

DROSOPHILA METTLERI AND SENITA CACTUS ALKALOIDS: FITNESS MEASUREMENTS AND THEIR ECOLOGICAL SIGNIFICANCE

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Abstract—1. *Drosophila mettleri* have been found feeding but not breeding on decaying stems of senita cactus, the normal host for *Drosophila pachea*.

2. Alkaloids were extracted from senita stems and used in tests of egg-to-adult viability, developmental rate, and adult longevity.

3. The results show that developmental rate is not appreciably affected by senita alkaloids.

4. In general, *D. mettleri* was less affected by the alkaloids with respect to egg-to-adult viability and adult longevity than *D. pachea* at concentrations which are fatal to other desert *Drosophila*.

5. Tolerance to alkaloids gives *D. mettleri* an ecological advantage.

INTRODUCTION

The Sonoran Desert has been a center for the study of *Drosophila* ecology for the last 20 years (Heed, 1978). One of the most interesting insect-host plant relationships in the Sonoran Desert is that of senita cactus (*Lophocereus schottii*) and *D. pachea*, one of the four endemic *Drosophila* species. Necrotic sections of senita cacti are used as both feeding and breeding substrates for *D. pachea* (Fellows & Heed, 1972). *Drosophila pachea* is restricted to senita cactus due to its absolute requirement of Δ^7 -sterols which are necessary for larval viability and female fertility (Heed & Kircher, 1965; Heed & Jensen, 1966). These sterols are intermediates in phytosterol biosynthetic pathways and accumulate in senita tissue due to interruptions of these pathways (Campbell & Kircher, 1980).

Senita cacti are rich in alkaloids. Total alkaloid concentration ranges from 3 to 15% of the dry weight of the plant depending on the maturity of the arm being sampled and the sample location with respect to epidermis or cortex (Kircher, 1969). Two alkaloids, lophocereine and its trimer, pilocereine, have been shown to be toxic to eight species of *Drosophila* that typically inhabit the Sonoran Desert (Kircher *et al.*, 1967). These species are: *D. aldrichi*, *D. arizonensis*, *D. hamatofila*, *D. longicornis*, *D. melanogaster*, *D. mojavensis*, *D. nigrospiracula*, and *D. pseudoobscura*. *Drosophila pachea* was the only species tested that could tolerate these compounds. Thus, alkaloids, particularly pilocereine, are considered to be primarily responsible for the absence of larvae of other desert-adapted *Drosophila* in rotting stems of senita—a fact which is supported by extensive rearing records. Alkaloids have also been suggested as the inhibitors involved in the accumulation of Δ^7 -sterols in senita tissue (Campbell & Kircher, 1980).

In 1977, Heed published the formal description of *Drosophila mettleri*, a cactus-feeding but soil-breeding desert drosophilid. Before this date, *D. mettleri* had been reported in the literature as "species M". Soils

which have been soaked with cardón (*Pachycereus pringlei*) rot juice or saguaro (*Carnegieia gigantea*) rot juice serve as the major larval substrates while the adults feed on both the cactus rot itself and soaked soil. *D. mettleri* was not, however, one of the eight species originally tested for alkaloid tolerance. An examination of our records showed that *D. mettleri* were frequently collected from decaying senita stems in the field, sometimes in relatively high proportions. This report investigates the effect of senita alkaloids on several fitness parameters in *D. mettleri* in order to ascertain whether this species, like *D. pachea*, is resistant to their toxicity. The fitness parameters that were measured include adult longevity, egg-to-adult viability and larval developmental rate. *Drosophila pachea* and *D. nigrospiracula*, another endemic desert species used in previous tests, were also tested as controls since their response to alkaloids is known.

MATERIALS AND METHODS

Strain derivation

Drosophila pachea were obtained from a laboratory strain of unknown origin. They were maintained in the laboratory on standard banana-agar media supplemented with either senita tissue or dried senita powder. Both *D. mettleri* and *D. nigrospiracula* were obtained from multi-female stocks which were initiated by rearing flies from natural substrates in the Tucson vicinity. Both species had been maintained in the laboratory on standard banana-agar media for about 1½ years.

