Behavioral Differentiation between Two Species of Cactophilic Drosophila. II. Pupation Site Preference
Author(s): James C. Fogleman and Therese A. Markow
Source: *The Southwestern Naturalist*, Vol. 27, No. 3 (Aug. 20, 1982), pp. 315-320
Published by: Southwestern Association of Naturalists
Stable URL: http://www.jstor.org/stable/3670881
Accessed: 10/08/2009 17:37

THE SOUTHWESTERN NATURALIST 27(3):315-320 AUGUST 20, 1982

BEHAVIORAL DIFFERENTIATION BETWEEN TWO SPECIES OF CACTOPHILIC *DROSOPHILA*. II. PUPATION SITE PREFERENCE

JAMES C. FOGLEMAN AND THERESE A. MARKOW

ABSTRACT.—Pupation site preferences (PSP) with respect to light, temperature, and gravity were studied in two species of cactophilic *Drosophila*, *D. nigrospiracula* and *D. mettleri*. Although specific preferences were exhibited, no significant difference was found between the species in PSP for light. A small but significant difference exists for temperature preference. The two species were found to be behaviorally differentiated in their PSP with respect to gravity. Differentiation in this parameter was anticipated based on the known differences in the primary larval niches of these species. Correlations between larval and adult behavior are discussed..

The novel ecological adaptations of one of the cactophilic *Drosophila* of the Sonoran Desert, *D. mettleri*, provide an interesting and unique opportunity to study several aspects of insect behavior. Adult *D. mettleri* and a morphologically similar species, *D. nigrospiracula*, feed on and mate in close proximity to necrotic sections of cardón cacti (*Pachycereus pringlei*) on the Baja California peninsula and saguaro cacti (*Carnegiea gigantea*) on mainland Mexico and in southwestern Arizona. Typically, the larval niches are spatially separate. *D nigrospiracula* larvae utilize the rotting cactus tissue while *D. mettleri* larvae live in the soil inundated with rot juices (Heed, 1977, 1978). Despite the general separation of larval niches, rearing records indicate that larvae of both species occasionally co-exist in the same cactus rot (Mangan, 1978; Heed, pers. comm.) or at the interface of cactus and soil substrates (Fogleman, pers. observ.). When this occurs, the percentage of *D. mettleri* which eclose from these secondary substrates has been reported to range up to 15% (Mangan, 1978).

Thus, under certain circumstances, there is considerable larval niche overlap and interspecific competition may occur during any of the pre-imaginal stages. The site at which larvae pupate undoubtedly has an effect on their survival, especially in the extreme conditions of the desert. Habitat selection would reduce competition, and behavioral differentiation with respect to pupation site preference (PSP) may act in this manner for these species. In order to define potential factors which separate the niches of the two species, we have been testing the behavioral responses of their larvae and adults to a series of environmental variables. Markow and Fogleman (1981) reported on adult responses to light and to gravity. Here, we report an investigation of the effects of light, temperature, and gravity on the pupation site preferences of late third-instar larvae of these two species.

MATERIALS AND METHODS.—Strains of *D. nigrospiracula* and *D. mettleri* were obtained by mass mating flies that had been reared from natural substrates (rotting saguaro and soaked soil) in May 1979. These multi-female lines were subsequently maintained in the laboratory on standard banana media.

PSP: Light vs. Dark.—Pupation site preference tests for lighted versus darkened areas were performed in Wheaton glass staining dishes with covers (see Markow, 1980). *Drosophila* media was layered onto the floor of each dish. For each replication, 300 second instar larvae were introduced along a line in the central axis of the dish. One half of each dish was darkened by a doubled layered jacket of black felt. The other half was left uncovered. Dishes were then put into light-tight incubators having an internal temperature of 25°C. Flourescent lights in the incubators provided approximately 15 f.c. of illumination at the media surface. No temperature differences between the light and dark parts of the dishes were detectable. After 8 days, the numbers of pupae in the light and dark sections were recorded. Control experiments were conducted in both completely lighted and completely darkened dishes.

PSP: Temperature.—Pupation site preference for temperature was measured using collapsible, plexi-glass boxes (internal dimensions: 2.54 cm high by 2.54 cm wide by 101.6 cm long; see Foleman, 1978). Three layers of Kimwipe tissues (2.54 cm wide) were placed along one side for the length of each box. The tissue layer was wetted to provide moisture for the larvae and to maintain a high humidity in the boxes. Two hundred third-instar larvae were used in each run and were evenly distributed: 10 larvae every 5.08 cm. Experimental boxes were then put on a temperature gradient device (Fogleman, 1978) which produced a linear temperature gradient in the boxes. Experiments were performed in complete darkness, the boxes being covered by several layers of newspaper and dark cloth, in order to eliminate the effect of larval phototaxis on the distribution of pupae. At the conclusion of a replicate (48 h), the box was taken apart and the number of pupae per 5.08 cm segment was recorded. Simultaneous control replicates were identically prepared boxes placed in light-tight chamber at 25°C.

