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PROLONGED DIAPAUSE AND PUPAL SURVIVAL OF *PAPILIO ZELICAON* LUCAS (LEPIDOPTERA: PAPILIONIDAE)

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ABSTRACT. Pupae of *Papilio zelicaon* are capable of at least a two-year diapause. Univoltine populations utilizing ephemeral native host plants had a greater incidence of prolonged diapause than multivoltine populations using the introduced *Foeniculum vulgare*. A laboratory strain, derived from multivoltine individuals and selected for decreased diapause, exhibited a corresponding loss of prolonged diapause. Mortality was high among pupae overwintering twice and complete in three-year-old individuals. Pupal water and dry material (fat body, etc.) weight loss was gradual over a 541 day period and paralleled survivorship. Prolonged diapause is regarded as an adaptive response to environmental uncertainty, especially that related to the phenology of host plants associated with univoltine and multivoltine populations.

Univoltine (one brood/year) populations of the swallowtail butterfly, *Papilio zelicaon* Lucas, typically occur in rocky or exposed areas at mid to high elevations in the California Coast Range and Sierra Nevada. These populations feed on native species of Umbelliferae (especially *Angelica*, *Lomatium*, *Pteryxia*, and *Tauschia* spp.) that are suitable for larval development approximately 2 to 4 months each year (Emmel & Shields, 1978). Multivoltine (several broods/year) populations occur in California coastal, Sacramento, and San Joaquin Valley areas, where they feed primarily on the introduced weed *Foeniculum vulgare* Mill. (Shapiro, 1974a, b) but occasionally attack *Citrus* spp. (Coolidge, 1910; Horton, 1922; Shapiro & Masuda, 1980). The temporal suitability of *Foeniculum* and *Citrus* as larval hosts varies among individual plants and location but, based on the annual availability of new foliage and succulent leaves, host suitability ranges from 8 to 12 months (Emmel & Shields, op. cit.).

P. zelicaon undergoes a photoperiod-induced pupal diapause, the incidence of which is modified by temperature and host plant species (Sims, 1980). The intensity of pupal diapause (=duration under specified environmental conditions) varies between individuals and among populations; individuals from a lab strain have reduced response to diapause-inducing conditions (Sims, in press). Some southern California populations (Oliver, 1969) appear to have reduced chilling requirements for diapause termination compared to central California populations. Some pupae remain in a diapause of at least two years'

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duration, either under artificial indoor conditions (Hynes, 1949) or outdoors exposed to naturally varying temperature and photoperiod (Emmel & Shields, op. cit.; Emmel, pers. comm.). The purpose of this study was to document the occurrence of prolonged diapause in several univoltine and multivoltine *P. zelicaon* populations and to obtain estimates of overwintering survival in normal and prolonged diapause pupae. Observations were made on progressive pupal weight loss as one possible mortality agent of diapausing pupae.

METHODS

Ova were obtained from females field-collected in univoltine (Butts Canyon, Napa Co., CA, el. 500 m, and Donner Pass, Placer Co., CA, el. 2100 m) and multivoltine (Suisun Marsh, Solano Co., CA, el. <10 m and Rancho Cordova, Sacramento Co., CA, el. <30 m) populations. Techniques used to establish and maintain colonies were similar to those of Sims (1980). Larvae were reared on *Foeniculum* under a 14 h light/10 h dark (LD 14:10) photoperiod at 23.5°C. Diapause pupae reared under similar conditions were also obtained from the F_3–F_5 generations of a laboratory stock which originated from the two multivoltine populations. Within 90 days of pupation, diapause pupae (in 1 pt. cardboard containers) were placed in a sheltered outdoor cage exposed to normal fluctuations of temperature, humidity, and photoperiod at Davis, Yolo Co., CA, el. 10 m, during the years 1975–1977. Emergence and mortality data were recorded daily during spring and summer and weekly during the remainder of the year.

Desiccation tolerance of diapausing pupae was studied by recording survivorship and wet weights of 24 pupae from Donner Pass over a 541 day period. Pupae were maintained at LD 15:9, 26.5°C with a relative humidity of 35 ± 5%. The laboratory conditions used subjected the pupae to much more desiccation stress than they are likely to experience in their normal habitat. Mortality in pupae was indicated by greatly accelerated water loss. The presence of unidentified disease in many dead pupae was suggested by abdominal swelling and exposure of the intersegmental membrane revealing discolored internal body fluids. Water and dry weight loss over the 541 days was estimated by weighing samples of pupae at the beginning and end of this period. Weighed living pupae were slit open, dried at 90°C for 48 h in a vacuum oven, and reweighed.

