Longest Insect Dormancy: Yucca Moth Larvae (Lepidoptera: Prodoxidae) Metamorphose After 20, 25, and 30 Years in Diapause

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ABSTRACT Fully grown larvae of *Prodoxus y-inversus* (Riley) undergo diapause in hardened cysts in the sterile tissue of *Yucca baccata* Torrey fruits. Dormancy normally lasts from early summer until the following May, but when optimal climatic cues, particularly winter chilling, are not received, the diapause is maintained. Larvae of the 1969 generation were collected in April 1970 in southern Nevada and held in winter conditions that varied but were warmer than those to which the population was adapted in Nevada. More than 180 individuals emerged synchronously following the 16th and 17th winters. Temperature data suggested that the intensity of winter chilling is the primary factor initiating diapause development and that after many years in diapause larvae are conditioned to respond to temperature regimes that were not acceptable for development in the early years of diapause. To test these hypotheses, larvae were held in constant warm temperatures for 4–5 yr, then exposed to a variety of winter conditions. Additional large groups eclosed following their 20th (151 adults) and 25th (125 adults) winters. X-rays before the 30th winter indicated that few larvae remained, and 14 moths emerged following 30 yr in diapause.

KEY WORDS *Prodoxus y-inversus*, Agavaceae, *Yucca baccata*, prolonged diapause, Nevada

Diapause in insects is a state of arrested development, regulated by hormones, which enables survival during unfavorable times (e.g., dry season, winter). In temperate zone species this normally occurs on an annual cycle, but diapause may be prolonged to a second or later year as an adaptation to resources that are limited to a specific season but are erratic in availability or abundance from year to year. Examples include seed-feeding insects that depend on crops that are biennial or unpredictably infrequent, ground nesting bees that depend on annual plants, gall gnats, beetles, flies and wasps that are parasitoids in sawfly and moth larvae, and both phytophagous and predaceous desert species, which are subject to long-term fluctuations in seasonal climate (Powell 1974, Sunose 1983, Ushatinskaya 1984). Here I report the longest dormancy known for an insect, a desert-dwelling moth that can survive up to 30 yr in diapause. The time lapse is successfully terminated by seasonally synchronized development of numerous adults. Individual wood-boring beetles (Buprestidae and Cerambycidae) have metamorphosed sporadically after 20–50 yr, but the longevity is believed to result from a low basal metabolic rate and slow growth in an oxygen poor environment, not as a consequence of lengthy diapause (Crowson 1981).

Prodoxid moths of four genera are specialist feeders on *Yucca* and *Agave* (Agavaceae), and their seasonal activities (adult emergence, oviposition, larval feeding and dormancy, pupation) are closely synchronized with the development of infloresences of the host plants (Powell 1992, Davis 1999), which vary in abundance depending on seasonal climatic conditions. As an adaptation to such variables, metamorphosis may be delayed by prepupal larvae in diapause (i.e., larvae that are fully developed, ready to pupate, which hereafter are referred to simply as larvae). Emergence of adults of these prodoxids sometimes occurs 2–10 yr after feeding (Powell 1984a, 1984b, 1987). These larvae are not in a cryptobiotic state as defined by Hinton (1960) for larvae of chironomid flies, which survived up to 10 yr dehydration. *Prodoxus* larvae react by moving the mandibles and thoracic segments when prodded or exposed to a high intensity microscope lamp. Yet they remain in an obligate diapause state if winter temperatures are not encountered, so long as cocoons remain intact preventing desiccation. Larvae did not diminish in size with successive years. (Powell 1989).

The longest dormancy was recorded in *Prodoxus y-inversus* Riley, when >180 moths emerged in synchronized periods of 15 and 16 d, following 16 and 17 yr in diapause (Powell 1989). Larvae were collected in April 1970, and no adults developed that season, following an unusually mild winter that promoted diapause maintenance in several species of *Prodoxus* in the Mojave Desert (Powell 1987). After storage in constant, warm temperature during the second winter, larvae were exposed to a much colder winter in the third year, and 12 moths metamorphosed. Similar conditions during year four failed to promote diapause development, as did several subsequent winters in

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Table 1. Development of larvae to adult *Prodoxus y-inversus* in successive years