Alkaloids

The alkaloids were extracted from fresh senita tissue in a manner similar to that described by Kircher (1969). Total alkaloids were found to represent approximately 7% of the tissue on a dry weight basis. The alkaloids were dissolved in ethanol at a concentration of 1 g per 3 ml. Alkaloids (in ethanol) were added to regular banana-agar *Drosophila* media in concentrations of 1, 5 and 10% on a dry weight basis. Ethanol without alkaloids was added to media representing controls (0%). All media contained 0.25% (dry wt)

7-cholesterol, which fulfils the requirement of *D. pachea* for a Δ^7 -sterol. Propionic acid was added as a mold inhibitor. All experiments were carried out at ambient laboratory temperature (approx 25 C) and humidity. Although these parameters varied during the study, all replicates were subject to identical conditions.

Egg-to-adult viability tests and developmental rates

Male and female *Drosophila* were put into petri plates containing yeasted *Drosophila* medium in order to obtain large numbers of eggs. Eggs were removed from the plates by washing them from the medium with water squirted from a #25 gauge syringe needle. They were collected on a fine mesh screen and transferred onto filter paper in a Buchner funnel. Eggs were washed several times with 0.7% sterile saline and distributed randomly over the filter paper. The filter paper was then cut into equal sections and the sections put into half-pint milk bottles containing about 30 cm³ of the test media. Replicate sets were produced such that they contained sections from the same filter paper and therefore approximately the same number of eggs. A replicate set consisted of four bottles containing 0, 1, 5 and 10% alkaloids respectively. At least four replicate sets were produced for each species. Adults which eclosed in each bottle were counted and removed on a daily basis until eclosion ceased. The viability measurements were normalized by dividing the total number of flies eclosed in each bottle of a replicated set of four bottles by the largest number of eclosed flies/bottle. This procedure yields relative per cent viability and makes comparisons possible between replicate sets even though they were initiated with slightly different numbers of eggs. Developmental rate was measured by calculating the mean development time for each treatment bottle and by averaging the means from all replicate bottles.

Adult longevity

Vials (100 × 35 mm) containing *Drosophila* media and different concentrations of alkaloids (0, 1, 5, 10%) were set up with 25 adult flies that had been aged 7–10 days. Males and females were tested separately and two replicate vials were set up for each sex of both *D. mettleri* and *D. pachea*

at each of the four alkaloid concentrations. The flies were transferred to new vials twice a week, and the number of dead flies in each vial was recorded every day. These experiments were terminated after 25 days. *D. nigrospiracula* was not tested for longevity.

Field test

Senita tissue was frozen, thawed and artificially rotted in the lab for 7–10 days. Juice was obtained from the rot by cloth filtration and was used to wet several kilograms of desert soil in a metal pan (22.9 cm × 30.5 cm × 5.1 cm). The soaked soil was positioned under palo verde tree in a desert area in the Tucson vicinity. The substrate was left exposed for 7 days and additional senita rot juice was added when necessary to keep the soil moist. After 1 week, the pan was brought back to the lab and placed in a sealed aquarium in order to detect eclosed *Drosophila* adults.

RESULTS

Selected collection records of adult *Drosophila* from natural senita rots are presented in Table 1. These collections provide evidence that *D. mettleri* can be present in significant proportions, sometimes even outnumbering *D. pachea*.

The effect of alkaloids on egg-to-adult viability of the three species tested can be seen in Table 2. *Drosophila nigrospiracula* are greatly affected by high concentrations of senita alkaloids. The average per cent viability is quite reduced at 5% and is zero at 10%. One-way analysis of variance (ANOVA) indicates that the effect is statistically significant ($F_{(3,12)} = 7.306$, $P < 0.01$). The other two species, *D. pachea* and *D. mettleri*, are not significantly affected by the alkaloid concentrations used in these experiments. Decreases in viability going from 5% to 10% alkaloids in *D. pachea* (14.6%) and *D. mettleri* (7.2%) are noticeable but not statistically significant.

The mean developmental rates of the three species for each alkaloid treatment are given in Table 3.

Table 1. Selected collection records showing the number of *D. pachea* and *D. mettleri* adults feeding upon rotting senita cactus

Location	<i>D. pachea</i>		<i>D. mettleri</i>		% <i>D. mettleri</i>
	Males	Females	Males	Females	
Caborca, Sonora Mexico	5	7	5	1	33.3
Cataviña, Baja California	15	17	6	22	46.7
Caborca, Sonora Mexico	7	14	1	3	16.0
Organpipe National Monument, AZ	29	38	101	44	68.4
Total	56	76	113	70	58.1

Table 2. Average % egg-to-adult viability* with increasing concentrations of senita alkaloids

Species	Reps.	% Alkaloids				F_s
		0	1	5	10	
<i>D. pachea</i>	4	70.8 ± 13.5	90.4 ± 8.8	71.3 ± 5.8	56.7 ± 14.5	1.513
<i>D. mettleri</i>	10	61.9 ± 5.8	76.4 ± 6.1	86.2 ± 4.5	79.0 ± 8.6	2.332
<i>D. nigrospiracula</i>	4	84.2 ± 13.4	77.6 ± 11.6	28.2 ± 24.0	0.0 ± 0.0	7.306†

F_s indicates significance for each species (one-way ANOVA).

