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Involvement of cytochrome P450 in host-plant utilization by Sonoran Desert *Drosophila*

(polysubstrate monooxygenase/allelochemical detoxication/alkaloid resistance/alkaloid metabolism/larval viability)

MICHAEL R. FRANK AND JAMES C. FOGLEMAN*

Department of Biological Sciences, University of Denver, Denver, CO 80208

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ABSTRACT **The four *Drosophila* species endemic to the Sonoran Desert (*Drosophila mettleri*, *Drosophila mojavensis*, *Drosophila nigrospiracula*, and *Drosophila pachea*) utilize necrotic cactus tissue or soil soaked by rot exudate as breeding substrates. Each *Drosophila* species uses a different cactus species as its primary host. *D. pachea* is limited to senita cactus by a biochemical dependency on unusual sterols available only in that cactus. For the other *Drosophila* species, no such chemical dependencies exist to explain the relationships with their primary host plants. Each cactus species has a different array of allelochemicals that have detrimental effects on nonresident fly species. We have hypothesized that the desert fly–cactus associations are due, in part, to differences between the fly species in their allelochemical detoxication enzymes, the cytochrome P450 system. To test whether P450s are involved in the detoxication of cactus allelochemicals, several experiments were done. (*i*) The effect of a specific P450 inhibitor, piperonyl butoxide, on larval survival through eclosion on each cactus substrate was investigated. (*ii*) *In vitro* metabolism of cactus alkaloids was determined for each *Drosophila* species. The effects of specific inducers and inhibitors were included in these experiments. (*iii*) The basal and induced content of cytochrome P450 in each species was determined. The results support the hypothesis that P450 enzymes are involved in host-plant utilization by these Sonoran Desert *Drosophila* species.**

For >20 yr, the interrelationships of columnar cacti and *Drosophila* of the Sonoran Desert of the southwestern United States and northwestern Mexico have provided an excellent model system with which to study relevant questions in evolution, ecological genetics, and chemical ecology. These cactophilic *Drosophila* use the necrotic pockets that form in the stems of the columnar cacti or in soil soaked by rot exudate for all stages of their life cycle. To do this, the flies must be able to locate suitable rot pockets, assimilate required nutrients, and tolerate the array of toxic allelochemicals in the cactus tissue. Chemical interactions between the cacti and the flies, therefore, are of major importance in determining the pattern of host-plant–*Drosophila* relationships that exist in the Sonoran Desert (1).

The four *Drosophila* species that are endemic to the Sonoran desert are *Drosophila nigrospiracula*, *Drosophila mettleri*, *Drosophila mojavensis*, and *Drosophila pachea*. The four species are not closely related phylogenetically, although all are members of the major virilis-repleta radiation of the genus *Drosophila*. These species appear to have adapted independently to the desert environment (2). The major population center of the nearest relative of each species is found outside the desert or overlaps only slightly with the desert. Each of the endemic species also uses a different cactus species as a host, in a nearly one-to-one relationship, as demonstrated by extensive rearing records and collection of adults from naturally occurring rots (1). In any particular location, each *Drosophila* species generally utilizes the necrotic tissue of only one cactus species. Due to differences in the geographic distribution of the cacti and one case of behavioral preference, three of the four fly species exhibit a shift in host plants between the Baja Peninsula and the mainland Sonoran Desert. *D. nigrospiracula* uses saguaro (*Carnegiea gigantea*) on the mainland and cardón (*Pachycereus pringlei*) on the peninsula. Saguaro cactus is only found on the mainland, and cardón is primarily restricted to the Baja peninsula. The two cacti are morphologically and chemically very similar and have low concentrations (≈1% dry weight) of simple isoquinoline alkaloids (e.g., carnegine and gigantine in saguaro) as their primary allelochemicals. The host plants for *D. mojavensis* are agria (*Stenocereus gummosus*) and organ pipe (*Stenocereus thurberi*), which are sympatric on the peninsula. *D. mojavensis* shows a clear preference for agria and utilizes organ pipe on the mainland, where agria is all but absent. Agria and organ pipe do not contain alkaloids, but both have sterol diols, medium-chain (C_8–C_{12}) fatty acids, and triterpene glycosides. These compounds have been shown to reduce fitness as measured by larval viability, development time, adult longevity, and/or thorax size (3–5). *D. pachea* has a nutritional dependency on unusual sterols found only in senita cactus (*Lophocereus schottii*) and so undergoes no host shift (3). Senita contains fairly high concentrations (3–15% dry weight) of more complex isoquinoline alkaloids (lophocereine and its trimers, pilocereine and piloceredine) that are toxic to both adults and larvae of *D. nigrospiracula*, *D. mojavensis*, and to species found on the margin of the desert, including *Drosophila melanogaster* (6).

