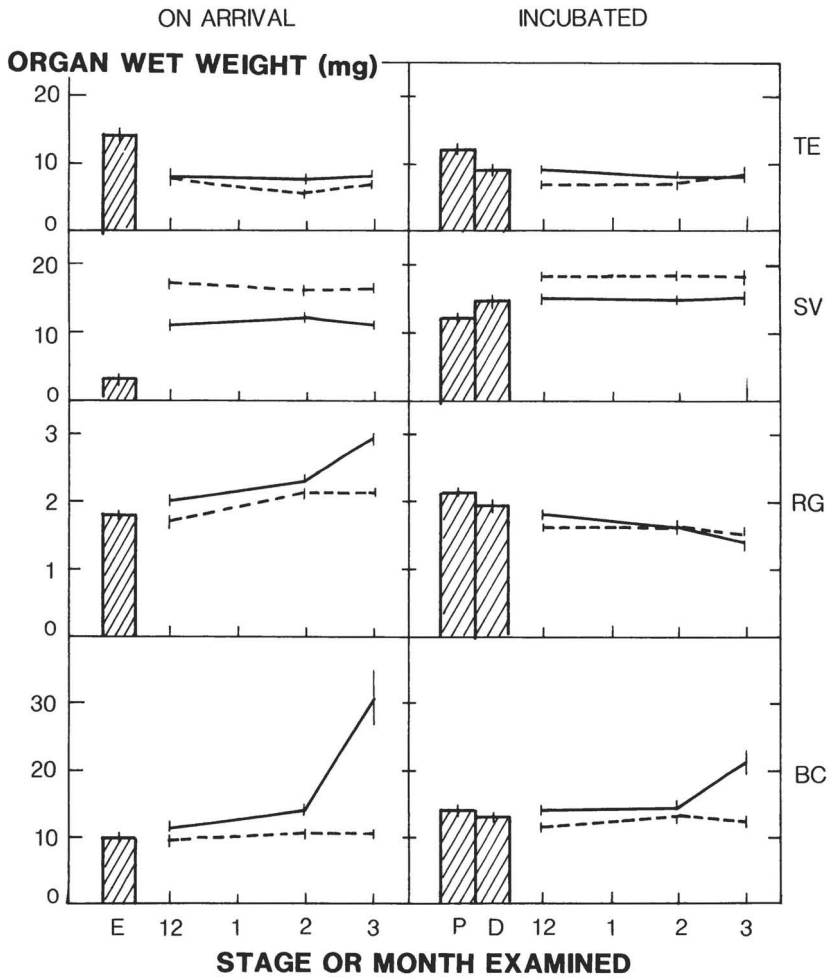

FIG. 3. Wet weights of testes (TE) and seminal vesicles–vas deferens complexes (SV) from males, and of receptacle glands (RG) and bursae copulatrix (BC) from females, collected in Californian and Mexican colonies. Other abbreviations, data presentation, and n values in Figs. 1 and 2.

overwintering colonies. Females from both populations possess OV and CG that are nearly identical in December and February, and only slightly different in March. In addition, these two organs are indistinguishable in December and February, in both groups of monarchs, from those of insects emerging in summer in Minnesota. Female RG and BC are also indistinguishable in December and similar in February in the two populations, but both are larger in Californian females in March.

We believe the differences noted in weights of the female reproductive organs of the two groups in March may be due to a higher percentage of mating in the Californian animals. This conclusion is supported by reports that mating increases juvenile hormone levels in female monarchs (Herman & Barker 1977), and that juvenile hormone stimulates the development of all four organs in this species (Herman 1985). Moreover, the considerable difference in BC weights appears to be due principally to the greater number of Californian females carrying spermatophores in their BC.

Female OV and CG also exhibit qualitatively similar responses to incubation in summer-like conditions, that is, both groups of animals exhibit their lowest response in December and their highest in March. Again, we believe the best explanation for the quantitatively greater response of these organs in Californian females is the higher percentage of mated females in the Californian colonies. Response of female RG to incubation mimics that of posteclosion animals in both groups, that is, the glands diminish in weight after exposure to summer-like conditions for 10 days (Herman et al. 1981). Response of BC in both groups is comparable to that of both prediapause and diapause monarchs: the weights of these organs after incubation are usually slightly above those recorded at eclosion. The relatively high weight of BC in incubated Californian females we again attribute to a higher proportion of mated females.

For most of the overwintering period, the tracts of both groups of females resemble those of females at eclosion in Minnesota. Both groups of monarchs also show remarkable similarity in the response of the female reproductive tract to incubation. Our data do suggest, however, that diapause may last longer in Mexican females than in Californian, since a response of the OV and CG to incubation similar to that of diapause animals is found only in Mexican females in December.

Males from the two colonial sites also exhibit strong similarities and some differences. Both groups of animals have AG, TG and ED of similar size and significantly above eclosion values in both December and March. However, the increased weight noted in Californian males in February was delayed until March in Mexican males. On arrival, both groups also possessed TE that were comparable and smaller than those of newly emerged males. The SV of Californian animals were significantly smaller on arrival, perhaps resulting from the additional mating occurring in the Californian colonies.

The response of male organs to incubation was also similar in both groups. AG, TG and ED exhibited pronounced responses that were comparable in February and indistinguishable in March. Mexican males,

however, exhibited a reduced response of those three organs in December, suggesting that male diapause might also last somewhat longer in the Mexican colonies. No striking changes were observed in the TE or SV from either group after incubation. Thus, male monarchs from the two colonial locations exhibited only minor differences in the weight of their reproductive organs on arrival, and in the response of those organs to incubation. The organs were, with the notable exception of the SV, frequently indistinguishable on arrival and they normally exhibited similar responses to summer-like conditions.

The above differences in Californian and Mexican monarchs do not appear to be due to size differences of monarchs in the two populations. As indicated by our data on wing length, Mexican monarchs were somewhat larger than Californian, but exhibited similar or smaller reproductive organs on arrival.

We conclude that overwintering monarchs of both sexes in both California and Mexico maintain similar and low levels of reproductive tract development through most of the overwintering period, and that monarchs from both populations become more responsive to summer-like conditions as the overwintering period progresses. The data suggest that the postdiapause response of the reproductive tract to summer-like conditions may be delayed in both sexes in Mexico, that is, reproductive diapause may last longer in monarchs in the Mexican colonies. The observed quantitative differences in the condition of the tracts, and of their responses to incubation, may be due principally to the greater percentage of mated females observed in Californian animals. Why such a difference in mating exists in the two locations remains to be determined.

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