* ± SE.

† $P < 0.01$.

Table 3. Mean development rate (in days) averaged over four replications (\pm SE) with increasing concentrations of senita alkaloids

Species	% Alkaloids				Total flies	F_s
	0	1	5	10		
<i>D. pachea</i>	20.4 \pm 0.6	20.0 \pm 0.8	19.8 \pm 1.3	18.9 \pm 0.3	3200	0.519
<i>D. mettleri</i>	23.9 \pm 0.4	22.3 \pm 0.5	22.9 \pm 0.3	22.5 \pm 0.6	3535	1.918
<i>D. nigrospiracula</i>	18.4 \pm 1.0	19.7 \pm 0.8	20.0*	—	388	0.791

F_s indicates significance for each species (one-way ANOVA).

* Only one of the four replicate bottles had sufficient progeny to calculate mean development time.

None of the F -statistics which resulted from one-way analyses of variance of the developmental rate data were statistically significant. Therefore, developmental rate is not appreciably affected by alkaloid concentration of up to 10%.

The effect of senita alkaloids on adults of *D. pachea* and *D. mettleri* is shown in Fig. 1. The per cent mortality after 25 days of exposure to the alkaloid media was calculated for each vial and replicate vials were averaged. The figure indicates that a much higher percentage of females died than males in both species and that *D. pachea* had a higher percentage mortality overall than *D. mettleri*. The data were analyzed with a three-factor factorial analysis of variance with replication (Snedecor & Cochran, 1967, pp. 361–364). This analysis indicates that all of the main effects (alkaloids, species and sex), as well as several of the interactions, have a statistically significant effect on adult longevity. However, two of the main effects, species and sex, are by far the largest contributors to the variance in the longevity data. The F -statistics for these main effects are 29.304 and 54.802 respectively ($df = 1, 15$; $P \ll 0.001$). The effect of alkaloids on longevity of these two species is statistically significant ($F_{(3,15)} = 4.431$, $P < 0.05$) but comparatively minor. *D. mettleri* is less affected than *D. pachea*. The interactions which are statistically significant are also minor in comparison to the main effects of species and sex.

During the 7-day field test, *Drosophila* adults were observed on and around the senita-soaked soil substrate. A small sample of those flies was taken for species identification and consisted entirely of *D. mettleri*. No *D. pachea* were found or expected since Tucson is not in the geographical distribution of this cactus. After the 7-day exposure period, cursory examination of the soil produced 39 *D. mettleri* larvae which were removed and transferred to food vials. An additional 44 *D. mettleri* adults subsequently eclosed from the senita-soaked soil. Both males and females of this group were fertile.

DISCUSSION

Although Table 1 shows that *D. mettleri* can represent more than 50% of the adult *Drosophila* collected from natural senita rots, the intent of presenting these data was to demonstrate what is possible rather than what is typical. The actual number of *D. mettleri* would depend heavily on the collection location (nearness to cacti which serve as the major substrates of this species) and season. A typical senita collection record generally contains less than 10% *D. mettleri*. Data of Fellows & Heed (1972) show that when given a choice between five artificially rotted cacti (agria, organpipe, senita, saguaro and cina), 28% of all *D. mettleri* adults collected were collected from senita. Furthermore, 14% of the 1092 *D. mettleri* adults collected from natural rot pockets of the same five cacti species were caught on senita rots. Thus, collection records clearly illustrate the fact that *D. mettleri* regularly feed on senita. However, this species has never been reared from decaying senita brought in from the field.

In *Drosophila*, egg-to-adult viability and mean development time are fitness parameters which are generally sensitive to environmental conditions. Previous viability tests on alkaloid toxicity showed that eight of the nine species tested were affected by either purified pilocereine, when present at a concentration of 1% of the dry ingredients of the diet, or sufficient senita tissue to yield approximately 1% crude alkaloids (Kircher *et al.*, 1967). In these tests, manifestation of toxicity included larval death, apparent F_1 sterility, or drastically reduced numbers of F_1 (and F_2) compared to controls. *Drosophila pachea*, on the other hand, was able to withstand alkaloid concentrations of up to 14% of the dry weight of the medium. Although fewer progeny were produced at the higher concentrations, fertility was not affected.