D. mettleri exhibits a trait that is very unusual in the genus *Drosophila*: oviposition in soaked soil. Only two other species display this behavior. *Drosophila heedi* is a known soil breeder and is found in a xeric area of the island of Hawaii. *Drosophila micromettleri*, which is the closest relative of *D. mettleri*, is thought to utilize soaked soil produced by columnar cacti in the Caribbean (2, 7). Because *D. mettleri* oviposits in soil soaked by rot exudate and this situation occurs more frequently with saguaro and cardón than with the other cacti due to differences in the size of the cactus stems, *D. mettleri* exhibits the same host-plant association and host shift as *D. nigrospiracula*. *D. mettleri* can tolerate the allelochemicals found in senita, agria, and organ pipe but is limited to soil breeding by a strong behavioral preference (7). In the soil, this species may encounter all the secondary plant compounds in concentrations significantly higher than in the necrotic tissue due to water evaporation (8, 9). Although *D. nigrospiracula*, *D. mojavensis*, and *D. pachea* can tolerate the secondary plant compounds in the concentration

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Abbreviation: PBO, piperonyl butoxide.

*To whom reprint requests should be addressed.

encountered in the tissue of their respective hosts, none can tolerate the higher concentrations found in soaked soil.

In contrast to the ability to tolerate these diverse allelochemicals that the Sonoran *Drosophila* exhibit, *D. melanogaster* is unable to survive in the necrotic tissue of agria, organ pipe, senita, or saguaro cactus (6). Furthermore, *D. melanogaster* has never been reared out of naturally occurring rots of the first three cacti, and only a few adult *D. melanogaster* have been reared from saguaro (10).

The response of insects to toxic allelochemicals may be behavioral adaptations, modified physiological processes, or biochemical resistance mechanisms. Although the first two are important, there is reasonable agreement that biochemical resistance mechanisms are of primary importance in the development of patterns of host utilization by insects (11). Within this category, there are several enzyme systems that have been implicated in the detoxication of allelochemicals. Of these, the most intensively studied is the cytochrome P450 enzyme system, considered to be the major detoxication system, due to the ability of cytochrome P450s to metabolize an extremely broad range of substrates. The cytochrome P450 enzyme system is also called the polysubstrate monooxygenase system or the mixed-function oxidase system.

The cytochrome P450 enzymes, made up of the terminal oxidase cytochrome P450 and NADPH-cytochrome P450 reductase, are located in the endoplasmic reticulum. They function primarily by converting lipophilic compounds into more hydrophilic forms that are more easily metabolized or excreted by the insect. P450s are involved in processing pheromones, such as disparlure and monocrotaline, and steroids, such as ecdysone (12). The same enzyme system is also involved in the metabolism of insecticides and drugs and in the metabolism and/or activation of mutagens and carcinogens (13–16). In insects, the system has been well-characterized in houseflies (*Musca domestica*) and many economically and agriculturally important insects, especially lepidopterans. For excellent reviews of this aspect of the cytochrome P450 system, see Hodgson (17) and Brattsten and Ahmad (18). In terms of host–plant utilization, cytochrome P450s have been shown to metabolize many secondary plant compounds implicated in plant-defense mechanisms against herbivory—such as alkaloids, phenolics, quinones, sterols, and terpenes (19). The cytochrome P450 enzymes are specified by multiple genetic loci and are differentially inducible. Exposure to a substrate or other inducer may result in elevated levels of a particular isozyme or elevated activity or both (20–23). In *Drosophila*, only the cytochrome P450 system of *D. melanogaster* has been studied, and the emphasis for that species has been on pheromone (ecdysone) processing (24, 25), insecticide resistance, and mutagen metabolism and activation (14–16, 26–38).