Year	20	21	22	23	24	25	26	27	28	29	30
Lot D21:											
Site	L	L	L	L	L	I	L	L	L	L	D
HDD	0	0	0	0	0	1953	0	0	0	0	1170
Moths	0	0	0	0	0	72	0	0	0	0	0
Lot D21.1:											
Site	B	L	L	L	L	B	L	L	L	L	I
HDD	1100	0	0	0	0	977	0	0	0	0	1925
Moths	77	0	0	0	0	30	0	0	0	0	2
Lot D21.2:											
Site	E	L	L	L	L	C	L	L	L	L	B
HDD	2272	0	0	0	0	1670	0	0	0	0	1018
Moths	74	0	0	0	0	23	0	0	0	0	0
Lot D21.3:											
Site	B′	B	B	B′	B′	B	L	L	L	L	C
HDD	1100	1039	1037	857	1056	977	0	0	0	0	1700
Moths	1	32	0	0	1	0	0	0	0	0	12

HDD = heating day degrees; sites: B, B′ = Berkeley (cage, unheated mobile lab); C = Orinda; D = Davis; E = Blodgett Forest; I = Big Pine; L = Lab, U.C. campus (see *Methods*).

milder temperatures (Powell 1989). Then, 120 individuals developed following their 16th winter, which had been 16–37% colder than the preceding 5 yr (measured by cumulative heating day degrees [HDD], see *Materials and Methods*). In the 17th winter, two of four larval groups were exposed to colder conditions than in the 16th yr (37–43% greater HDD), and 61 more moths eclosed (Powell 1989). Based on analysis of temperature data, I postulated that after many years of extended diapause, development was influenced by exposure to a relatively cold winter and that larvae had become acclimated to respond to warmer temperatures than they had experienced in their first few winters.

To test these hypotheses I held groups of larvae from the same population sample in constant temperature above 18°C for 4–5 yr, then exposed them to winters of varying intensity of chilling.

Materials and Methods

Dried fruits of *Yucca baccata* Torrey containing larvae of *Prodoxus y-inversus* from the 1969 season were collected at Kyle Canyon (≈20 km west of Highway 95, at 1,680 m elevation) in southern Nevada in early April 1970, 6–8 wk before the normal moth emergence period. Details of the biology and study methods have been reported (Powell 1984a). Larvae were numerous, >100/capsule. They caused hardened regions in the plant tissue surrounding their galleries, and these had coalesced, preserving large parts of the capsules intact, woody in consistency. After year 16, the yucca material containing the larvae was divided into four equal parts by weight and was stored in sealed, corrugated cardboard boxes during overwintering. Larval galleries of *P. y-inversus* terminate at two opaque silken caps, at the surface and recessed 1.5–2.5 mm (Powell 1984a); hence, larvae were exposed to extremely subdued photoperiod. Boxes were housed in the laboratory or in shaded, open-sided sheds or roofed cages preventing direct rainfall contact, from November through March at the following California sites: Berkeley, Alameda County (Oxford Tract, UC campus) [B = outdoor cage; B′ = unheated mobile unit, which had similar temperature ranges but excluded rainfall]; Orinda, Contra Costa County [C]; Davis, Yolo County [D]; Blodgett Forest, El Dorado County in the Sierra Nevada [E]; Big Pine, Inyo County, east of the Sierra Nevada [I]; Lab (20 ± 2°C) (Wellman Hall, UC campus) [L]. After year 18–19 in mild winter temperatures (below the 10-yr average HDD in Berkeley) (Powell 1989), two groups were retained in B and B′, one was held in L as a control, and the fourth was deployed at E during winter 20. Subsequently, lots were held 4 yr in L, then deployed in outdoor temperatures for one winter, rotating them among the four localities, as indicated in Table 1.

In April all lots were returned to Berkeley, each box was provided with a 32-mm exit hole leading to a translucent vial, and emerging moths were harvested daily. At the end of each emergence season, small samples were examined for presence of larvae. However, opening a cocoon sentences the larva to desiccation. Consequently a census of the number of larvae in diapause could not be made without terminating the study, and development data are given as numbers of moths that eclosed and percent of total successful development rather than the percent maintaining diapause.