The results reported here confirm the previous results of Kircher in that *D. nigrospiracula* larvae are

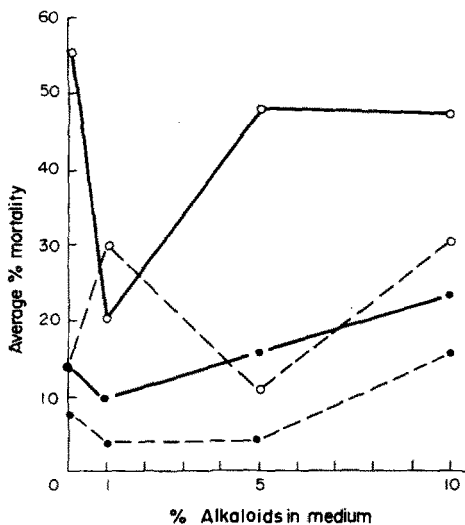


Fig. 1. Adult mortality (average of 2 replicates) on media alkaloids. *Drosophila pachea*: males ●—●, females ○—○; *D. mettleri*: males ●---●, females ○---○.

greatly affected by senita alkaloids while larvae of *D. pachea* can withstand high concentrations. Contrary to previous reports, *D. pachea* is not the only desert-adapted species to possess this ability. *D. mettleri* larvae can certainly tolerate senita alkaloids and, ostensibly, *D. mettleri* could use necrotic senita stems as a larval substrate in nature.

One of the noteworthy results reported by Kircher *et al.* (1967) was the effect of alkaloids on adult longevity. Except for *D. pachea*, all of the adults of the species tested exhibited significantly greater mortality on media containing 1% pilocereine than on control media without alkaloids. Six species showed 100% adult mortality within 20 days. In both the previous and the present study, longevity of *D. pachea* adults was relatively unaffected by alkaloids (Fig. 1), even at concentrations of 10% or greater. With respect to this fitness parameter, *D. mettleri* were again shown to be as tolerant of senita alkaloids as *D. pachea*.

The ability of adults to utilize senita rots as feeding substrates is an obvious advantage to *D. mettleri*. Rather than being restricted to a single host plant, as is *D. pachea*, *D. mettleri* can (and probably does) use all necrotic cacti as feeding substrates. Since several different species of the columnar cacti are present in any given area of the Sonoran Desert, the feeding substrate density is greater for *D. mettleri* adults than for most other desert-adapted *Drosophila*.

Although the experiments reported here suggest that *D. mettleri* can use senita rots as larval substrates, they have never been reared from them. Fogleman *et al.* (1981) reported on the oviposition site preferences of *D. mettleri* and concluded that this species is behaviorally restricted to soil-breeding but not necessarily restricted to soil soaked by necrosis of a particular host plant. On the basis of their tolerance to senita alkaloids, it seems reasonable that *D. mettleri* use senita-soaked soil in nature when available. This contention is strongly supported by the results of the field test. However, the senita soil substrate is not as abundant in nature as the more typically used cardón or saguaro substrates because of the smaller physical size of senita cacti and the rarity of rots extensive enough to soak the soil. Thus, the population of *D. mettleri* is more likely governed by the availability of its major breeding substrates, cardón or saguarocactus-soaked soils, rather than its adult feeding sites. The rarity of senita-soaked soil also indicates that the major advantage of the tolerance of *D. mettleri* to senita alkaloids is to the adult rather than to the larval stage of the life cycle. This is a facet of the ecology of *D. mettleri* previously unrecognized. It also demonstrates the opportunistic and invasive character of this species which may reflect a greater colonizing ability than other desert-adapted *Drosophila*.

There is some question as to the biological function of plant alkaloids. Only a few alkaloids have been studied with respect to their role in insect-host plant relationships, and most of these have been described as either repellents, feeding inhibitors, or acute toxins (Beck & Reese, 1976). On the other hand, Robinson (1974) pointed out that although alkaloids are not inert end products of metabolism, there is little hard evidence to support the idea that their primary role is that of plant defense substances. Even less is known about the action of dietary alkaloids on insects.

Several investigators have suggested that alkaloids affect insects by blocking steroid metabolism or phyto-steroid assimilation (Schreiber, 1958; Harley & Thorsteinson, 1967).