PSP: Height.—Pupation site preference with respect to gravity was measured as the heigth of pupation in the plexi-glass boxes when oriented vertically. In all experiments, 200 late third-instar larvae were placed at the bottom end of a box located in an unlighted environmental chamber maintained at 25°C. After 48 h, the distance of each pupa from the point of introduction was measured. Control experiments consisted of identically prepared boxes positioned horizontally. Vertical and horizontal replicates were paired in order to eliminate potential differences between batches of larvae.

RESULTS.—The data obtained in the PSP tests of lighted versus darkened areas are presented in Table 1. Both species are significantly photopositive in this behavior, as about 75% of the pupae of both species were located in the lighted section of the boxes. The Chi-square values based on the hypothesis that the pupae should be distributed equally between the two areas (no preference) are 166.14 and 234.57 for *D. nigrospiracula* and *D. mettleri* respectively. With one degree of freedom, these statistics are highly significant. There was no statistically significant difference between the species (t = 0.700, $P > 0.5$). The results of the control (both light and dark dishes) experiments did not deviate signifcantly from expected values based on the random distribution of pupae.

All three replicates of the PSP tests with respect to temperature were combined and are depicted in Fig. 1. The means and standard errors of the pupae distribution of *D. nigrospiracula* and *D. mettleri* are 23.1 ± 0.2°C and 22.4 ± 0.3°C respectively. The difference (0.7°C) between the two means is statistically significant at the 0.001 level (t = 4.21; d.f. = 850). Pupal distribution curves from the control tests were u-shaped due to a "trap effect" at each end.

Table 2 shows the effect of gravity on PSP. The data from the control (horizontal) boxes support the contention that there was no difference between larvae of the two species in their ability or inclination to move away from the point of introduction. This is indicated by a non-significant t-statistic (t = 0.892, $P > 0.4$) comparing the two pooled estimates of the

TABLE 1.—*Pupation site preference of* D. nigrospiracula *and* D. mettleri; *light versus dark.*** = P < 0.001.

Replications	Number (percent) of *D. nigrospiracula* pupae in: Light	Dark	Number (percent) of *D. mettleri* pupae in: Light	Dark
1	240 (80%)	59 (20%)	190 (71%)	76 (29%)
2	176 (67%)	87 (33%)	248 (84%)	46 (16%)
3	181 (69%)	81 (31%)	209 (73%)	79 (27%)
Pooled	597 (72%)	227 (28%)	647 (76%)	201 (24%)
χ^2(L vs. D)	166.14**		234.57**	

mean distance traveled. The results from the vertically oriented containers show that larvae of *D. mettleri* are significantly more inclined to crawl upwards to pupate than are larvae of *D. nigrospiracula*. This difference is significant at the 0.01 level ($t = 4.867$, d.f. $= 4$).

DISCUSSION.—Behavioral differentiation in pupation site selection of various *Drosophila* species with respect to temperature, gravity, or light has been reported previously. Sokal et al. (1960) reported that temperature, moisture content of the medium, and larval density had an effect on pupation site selection in *D. melanogaster*. Peripheral pupation was prefered to central sites in the temperature range of 22 to 25°C. Our data show that both *D. nigrospiracula* and *D. mettleri* have very specific temperature preferences for