RESULTS

Progeny of four females from Rancho Cordova (combined n = 69) and three females from Suisun Marsh (n = 64) were compared to prog-

TABLE 1. Numbers of *P. zelicaon* pupae diapausing a second winter inside a field cage in Davis, CA.

Lab strain			Multivoltine			Univoltine		
Population/ female	# diapause/ total alive	% diapause	Population/ female	# diapause/ total alive	% diapause	Population/ female	# diapause/ total alive	% diapause
F_3/1	0/8	0.0	Rancho Cordova/1	11/17	68.4	Donner/1	4/4	100.0
F_3/2	0/8	0.0	Rancho Cordova/2	4/16	25.0	Donner/2	8/12	66.7
F_3/3	0/12	0.0	Rancho Cordova/3	1/28	3.6	Donner/3	1/3	33.3
F_3/4	0/8	0.0	Rancho Cordova/4	0/8	0.0	Butts Canyon/1	6/13	46.1
F_4/1	0/11	0.0	Suisun/1	5/9	55.6			
F_4/2	0/5	0.0	Suisun/2	4/17	23.5			
			Suisun/3	0/38	0.0			
Total[a]	0/52	0.0	Total	25/133	18.8	Total	19/32	59.4

[a] Univoltine total is significantly greater than laboratory or multivoltine (Duncan's Multiple Range Test, $P < 0.05$).

eny of three Donner Pass and one Butts Canyon female (n = 32). Six females from the multivoltine-derived laboratory colony provided 52 pupae for comparison with the wild type populations. Results are given in Table 1. The proportion of living non-emerged pupae of those overwintering for the first year was arc-sine transformed using the tables provided by Mosteller and Youtz (1961). Transformed data were analyzed using ANOVA procedures (Sokal & Rohlf, 1969) and Duncan's Multiple Range Test (DMR). Differences among the populations within the uni- and multivoltine areas and between the F_3 and F_4 generations of the laboratory strain were not significant; therefore, a comparison was made between the combined laboratory strain, multivoltine, and univoltine populations. The difference between these groups was significant ($F_{2,14} = 10.26$, $P < 0.002$). No pupae from the laboratory strain overwintered a second time; whereas, 19% of the multivoltine and 59% of the univoltine individuals did so. The difference between the latter two groups is significant ($P < 0.05$, DMR).

Emergence and survivorship of diapausing pupae older than one year was examined in 97 laboratory strain (generations F_3–F_5) individuals that had been previously chilled from November 1974 to March 1975 for 126 days at 2°, 5°, 11°, or 16°C at LD 0:24. Of 97 pupae maintained in an outdoor cage over the 1975–1976 and 1976–1977 winters, 24 ♂♂ and 27 ♀♀ developed the first spring. Fifteen of these adults were either dead inside the pupa or emerged very weak and/

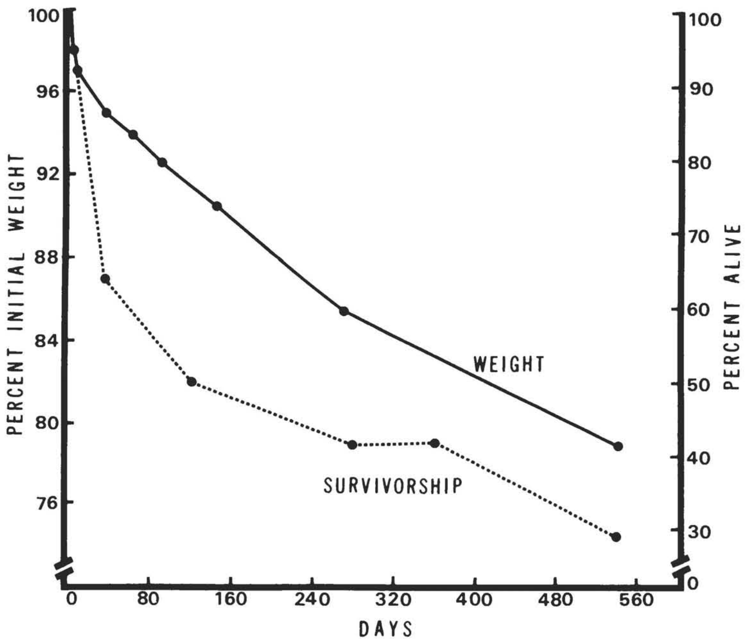

FIG. 1. Weight loss (percent of initial weight remaining) and survivorship of 24 *Papilio zelicaon* pupae under LD 15:9, 26.5°C conditions.