Although P450 activity and inducibility were initially thought to correlate with degree of polyphagy in insects (12, 39, 40), recent investigations have indicated that, at least in lepidopterans, activity of P450 more closely correlates with the classes and content of allelochemicals of the host plant (41). The Sonoran Desert *Drosophila* represents a good model system with which to test whether cytochrome P450 activity is involved in host–plant utilization via the detoxication of allelochemicals. Furthermore, because the repleta group species are generally cactophilic, the P450 system in this species group and flies ancestral to it may contain specific features that enabled them to colonize the cactus niche. Comparing the desert flies to *D. melanogaster*, which is in a different subgenus of *Drosophila*, provides an evolutionary context.

The experiments reported herein were designed to investigate the role of P450 in host use by desert *Drosophila*. (*i*) Larval viability on each of the cactus substrates was investigated in the presence of various concentrations of piperonyl butoxide (PBO), a specific cytochrome P450 inhibitor. (*ii*) *In vitro* metabolism of the relevant allelochemicals by cytochrome P450, both induced and uninduced, was evaluated. (*iii*) Determination of basal cytochrome P450 content and inducibility by phenobarbital and cactus allelochemicals were determined.

MATERIALS AND METHODS

***Drosophila* Strains and Larval Induction.** Stocks of *D. nigrospiracula, D. mettleri, D. mojavensis*, and *D. melanogaster* (strains Canton-S and Hikone-R) had been maintained in the laboratory for >4 yr. *D. nigrospiracula, D. mettleri*, and *D. mojavensis* were multi-female lines collected from the mainland section of the Sonoran Desert. Due to the difficulty of maintaining *D. pachea* in the laboratory, it was excluded from the present study. All species were raised on yeasted instant *Drosophila* medium at ambient temperature and humidity. Larval induction was accomplished by adding 1.0 g of powdered cactus tissue to the medium surface, rehydrated by adding deionized water. This amount of rehydrated tissue produced a layer ≈6 mm thick on the medium surface. Induction by phenobarbital was similarly accomplished by adding 1 ml of 2% sodium phenobarbital (pH 9). Induction was carried out for a period of 48 hr before harvest. Induction of *D. nigrospiracula* by senita tissue was not done due to the extreme toxicity of senita alkaloids to *D. nigrospiracula* larvae.

Microsomes. Microsomes were obtained from mid-third-instar larvae. Larvae were collected by flotation in 20% sucrose and rinsed in deionized water. Twenty-gram portions of larvae were used to prepare microsomes by standard methods (27, 42). Microsomal protein concentration was determined with the Pierce Coomassie protein assay reagent kit and resuspended at 2 or 5 mg of microsomal protein per ml. P450 concentration was determined spectrophotometrically by the method of Omura and Sato (43), except reduction was by NADPH rather than by sodium dithionite. Cytochrome P450 content was expressed as nmol of P450 per mg of microsomal protein.

Alkaloid Extraction. Alkaloids were extracted from senita or saguaro tissue by using standard techniques (9, 44), and alkaloid identities were verified by mass spectrometry.

Larval Viability. Cactus substrates were prepared by adding PBO in ether to dried cactus powder, such that after rehydration the concentrations were 0.01, 0.05, 0.1, 1.0, and 10.0 mg of PBO per g of tissue. Control plates without PBO were made similarly. After evaporation of ether, 4 g of the PBO-treated powder was rehydrated in a plastic Petri plate with 16 ml of a microbial suspension containing cactophilic yeast and bacteria originally isolated from natural rots. Three replicate sets were prepared with various concentrations of PBO. All concentrations were not used for each species. First-instar larvae were obtained from oviposition plates (2% water agar with a 1-cm spot of yeast paste in the center). One hundred first-instar larvae were transferred to a 1-cm^2 piece of filter paper in the middle of each substrate plate, and larvae killed in transfer were replaced. In most cases, the same batch of larvae was used to set up all replicates of a concentration series for a particular *Drosophila* species. Eclosion rates were recorded, and the mean eclosion of each replicate set (of a specific PBO concentration) was expressed as percent of the mean eclosion in the control (no PBO) plates.