The intensity of winter chilling was estimated by a sum of HDD for 5 mo, November through March. HDD were calculated by subtracting the mean of daily maximum and minimum temperatures from 18.3°C (65°F). HDD <0 were converted to 0. Berkeley HDD were calculated using data recorded by a thermograph at the overwintering site or from the University of California Weather Station. The latter data were published by the National Oceanic and Atmospheric Administration (NOAA 1988–1999); temperatures at Blodgett Forest were recorded by a thermograph on site; HDD from Big Pine were estimated by NOAA data from Bishop, 24 km to the north; those at Orinda were estimated from 7-yr means at the Russell Reserve, 6 km to the NW (Powell 1989) and from con-

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current Berkeley temperatures; and at Davis temperatures were recorded by a HOBO 2K recorder and BoxCar Pro Software. At the beginning of year 30, representative samples consisting of 33–40% by weight of each lot were subjected to x-ray imaging.

Results

After the four groups of larval *Prodoxus y-inversus* were exposed to different sequences of winter and laboratory during winters 17–20, 152 *Prodoxus* metamorphosed during 12- and 13-d periods in April–May 1989 in year 20 (Table 1). This was 29% of the ultimate total emergence, but only one moth eclosed from a group held at Berkeley, which had been exposed to winter at Blodgett Forest in the Sierra Nevada three years earlier (Powell 1989). In year 21, 32 adults from that group eclosed following two winters in an outdoor cage at Berkeley with above average HDD (Table 1).

In 1994, following 4–5 yr in constant temperature, then a winter in outdoor climates, 125 *P. y-inversus* developed successfully in year 25 (Table 1), 24% of the total emergence. The moths' emergence was again synchronized seasonally, occurring during a 20-d period in May. Among these, 30 eclosed at Berkeley, even though the winter was mild, whereas no metamorphosis occurred in a second group that had been exposed to Berkeley winters for eight consecutive years. Finally, in April–May 1999, after another 4-yr delay in constant temperature, 14 individuals emerged, following 30 yr in diapause (Table 1).

In summary, 97% of the 520 *Prodoxus y-inversus* that successfully developed did so after year 15, 62% after year 17, in response to sequences of several years warmer winter regimes, followed by a colder one (Powell 1989 and Table 1).

X-ray images before winter of year 30 revealed almost all larval galleries to be empty. A few had faintly visible contents that were difficult to identify owing to various angles in which the galleries were situated. After the last emergence period, examination of 200 larval galleries (50 in each lot) by scraping the surface of pod fragments revealed only five with intact silken cocoon caps. One contained a desiccated *Prodoxus* larva, and the other four had been destroyed from below, probably by anobiid beetle larvae in the early years after collection. Hence the diminished emergence in year 30 is attributable to few larvae surviving.

Discussion

The region at the source population, Kyle Canyon (1,680 m elevation) in southern Nevada, is arid, desert habitat. Climatological data are not available for the site but can be estimated from those recorded at the Kyle Canyon Ranger Station (U.S. Forest Service), Spring Mountains, 10 km to the west (2,140 m elevation), from temperatures at the Las Vegas Airport, 38 km to the SE (1,300 m elev.), and from isotherms plotted by Brown (1974). Annual rainfall averages only 108 mm at Las Vegas and about 500 mm in the Spring Mountains. Precipitation is seasonally sporadic, with monthly average highs in both summer (July–August) and winter (December–February) and lowest in April–June, when *Yucca baccata* blooms. Mean maximum temperatures at Kyle Canyon in January are estimated to range between 7 and 11°C and minima from −7 to −4°C, contrasted with means of 13 and 0°C recorded at Las Vegas. Therefore, average total HDD for November–March at Kyle Canyon is appreciably greater than the mean 2,384 at Las Vegas, and winter temperatures to which the insect population is adapted are considerably cooler than any to which the larvae were exposed during this study.

When larvae were moved from Berkeley (average 956 HDD two preceding years) to Blodgett Forest (2272 HDD), 74 individuals metamorphosed in year 20; when moved after 4–5 yr at constant temperature (0 HDD) to widely differing winter regimes, 125 *P. y-inversus* responded by successful development in winter 25, including 30 after exposure to only a moderate winter (977 HDD) at Berkeley (Table 1). After another 4 yr in the laboratory at 0 HDD, 14 larvae metamorphosed in response to winter 30. The results affirm my speculation derived from earlier years: long-term dormant larvae respond by diapause development when exposed to a colder winter than encountered in the immediate few preceding years. A series of winters perceived by larvae as too mild conditions them to develop more readily, even to considerably less intense chilling than circumstances they had experienced during earlier years of their dormancy. Metamorphosis of one, then 32 *Prodoxus* at Berkeley in year 20 and 21 (Table 1) suggests that diapause development may begin one year and continue the next. The data corroborate previous conclusions, based on studies of 10 species of *Prodoxus* and *Agavenema* (Powell 1984a, 1984b, 1987): larvae maintain the diapause state when exposed to ineffective winter conditions, and temperature is the primary factor for the initiation of diapause development.