or crippled. Thus, only 37.1% (36/97) of the pupae produced adults that appeared to have adequate vigor for normal reproductive activity. The 44 unemerged pupae (45.6%) died without showing signs of development, while 2/97 (2.1%) remained alive in diapause. These latter two pupae plus 13 surviving two-year-old uni- and multivoltine pupae died while overwintering a third time. There was no difference between percent emergence or mortality of individuals previously chilled at the different temperatures. Prolonged diapause in the populations of *P. zelicaon* studied, therefore, appears to be normally of two years' maximum duration. Mortality is high in second-year pupae and complete in three-year-old individuals.

Figure 1 illustrates weight loss and survivorship of 24 *P. zelicaon* pupae over a 541 day period. A steep decline in weight, corresponding to water loss during hardening of the pupal integument, occurred during the first week of pupation. Subsequent weight loss was gradual and nearly linear over time. The survivorship curve in Figure 1 roughly parallels the progressive loss of pupal weight. The highest mortality rate (50%) occurred during the first 120 days; the remaining pupae had a high probability of survival (83%) to day 360. Although weight

TABLE 2. Depletion of water and dry weight of *P. zelicaon* pupae over a 541 day period at LD 15:9, 26.5°C conditions and RH = 35 ± 5%.

Pupal age (days)[a]	Total water (g)	Total dry weight (g)	Water/dry weight	Percent dry weight loss	Percent water loss	Total percent weight loss
1 (10)	0.814	0.238	3.419	—	—	—
541 (6)	0.593	0.160	3.700	32.68	27.14	28.39

[a] Number in parentheses indicates numbers of pupae sacrificed.

loss and survivorship are probably not related in a simple way, it is possible that mortality of some pupae, especially those older than one year, occurred due to water or energy reserve depletion. The six surviving pupae at day 541 were 84% ($\bar{x}$ day 1 = 0.955 g; $\bar{x}$ day 541 = 0.802 g) of their own day 1 weight.

Table 2 provides an analysis of water and dry weight loss over 541 days. Rates of weight loss of both water (27.1%) and dry material (fat body, etc.) (32.7%) are similar, although the actual weights of water (0.221 g) and dry matter (0.078 g) lost differ greatly. Similar initial and final ratios of water to dry weight (3.419 to 3.700) suggest homeostatic maintenance of water/fat body quantities by the pupae.

DISCUSSION

The data presented here provides evidence for both individual female brood variation and population differentiation in the incidence of prolonged pupal diapause in *P. zelicaon*. Prolonged diapause was most frequent in univoltine populations and least frequent in a laboratory strain founded from multivoltine individuals.

The laboratory strain of *P. zelicaon* was selected for continuous development at LD 14:10, 23°C; thus, pupae diapausing under these "long-day" conditions were eliminated from the culture. By the F_3 generation this procedure had significantly decreased the incidence of diapause (Sims, in press) as well as eliminated any manifestation of prolonged diapause (Table 1). This dual response to selection suggests that the regulation of both diapause initiation and diapause intensity is under genetic control.

Comparison in Davis of diapause intensity in *P. zelicaon* from low elevation (up to 500 m) coast range, coastal, and central valley populations seems justified by the similarity of winter temperature monthly means (8–11°C) in these areas. Higher elevation (750–2100 m) Sierra Nevada populations experience much lower winter temperature means, often spending several months under snow cover with temperatures at or near 0°C. Despite this, pupae from populations at all elevations examined exhibit a maximum diapause termination response (under constant temperatures) at 11°C (Sims, 1980). Thus, overwintering of higher elevation pupae under lower elevation valley conditions should provide an adequate approximation of prolonged diapause frequency.

Prolonged diapause has been found in a number of other temperate zone Lepidoptera that overwinter as pupae (Biliotti, 1953; Rabb, 1966; Powell, 1974; Prentiss, 1976; Shapiro, 1980) although it is not limited to the pupal stage (Yothers & Carlson, 1941; Surgeoner & Wallner, 1978).