Nevertheless, *D. pachea* and *D. mettleri* have adapted to a substrate containing high concentrations of alkaloids. One question currently being examined is how *D. mettleri* developed its tolerance to senita alkaloids when its use of this cactus compared to saguaro and cardón is relatively minor. Our speculations include two possibilities. One is that desert soil typically contains biologically significant amounts of alkaloids which have accumulated over evolutionary time due to the death and decay of alkaloid-containing plants. Many plants in the desert including certain legumes, creosote bushes, and numerous species of cacti are known to contain alkaloids (Brown *et al.*, 1968). The movement of *D. mettleri* into the soil niche was, therefore, concurrent with tolerance to alkaloids in general. Another possibility is that the typical breeding substrates of *D. mettleri* represent situations where chemicals in the plant are concentrated. Evaporation of water from the soaked-soil substrate could easily produce this phenomenon. Saguaro tissue has been found to contain 1.0–1.5% alkaloids (dry weight basis) of which carnegine and gigantine are the major constituents (Brown *et al.*, 1972). The concentration of alkaloids in saguaro-soaked soils has never been determined. These speculations are not mutually exclusive and should certainly be pursued.

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REFERENCES

- BECK S. D. & REESE J. C. (1976) Insect-plant interactions: nutrition and metabolism. *Rec. Adv. Phytochem.* **10**, 41–92.
- BROWN S. D., MASSINGILL J. L. JR. & HODGKINS J. E. (1968) Cactus alkaloids. *Phytochemistry* **7**, 2031–2036.
- BROWN S. D., HODGKINS J. E., MASSINGILL J. L. JR. & REINECKE M. G. (1972) The isolation, structure, synthesis and absolute configuration of the cactus alkaloid gigantine. *J. Org. Chem.* **37**, 1825–1828.
- CAMPBELL C. E. & KIRCHER H. W. (1980) Senita cactus: a plant with interrupted sterol biosynthetic pathways. *Phytochemistry* **19**, 2777–2779.
- FELLOWS D. P. & HEED W. B. (1972) Factors affecting host plant selection in desert-adapted cactophilic *Drosophila*. *Ecology* **53**, 850–858.
- FOGLEMAN J. C., HACKBARTH K. R. & HEED W. B. (1981) Behavioral differentiation between two species of cactophilic *Drosophila*. III. Oviposition site preference. *Am. Nat.* **118**, 541–548.
- HARLEY K. L. S. & THORSTEINSON A. J. (1967) The influence of plant chemicals on the feeding behavior, development and survival of the two-striped grasshopper, *Melanoplus bivittatus* (Say). Acrididae: Orthoptera. *Can. J. Zool.* **45**, 305.
- HEED W. B. (1977) A new cactus-feeding but soil-breeding species of *Drosophila* (Diptera: Drosophilidae). *Proc. Entomol. Soc. Wash.* **79**, 649–654.
- HEED W. B. (1978) Ecology and genetics of Sonoran Desert *Drosophila*. In *Ecological Genetics: The Interface* (Edited by BRUSSARD P. F.), pp. 109–126. Springer, New York.
- HEED W. B. & KIRCHER H. W. (1965) Unique sterol in the

- ecology and nutrition of *Drosophila pachea*. *Science* **149**, 758-761.
- HEED W. B. & JENSEN R. W. (1966) *Drosophila* ecology of the senita cactus, *Lophocereus schottii*. *Dros. Inf. Serv.* **43**, 94.
- KIRCHER H. W. (1969) The distribution of sterols, alkaloids and fatty acids in senita cactus, *Lophocereus schottii*, over its range in Sonora, Mexico. *Phytochemistry* **8**, 1481-1488.
- KIRCHER H. W., HEED W. B., RUSSELL J. S. & GROVE J. (1967) Senita cactus alkaloids: their significance to Sonoran Desert *Drosophila* ecology. *J. Insect. Physiol.* **13**, 1869-1874.
- ROBINSON T. (1974) Metabolism and function of alkaloids in plants. *Science* **184**, 430-435.
- SCHREIBER K. (1958) Über einige Inhaltsstoffe der Solanaceen und ihre Bedeutung für die Kartoffelkäferresistenz. *Ent. Exp. Appl.* **1**, 28-37.
- SNEDECOR G. W. & COCHRAN W. G. (1967) *Statistical Methods*. Iowa State University Press, Ames.