D. melanogaster was tested in a similar manner on untreated cactus substrates rotted with the same microbial community. Additionally, to determine whether or not the alkaloids are the toxic component of saguaro and senita cactus, *D. melanogaster* Canton-S larvae were transferred to plates containing artificial medium treated with 0.0%, 1.0%, or 5.0% purified senita alkaloids. All *D. melanogaster* ex-

Table 1. Average (± SD) percent viability of resident cactophilic Sonoran *Drosophila* and *D. melanogaster* larvae on cactus substrates

Substrate	Normal resident *Drosophila* species	Average larval viability of normal resident,* %	Average larval viability of *D. melanogaster*, %
Saguaro	*D. nigrospiracula*	68.4 ± 9.8	6.2 ± 1.6
Agria	*D. mojavensis*	73.0 ± 5.7	0.2 ± 0.5
Organ pipe	*D. mojavensis*	77.2 ± 13.0	1.6 ± 1.7
Senita	*D. pachea*	71.4 ± 6.0	0.0 ± 0.0
Control medium			89.6 ± 3.7

Five replicates of 100 larvae were used in each case.
*Data are from J. Fogleman (45).

periments were done with five replicates per treatment. Eclosion data were collected and analyzed as above.

***In Vitro* Metabolism of Alkaloids.** One-milliliter reaction aliquots were standardized to 1.6 mg of microsomal protein. Microsomes containing cytochrome P450 enzymes were incubated with 50 μM (approximate final concentration) saguaro or senita alkaloids (delivered in 7 μl of ethylene glycol monomethyl ether), 167 μl of 50 mM Tris·HCl/50 mM potassium phosphate/1 mM EDTA/1 mM magnesium chloride buffer, pH 7.4, and 500 μM NADPH for 2 hr at 37°C with shaking. Both minus-NADPH and minus-alkaloid controls were set up in parallel to the experimental replicates. Inhibition of alkaloid metabolism was investigated by the addition of PBO (1.0 μM, final concentration) in some experiments. Each treatment was always done in triplicate. After incubation, protein was precipitated by adding 25 μl of 72% trichloroacetic acid and then centrifuging for 15 min in a bench-top centrifuge. The supernatant was added to 200 μl of strong ammonia solution. The unmetabolized substrate was extracted with ether (two extractions of 2.0 ml each, pooled) and then dried under N_2. The extract was resuspended in 100 μl of chloroform, and the alkaloids were quantified by capillary gas chromatography. A Hewlett–Packard 5890A gas chromatograph equipped with a flame ionization detector and a Hewlett–Packard Ultra 2 column (crosslinked 5% phenylmethyl silicone, 25 m × 0.2 mm × 0.33-μm film thickness) was used. The carrier gas was hydrogen at 9 lb of head pressure (1 psi = 6.9 kPa). Injector and detector temperatures were 300°C, and the sample was run for 8 min at 220°C. Peak identity of carnegine was confirmed by comparison of the retention time of a commercial standard (Pfaltz & Bauer). The amount of alkaloid metabolized was determined by subtracting the area count of the alkaloid peak of the experimental tube from the area count of the same peak in the minus-NADPH control tube. These values were expressed in terms of area counts metabolized per mg of microsomal protein. The minus-alkaloid controls served to ensure that the alkaloid peaks did not overlap with peaks representing coextracted compounds. The induced versus uninduced and inhibited versus uninhibited cases were tested by one-way ANOVA. When appropriate, the Student–Newman–Keuls procedure was also used.

RESULTS AND DISCUSSION

***In Vivo* Inhibition of Allelochemical Detoxication.** If cytochrome P450 enzymes are involved in larval tolerance to cactus allelochemicals, addition of the P450 inhibitor PBO to the cactus substrate should reduce viability. The viability of each species on its normal host is shown in Table 1 and is relatively high, averaging ≈70%. All of the cactus substrates are toxic to *D. melanogaster*, as can be seen in that table.

Because cytochrome P450s are involved in essential physiological pathways (e.g., steroid processing), high PBO concentrations are likely to result in reduced larval viability, even in the absence of allelochemicals. Fig. 1 shows that none of the larvae survived in substrates containing the highest PBO concentration (10 mg of PBO per g of substrate), and, in general, the lowest concentration (0.01 mg/g) did not reduce viability of the larvae. The exception to this was *D. mettleri* on senita, where the lowest concentration resulted in only 10% larval viability. The most interesting effects were seen at the PBO concentration of 1 mg/g (log 1 = 0). At this concentration, both *D. mojavensis* and *D. mettleri* had zero viability on saguaro cactus, but their viability in organ pipe cactus was not significantly reduced compared with control values. The main difference between these two cacti is that saguaro contains isoquinoline alkaloids, whereas organ pipe contains other allelochemicals that are unrelated to alkaloids. *D. mettleri* was also tested on senita cactus, which contains much higher concentrations of alkaloids than saguaro, and the effect of PBO was correspondingly more extreme. The larval viability of *D. mettleri* was zero at all concentrations >0.01 mg/g of PBO in senita. *D. nigrospiracula* was only tested on treated saguaro, as it cannot survive on untreated senita, organ pipe, or agria cactus. On saguaro cactus, *D. nigrospiracula* had zero viability at 0.1 mg/g of PBO but had nearly 100% viability at 0.01 mg/g of PBO.