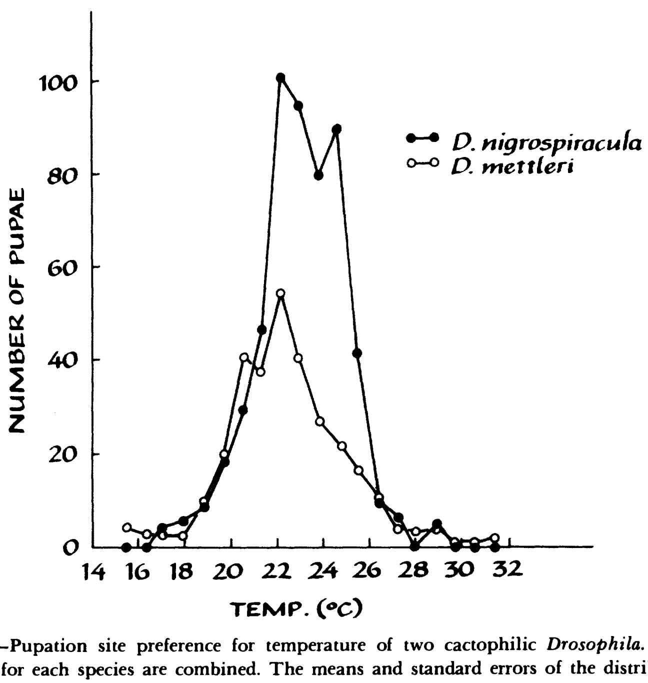

FIG. 1.—Pupation site preference for temperature of two cactophilic *Drosophila*. The three replicates for each species are combined. The means and standard errors of the distributions are 23.1 ± 0.2°C and 22.4 ± 0.3°C for *D. nigrospiracula* and *D. mettleri* respectively.

TABLE 2.—*Pupation site preference of* D. nigrospiracula *and* D. mettleri: *Pupation height. Mean distance traveled in centimeters* (±SE) *from point of introduction.* **=$P<0.01$.

	N	Vertical (experimental)	N	Horizontal (control)
D. nigrospiracula				
Rep. 1	193	5.1±0.2	200	35.2±1.9
Rep. 2	167	7.1±0.6	204	33.0±2.2
Rep. 3	178	5.4±0.3	184	27.8±2.2
Pooled	538	5.8±0.2	588	32.1±1.2
D. mettleri				
Rep. 1	68	19.9±2.2	99	29.8±2.6
Rep. 2	77	24.9±2.6	126	34.3±2.6
Rep. 3	163	15.1±1.0	171	46.4±2.5
Pooled	308	18.6±1.0	396	38.4±1.5
t (*D.n.* vs. *D.m.*)		4.867**		0.892

pupation sites (Fig. 1). Although the means of the pupal distributions are significantly different statistically, the biological significance of a difference of this magnitude (0.7°C) is unclear. Substrate temperature fluctuations are quite pronounced in the desert on a daily cycle. Because of this, it is somewhat surprising that *D. nigrospiracula* and *D. mettleri* should show such a high degree of specificity. Their specificity does not appear to be associated with any temperature effects on eclosion (Fogleman, unpubl. data) since the temperature versus percent eclosion curves for both species are comparatively broad. Since the temperature preferences of adult *D. nigrospiracula* and *D. mettleri* are not presently known, a comparison of adult and larval behavior is not possible.

While pupation height has been demonstrated to have a genetic basis (Markow, 1979), larvae probably are responding to a number of variables when moving upward to pupate. Sameoto and Miller (1968) and Barker (1971) report that *Drosophila* larvae crawl to higher pupation sites in response to increasing water content of the medium and/or increasing larval density. Gravity may well be a factor, but its effect is clouded since the apparatus used in these experiments does not allow larvae to express a positive geotaxis—larvae can only move upward. Movement against gravity may be compounded by responses to larval density or moisture.

Drosophila nigrospiracula and *D. mettleri* are behaviorally differentiated wih respect to pupation height. This undoubtedly relates to preimaginal ecology. Rotting cacti, the typical substrate of *D. nigrospiracula,* does not restrict vertical larval movement. They are as likely to find suitable pupation sites by moving dowward as by crawling up. On the other hand, larvae of *D. mettleri* living in the soil have a severe directional constraint. They must pupate at the suface of the soil in order to successfully enclose as adults. Natural selection may have created a geonegative behavioral approach to pupation sites in *D. mettleri.*

These experiments on pupation site preference provide for several comparisons of adult and preadult behavior. Markow and Fogleman (1981) investigated adult geotaxis and phototaxis in these two *Drosophila* species. Their results indicated that *D. nigrospiracula* adults were geoneutral to slightly geopositive while *D. mettleri* were strongly geonegative. Assuming that there is a gravity component to pupation height, there appears to be a close correspondence between larval and adult behavior. Markow (1979) also found such a relationship in populations of *D. melanogaster* and *D. pseudoobscura* that had been previously selected for geopositive or geonegative behavior. Larvae from strains selected to walk upward as adults (geonegative) crawl significantly higher to pupate than larvae from geopositive strains. She proposed that selection for adult geotaxis may have utilized genetic variation affecting behavioral processes common to both larvae and adults.

There is little correspondence between larval and adult behaviors of the two desert species with respect to light. Adults of both species are strongly photonegative (Markow and Fogleman, 1981), while the PSP experiments show strong photopositve tendencies. A similar lack of correspondence between adult and third-instar behavior exists in *D. melanogaster*, where strains showing strong photopostive behavior as adults are highly photonegative with respect to PSP (Markow, 1981). That the observed pupation site preferences are behaviors restricted to late third-instars is suggested by several lines of evidence. Larvae of *D. mettleri* have been reported to be photonegative (Heed, 1977), but they pupate in lighted places. *D. melanogaster*, which shows PSPs for darkened areas, is slightly photopositive throughout larval life until late third instar (Manning and Markow, 1981). Its sibling species, *D. simulans*, which prefers lighted pupation sites, is photoneutral prior to pupation. In *D. mettleri* then, PSP for light could easily represent a behavioral process separate from the photoresponse of either adults or larvae.