Multiannual emergence patterns displayed by insects in artificial situations often involve a large number in year 1, then declining numbers or only sporadic individuals in the next two or three and later years. As a result, many investigators have proposed a genetically determined, obligate carryover of some individuals in diapause irrespective of seasonal conditions, a presumed bet-hedging strategy for population survival. In prodoxid moths the genetic variability is manifested in response to less than optimal winter conditions. There is not a genetically determined, mass metamorphosis in year 1 followed by fewer numbers in years 2 and 3, irrespective of climatic conditions. Instead, when winter conditions are optimal, all or nearly all individuals of a given population undergo development and metamorphose; whereas in adverse conditions, carryover larvae in varying proportions maintain the diapause state. One can promote 100% carryover of larvae in confinement merely by exposure to constant warm temperature, but it is almost impossible to promote 100% development in experimental conditions.

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The behavior by larvae of *P. y-inversus* suggests that insects adapted to arid climates and larval resources that vary in abundance from year to year can maintain diapause for decades, analogous to seeds of annual flowering plants and eggs of crustaceans that live in temporary and unpredictable aquatic habitats (Hairston 1996, Ellner et al. 1998, Philippi 1998). This has profound implications to insect inventory and monitoring populations for conservation studies and planning preserves in arid regions; to regeneration of conifer plantations and management of native bees as pollinators; and to predictions of the effects of widespread environmental changes, such as prolonged drought and global warming.

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References Cited

Brown, M. 1974. The climate of Nevada, pp. 779–793. *In* Officials of NOAA, Climates of the states, vol. 2. Western States. National Oceanic and Atmospheric Administration, Asheville, NC.

Crowson, R. A. 1981. The biology of the Coleoptera. Academic, London.

Davis, D. R. 1999. The Monotrysian Heteroneura, pp. 65–90. *In* N. P. Kristensen (ed.), Lepidoptera, moths and butterflies, vol. 1: evolution, systematics, and biogeography. Handbook of zoology, vol. 4, Arthropoda: Insecta, Part 35. W. de Gruyter, Berlin.

Ellner, S. P., N. G. Hairston Jr.,, and D. Babai, D. 1998. Long-term diapause and spreading of risk across the life cycle. Ergeb. Limnol. 52: 1–15.

Hairston, N. G., Jr. 1996. Zooplankton egg banks as biotic reservoirs in changing environments. Limnol. Oceanogr. 41: 1087–1092.

Hinton, H. E. 1960. Cryptobiosis in the larva of *Polypedium vanderplanki* Hint. (Chironomidae). J. Insect Physiol. 5: 286–300.

NOAA. 1988–1999. National Oceanic and Atmospheric Admin., Climatological Data, California; Asheville, NC.

Philippi, T. E. 1998. Prolonged diapause and models of species coexistence: a cautionary tale from annual plants in deserts. Ergeb. Limnol. 52: 19–31.

Powell, J. A. 1974. Occurrence of prolonged diapause in ethmiid moths (Lepidoptera: Gelechioidea). Pan-Pac. Entomol. 50: 220–225.

Powell, J. A. 1984a. Biological interrelationships of moths and *Yucca schottii.* U. Calif. Publ. Entomol. 100: 1–93.

Powell, J. A. 1984b. Prolonged diapause in yucca moths, p. 307. XVII International Congress on Entomology, Hamburg, Germany (abstr.).

Powell, J. A. 1987. Records of prolonged diapause in Lepidoptera. J. Res. Lepid. 25: 83–109.

Powell, J. A. 1989. Synchronized, mass-emergences of a yucca moth, *Prodoxus y-inversus* (Lepidoptera: Prodoxidae) after 16 and 17 years in diapause. Oecologia 81: 490–493.

Powell, J. A. 1992. Interrelationships of yuccas and yucca moths. Trends Ecol. Evol. 7: 10–15.

Sunose, T. 1983. Prolonged diapause in insects and its ecological significance. Kotai Gun Seitaigaku Kaiho 37: 35–48.

Ushatinskaya, R. S. 1984. A critical review of the superdiapause in insects. Ann. Zool. 21: 3–30.

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