The differences seen here in the larval viability of each *Drosophila* species on the different cactus substrates at the same concentration of PBO suggest that cytochrome P450 is involved in larval tolerance to the alkaloids of saguaro and senita. Furthermore, differences between *Drosophila* species with respect to alkaloid–PBO inhibitor interactions in saguaro follow known trends in general tolerance to allelochemicals—e.g., *D. nigrospiracula* is the least tolerant because it encounters only low concentrations of only one class of allelochemical (i.e., alkaloids). *D. mojavensis* and *D. mettleri* interact with more diverse host species and a wider array of allelochemicals and are much more tolerant. The

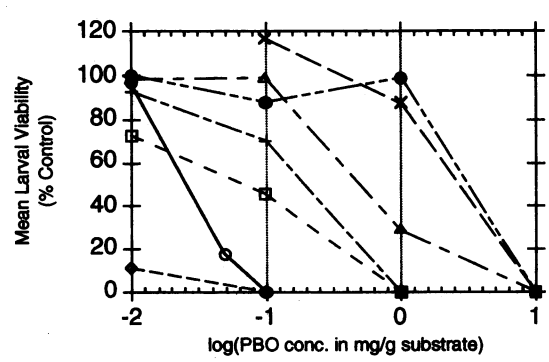

FIG. 1. *In vivo* inhibition of allelochemical detoxication by PBO. Mean larval viability (% control) of three replicates of 100 larvae per treatment. Species were as follows: *D. nigrospiracula* (Nig), *D. mettleri* (Met), and *D. mojavensis (Moj)*. Substrates were as follows: agria (Ag), organ pipe (Op), saguaro (Sag), and senita (Sen). Species–substrate data are listed as Nig–Sag (○), Met–Sag (□), Met–Sen (◇), Met-Op (X), Moj–Sag (+), Moj–Ag (△), and Moj-Op (●). PBO concentration is expressed as log (mg of PBO per g of substrate).

effect of PBO on larval viability of *D. mojavensis* in organ pipe and agria is not as pronounced as in substrates containing alkaloids. This can clearly be seen at the PBO concentration of 1 mg/g. The viability of *D. mojavensis* in organ pipe does not significantly differ from the control value. Although viability is reduced in agria, it is not reduced to zero, as it is in saguaro or senita. It is possible that tolerance to the allelochemicals in agria and organ pipe involve enzymes other than the cytochrome P450s or alternate mechanisms such as target-site insensitivity (46). For this reason, the measurement of *in vitro* metabolism with and without induction or inhibition was limited to activity on cactus alkaloids.

That the alkaloids of senita and saguaro are, in fact, the toxic components of these cacti is supported by the results of the experiment involving *D. melanogaster* Canton-S larvae on senita alkaloid-treated artificial medium. No larvae survived on the 5% senita alkaloid plates for >24 hr, and only a single individual, out of an initial 500 first-instar larvae, survived to eclosion on the 1% alkaloid plates (average viability ± SD = 0.4 ± 0.093). Thus, even relatively low concentrations of purified senita alkaloids are effective in reducing the viability of this species to <1% of the control mean.

***In Vitro* Metabolism of Alkaloids.** If cytochrome P450s are involved in the detoxication of alkaloids, incubating purified alkaloids with microsomal P450s and required cofactors *in vitro* should decrease alkaloid concentration as the alkaloids are metabolized. Additionally, induction and inhibition of this activity should be possible. These effects are demonstrated in Fig. 2. Basal (uninduced) metabolism of the saguaro alkaloids, carnegine and gigantine, was seen in all the species tested, and the differences in uninduced activity between the *Drosophila* species were statistically significant (one-way ANOVA; carnegine: $F_s = 14.757$; $df = 3,17$; $P < 0.001$; gigantine: $F_s = 4.202$; $df = 3,17$; $P < 0.05$). The trend in basal activity was *D. melanogaster* < *D. mojavensis* < *D. nigrospiracula* < *D. mettleri*.