Differences in photoperiod response and diapause intensity of latitudinally and altitudinally separated populations are commonly observed (Danilevskii, 1965; Beck, 1980; Sims, 1980) but interpopulation differentiation and temporal variability in the occurrence of prolonged diapause is less well known. Significant interpopulation variation in prolonged diapause has been found in sawflies (Prebble, 1941; Eichhorn, 1977), Cecidomyiidae (Sunose, 1978) and Lepidoptera (Surgeoner & Wallner, op. cit.). Prebble (op. cit.) obtained results similar to those of this study; uni- and bivoltine populations of *Gilpinia polytoma* (Hartig) (Hymenoptera, Diprionidae) were found to have considerably more prolonged diapause than multivoltine populations.

A major ecological difference between univoltine and multivoltine *P. zelicaon* populations is the growth phenology of the host plants (Emmel & Shields, op. cit.). Native perennial species of umbellifers used by lower elevation univoltine populations senesce rapidly with the advent of warm, dry weather in May and June. *Pteryxia terebinthina* (Hook.) C. & R., a host used by the Donner Pass and other high elevation Sierra Nevada populations (Emmel & Emmel, 1974), has a similar senescence in mid-July to August, placing a severe food limitation on occasional partial second broods unable to find or utilize alternate host species (Sims, 1980). Possibility of killing frosts in early fall increases the hazards for second brood individuals at higher elevations. The unpredictable quality and quantity of available food plants for larval development during the latter part of the growth season presents a considerable environmental risk for *P. zelicaon*. This food plant risk is partially buffered on *Foeniculum* since this "weedy" species has a longer growth season and occurs in a greater diversity of habitats than native hosts. In mesic areas *Foeniculum* may have tender young foliage throughout the season, and the plant commonly sends out fresh growth following disturbance. Variation in precipitation as it effects host plant growth is not the only possible environmental risk that may influence prolonged diapause in *P. zelicaon*.

Studies on other insects have demonstrated the adaptive value of prolonged diapause in the avoidance of biotic hazards such as disease, predators, and parasites (Price & Tripp, 1972; Minder, 1973).

Relatively dry areas of the temperate zone, including the Mediterranean climate of central California, tend to have particularly variable rainfall patterns (Rumney, 1969). Records from Sacramento provide a good example of this variability. Here, 120 years of data (1849–1969) show a mean seasonal rainfall of 45.5 cm with a range of 11.9–92.5 cm (Figgins, 1971). In seven of the 120 years the seasonal total was less than one-half the mean and the 1975–1976 and 1976–1977 seasons subjected populations to consecutive extreme drought years (15.6 and 19.5 cm rainfall, respectively). The effect of drought on host plants and population levels and/or population persistence through time is still poorly understood for *P. zelicaon*. For *Euphydryas editha* Boisduval, a species frequently co-occurring with *P. zelicaon* in central California, drought can reduce population size through larval starvation (White, 1974) or be a factor involved in local population extinction (Ehrlich et al., 1980).

Within the context of current thought on life history adaptations in insects, the presence of prolonged diapause in *P. zelicaon* represents a "bet-hedging" strategy in which individual females spread the risk of their reproductive effort over more than one season (den Boer, 1968; Stearns, 1976). This explanation implies, but does not demonstrate, the unpredictable risk of complete reproductive failure among the diapause progeny from a single female emerging in a given year. It is also possible that, even without the risk of a catastrophic season, the value of R_0 (net reproductive or replacement rate during a growth season) will be small enough in some years to select for the allocation of progeny into both normal and extended diapause categories (Istock, 1981). On an evolutionary scale the presence of prolonged diapause suggests that the long-term benefits of this response outweigh the disadvantages of the increased mortality rate demonstrated in this study.

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OBITUARY

DR. RENÉ LICHY (1896–1981)

Professor Lichy was an outstanding lepidopterist who lived and worked for many years in Venezuela. I first knew him in 1938, and we have exchanged papers and occasional letters ever since. He taught on the faculty of the Universidad Central de Venezuela, was a member of the Academia de Ciencias Fisicas-matematicas y Naturales de Venezuela, an honorary member of both the Sociedad Venezolana de Ciencias Naturales and Sociedad Venezolana de Entomologia. He was the honorary curator for Lepidoptera at the Museo de Ciencias Naturales in Caracas. He joined the Lepidopterists' Society soon after its foundation.

The last twenty years or so of his life were spent in France, the land of his origin. There he was associated with the Department of Entomology at the Museum d'Histoire Naturelle in Paris. He continued his great interest in Venezuelan Lepidoptera and furthered his world-wide studies of Sphingidae. René Lichy died on 6 April 1981. He is survived by three children and three grandchildren. His daughter and her three children live in France. Also, refer to Freiche & Lemaire (1981, Bull. Soc. Ent. France 86(9&10):313–314).

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