Rizki and Davis (1953) found that *D. melanogaster* and *D. willistoni* were different in their PSP for light and suggested that light intensity is one of the environmental determinants of the degree of interspecific competion in *Drosophila*. Their use of *D. melanogaster* was fortuitous in retrospect since it is the only species investigated to date which prefers to pupate in the dark (Markow, pers. observ.). Thus *D. nigrospiracula* and *D. mettleri* are similar to the majority of other *Drosophila* species. No hypothesis as to the biological significance of the general preference for pupating in the light has been adequately tested.

The environmental parameters which behaviorally distinguish *D. mettleri* from *D. nigrospiracula* with respect to pupation site preference are temperature and gravity. They do not differ with respect to their response to light. The biological significance of their differentiation with respect to temperature remains unclear due to the relatively small difference (0.7°C) in the mean response between species. They are clearly differentiated in their performance relative to pupation height, and this may be related to habitat selection. Under certain circumstances, the tendency of *D. mettleri* to craw upward might put them in an area unoccupied by *D. nigrospiracula*. However, this behavior is perhaps more realistically viewed as the result of natural selection on fossorial larvae. In short, the niche overlap of these two species may be insufficient to cause differentiation in these behavioral traits. This contention is supported by the results of some preliminary experiments on the oviposition site preference (OSP) of these two cactophilic *Drosophila* with respect to gravity, light, and substrate type. The results show *D.*

nigrospiracula and *D. mettleri* to be behaviorally differentiated in OSP in directions expected on the basis of the known larval habitats (Fogleman, unpubl. data). Thus, oviposition may be intrinsically involved in the maintenance of the larval niche separation.

This research was made possible by a NIH postdoctoral fellowship (GM06807) awarded to JCF and NIH grant (GM 25424) awarded to TAM.

Literature Cited

BARKER, J. S. F. 1971. Ecological differences and competitive interaction between *Drosophila melanogaster* and *Drosophila simulans* in small laboratory populations. Oecologia, 8:139-146.

FOGLEMAN, J. C. 1978. A temperature gradient bar for the study of *Drosophila.*Drosophila Inf. Serv., 53:212-213.

HEED, W. B. 1977. A new cactus-feeding but soil breeding species of *Drosophila* (Diptera; Drosophilidae). Proc. Entomol. Soc. Washington, 79:649-654.

———. 1978. Ecology and genetics of Sonoran Desert *Drosophila. In* Ecological genetics: the interface (P. F. Brussard, ed.). Springer-Verlag, New York.

MANGAN, R. L. 1978. Competitive interactions among host plant specific *Drosophila* species. Ph.D. thesis, Univ. Arizona, 190 pp.

MANNING, M., AND T. A. MARKOW. In press. Light dependent pupation site preference in *Drosophila.* II. *D. melanogaster* and *D. simulans.* Behav. Genet.

MARKOW, T. A. 1979. A survey of intra- and interspecific variation for pupation height in *Drosophila.* Behav. Genet., 9:209-217.

———. 1981. Light-dependent pupation site preference in *Drosophila*: Behavior of adult visual mutants. Behav. Neural Biol., 31:348-353.

MARKOW, T. A., AND J. C. FOGLEMAN. 1981. Behavior differentiation between two speices of cactiphilic *Drosophila.* I. Adult geotaxism and phototaxism. Experientia, 37:145-146.

RIZKI, M. T. M., AND C. G. DAVIS, JR. 1953. Light as an ecological determinant of interspecific competition between *Drosophila willistoni* and *Drosophila melanogaster.* Amer. Nat., 87:389-392.

SAMEOTO, D., AND R. S. MILLER. 1968. Selection of pupation site by *Drosophila melanogaster* and *Drosophila simulans.* Ecology, 49:117-880.

SOKAL, R., P. EHRLICH, P. HUNTER, AND G. SCHLAGER. 1960. Some factors affecting pupation site of *Drosophila.* Ann. Entomol. Soc. Amer., 53:174-182.

Address of authors: J. C. FOGLEMAN, *Dept. of Ecology and Evolutionary Biology, Univ. of Arizona, Tucson, AZ 85721;* T. A. MARKOW, *Dept. of Zoology, Arizona State Univ., Tempe, AZ 85281.*