The *in vitro* metabolism of saguaro alkaloids, by both *D. mettleri* and *D. mojavensis*, was significantly induced by senita tissue. In the most extreme case, a 21.0-fold increase in carnegine metabolism and a 49.2-fold increase in gigantine metabolism was observed for *D. mojavensis*. It is important to point out that this result represents cross-induction—i.e., exposure to senita alkaloids increases the metabolism of saguaro alkaloids. Significant induction of P450 activity by saguaro tissue was seen only for *D. mojavensis*. The differences in inducibility between saguaro and senita appear to be from the differences in alkaloid concentration between the two cacti (1% in saguaro vs. 3–15% in senita) because induction of *D. mojavensis* activity by 10-times concentrated purified saguaro alkaloids was highly significant and comparable to induction by senita alkaloids (Fig. 2). Significant induction of saguaro alkaloid metabolism by phenobarbital was observed in all three cactophilic *Drosophila* species.

When the P450 inhibitor PBO was included in the *in vitro* metabolism experiments, significant decreases in carnegine metabolism were observed in every case. In most cases, a similar decrease was observed in gigantine metabolism.

This pattern of significant induction by senita tissue and inhibition by PBO was also seen in experiments on metabolism of the senita alkaloid lophocereine by *D. mettleri*. A 3.8-fold induction was observed ($P < 0.001$), and inhibition by 1 mM PBO reduced metabolism to 67% of the average induced level ($P < 0.01$). No metabolism of the senita trimers, pilocereine and piloceredine, was detected under the gas chromatography conditions used.

Although metabolism of alkaloids in these experiments is seen through reduction in quantity over time, possible metabolites were not identified. At least two new unidentified peaks were seen in the senita-induced extracts (as compared to minus-NADPH controls), and these peaks may represent products of saguaro alkaloid metabolism.

Cytochrome P450 Content and Inducibility. The basal cytochrome P450 content, seen in Fig. 3, differs significantly in each of the species examined ($F_s = 37.704$; $df = 4,17$; $P < 0.001$). The species ranked by P450 content are as follows: *D. melanogaster* (Hikone-R > Canton-S) > *D. mojavensis* > *D. nigrospiracula* > *D. mettleri*. The value for *D. melanogaster* strain Canton-S is similar to published values of P450 content for this strain—e.g., 0.17 ± 0.03 (32). Exposure to phenobarbital resulted in highly significant increases in P450 content for all species, compared with uninduced controls. Exposure of *D. nigrospiracula* to saguaro tissue, its normal host, had no significant effect. Although P450 content in *D. mettleri* was significantly induced by saguaro and senita tissue, these increases are relatively minor compared with the increase from phenobarbital induction. P450 content in *D. mojavensis* was significantly induced by agria tissue, senita

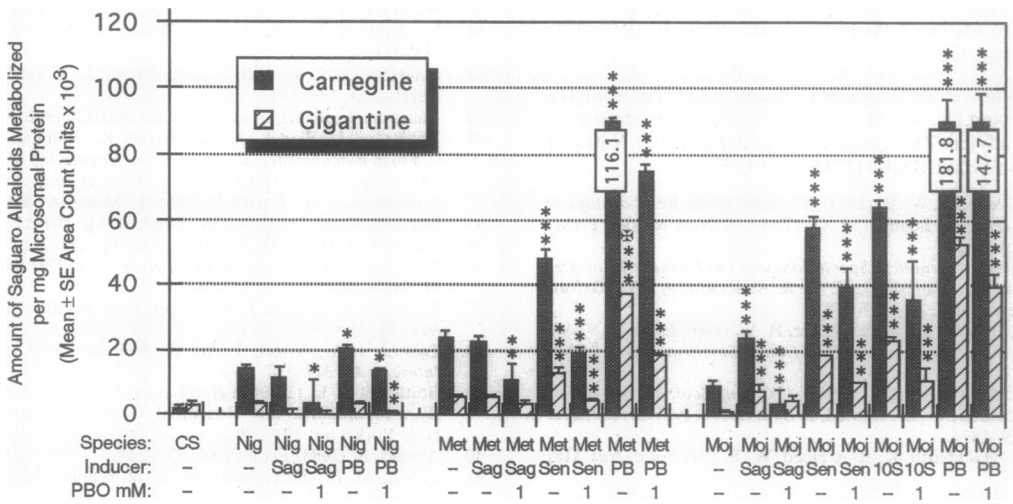

FIG. 2. *In vitro* metabolism of saguaro alkaloids. Asterisks above induced experiments indicate significant differences from the uninduced level in the same species (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$). Asterisks above inhibited experiments indicate significant differences from the uninhibited (induced) experiments. Species were as follows: *D. melanogaster* strain Canton-S (CS), *D. nigrospiracula* (Nig), *D. mettleri* (Met), and *D. mojavensis* (Moj). Inducers were as follows: saguaro (Sag), senita (Sen), 10-times-concentrated purified saguaro alkaloids (10S), and phenobarbital (PB). ✠, Truncated value due to complete metabolism of substrate.

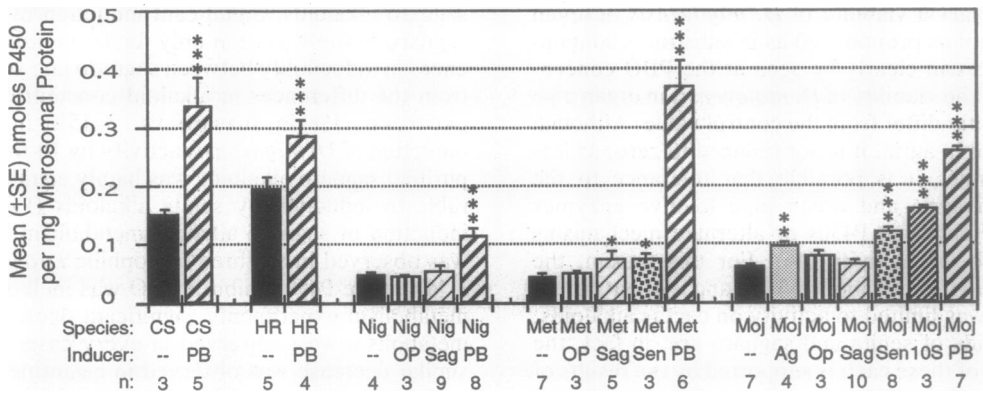

FIG. 3. Cytochrome P450 content of uninduced and induced Sonoran *Drosophila* third-instar larvae. Data were analyzed by one-way ANOVA ($F_s = 29.5965$; $df = 19,87$; $P < 0.001$) for the entire data set, and means were compared by Student–Newman–Keuls. Levels of significance indicated are treated vs. untreated within species (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$). Species were as follows: *D. melanogaster* strain Canton-S (CS), *D. melanogaster* strain Hikone-R (HR), *D. nigrospiracula* (Nig), *D. mettleri* (Met), and *D. mojavensis* (Moj). Inducers were as follows: agria (Ag), organ pipe (Op), phenobarbital (PB), saguaro (Sag), senita (Sen), and 10-times-concentrated purified saguaro alkaloids (10S).

tissue, and 10× concentration of saguaro alkaloids but was not induced by saguaro or organ pipe tissue. Interestingly, alkaloid-detoxication activity appears to negatively correlate with cytochrome P450 content, in that the species with the highest content (*D. melanogaster*) has the lowest activity, and the species with the lowest content (*D. mettleri*) has the highest basal activity. This result together with the fact that induction of activity does not, in all cases, include an increase in P450 content (and vice versa) suggests that a specific P450 isozyme may be involved in alkaloid detoxication.

In conclusion, three main results were obtained in these experiments: (*i*) a complete loss of larval viability in substrates that contain both alkaloids and the P450 inhibitor PBO at concentrations that had no effect on larval viability in other substrates (including those known to contain toxic allelochemicals), (*ii*) significant induction of cactus alkaloid metabolism (*in vitro*) by saguaro tissue, senita tissue, 10× saguaro-alkaloid concentration, and the known inducer, phenobarbital, and subsequent reduction of metabolism when the enzymes were inhibited by PBO and (*iii*) induction of total P450 content by saguaro tissue, 10-times-concentrated saguaro-alkaloids, senita tissue, and phenobarbital. These data, taken as a whole, strongly support the contention that P450 enzymes are involved in the detoxification of cactus alkaloids and, therefore, host-plant utilization by drosophilids in the Sonoran Desert.

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