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DIVERGENCE IN CACTOPHILIC *DROSOPHILA*: THE EVOLUTIONARY SIGNIFICANCE OF ADULT ETHANOL METABOLISM

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Four species of *Drosophila* endemic to the Sonoran Desert feed and breed on giant columnar cacti. Each species has an independent phylogenetic origin (Heed, 1982), yet all have invaded desert conditions by adapting to different host cacti. Necrotic cactus tissues ("rots") undergo microbial fermentation, providing nutrients such as alcohols, esters, and fatty acids for larvae and adults (Heed, 1978; Fogleman and Heed, 1989). Polyphagic *D. mojavensis* use the rots of several host cacti, including organ pipe (*Stenocereus thurberi*) and pitaya agria (*S. gummosus*) (Fellows and Heed, 1972; Heed and Mangan, 1986; Etges and Heed, 1987), that produce many volatile compounds which adults metabolize (Starmer et al., 1977; Etges and Klassen, 1989). Tissues of these cacti contain free monosaccharides (Kircher, 1982), which are necessary for adult survival (Sang, 1978), and relatively high concentrations of complex oligosaccharides, triterpene glycosides, which are available as nutrients only after microbial degradation. Fermenting agria and organ pipe tissues typically contain higher concentrations of volatile compounds, particularly ethanol, than *Opuntia* or other Sonoran Desert columnar cacti, because of the high tissue concentrations of fermentable simple and complex sugars (Starmer et al., 1986; Fogleman and Heed, 1989). Other sources of free carbohydrates, such as cactus fruits, are very seasonal or absent.

Adult *D. mojavensis* survive in these volatile-rich environments by assimilating atmospheric volatiles that increase longevity (Starmer et al., 1977; Batterham et al., 1982), metabolic rates, and lifetime fecundity (Etges and Klassen, 1989). The rate of ^{14}C-ethanol vapor assimilation is greater in females than in males, as is evident from assays of ^{14}C-containing metabolites in both whole body tissues and respired $^{14}CO_2$, which provide direct evidence that adults metabolize ethanol vapor and accumulate some ethanol metabolites in body tissues.

Of the other endemic species, *D. pachea* uses only senita cactus (*Lophocereus schottii*), and *D. nigrospiracula* and *D. mettleri* use saguaro (*Carnegiea gigantea*) or cardon (*Pachycereus pringlei*). Senita, saguaro, and cardon rots contain much lower concentrations of usable volatiles, and the drosophilid species that inhabit them do not show longevity increases due to ethanol vapor (Heed, 1978).

The degree to which *D. mojavensis* can metabolize volatiles such as atmospheric ethanol suggests that this species is physiologically adapted to the unique chemistries of its host cacti (Heed and Mangan, 1986). The closest relatives of *D. mojavensis* are two sibling species *D. arizonensis* and *D.* "Sp. N," an undescribed species from Navojoa, Sonora. Both are found primarily outside the desert. The last species breeds and feeds exclusively in fermenting cladodes of *Opuntia* cactus; *D. arizonensis* uses a variety of columnar cacti and platyopuntias throughout its range, and it is occasionally found in the same rots as *D. mojavensis* (Heed, 1982; Ruiz and Heed, 1988).

If the ability to metabolize volatiles such as ethanol vapor is an adaptation for survival in volatile-rich columnar cactus rots, then *D. mojavensis* and perhaps *D. arizonensis* should show greater adult survivorship in those environments than their *Opuntia*-breeding relatives. *Opuntia* pads contain moderate concentrations of free sugars and low concentrations of some volatiles and alkaloids (Kircher, 1982; Fogleman, 1982; Brazner et al., 1984; Ruiz et al., 1985). In this report, I show that *D. mojavensis* metabolizes ethanol vapor differently than its *Opuntia*-breeding relatives. The latter species store ethanol metabolites in body tissues and do not use them as an energy supply for increasing adult longevity to the degree that *D. mojavensis* does.

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TABLE 1. Collection information for the *Drosophila* species used in this study. Reference-stock numbers are those of W. B. Heed at the University of Arizona. Cina is the common name for *Stenocereus alamosensis*, the principal host plant of *D. arizonensis* in northwestern Mexico (Ruiz and Heed, 1988).

Species	Locality	Reference stock	Host cactus	Number of founders
D. mojavensis	Sil Nakya, Arizona	A900	Organ pipe	352
D. arizonensis	Las Bocas, Sonora	A891	Cina	550
D. "Sp. N"	Las Bocas, Sonora	A876	*Opuntia wilcoxi*	474
D. mulleri	Discovery Bay, Jamaica	ORV-25	*Opuntia stricta*	96

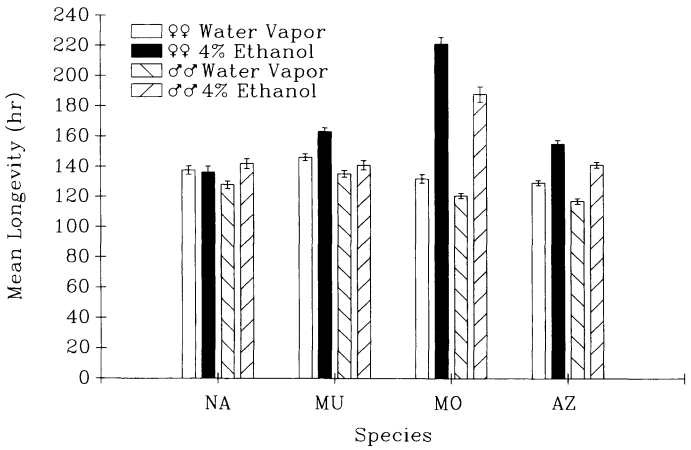

FIG. 1. Mean longevity (±SE) of adult *Drosophila* held over water vapor or 4% ethanol vapor. *D. mojavensis* (MO) and *D. arizonensis* (AZ) breed on columnar cacti, whereas *D.* "Sp. N" (NA) and *D. mulleri* (MU) are obligate *Opuntia* breeders.

MATERIALS AND METHODS

Outbred lab stocks of *D. mojavensis, D. arizonensis, D.* "Sp. N," and a more distantly related *Opuntia* breeder, *D. mulleri,* were used in longevity tests (Table 1). Each species was cultured in 8–10 half-pint milk bottles containing agar-malt-Karo-banana food at room temperature. Pupae of each species were collected on file cards inserted into the medium in each bottle. Prior to eclosion, all cards were removed, grouped by species, and transferred to empty bottles to avoid posteclosion feeding on lab food. All emerged adults of each species were collected each day. Ten adults of each sex were anesthetized with CO_2 and loaded separately into one-dram shell vials that were closed with several layers of cheesecloth secured with a rubber band. Each day, groups of vials containing each species were divided evenly and placed into sealed desiccators containing one liter of water (control) or 4% (volume/volume) ethanol in a 25°C incubator with a 14L:10D photoperiod. Thirty-two vials of both males and females of each species were assigned to each treatment (total $N = 512$ vials). Relative humidity in the desiccators was not assayed, but the air spaces were assumed to be nearly saturated. Adult longevity was measured by counting the number of dead adults every 12 hours, until all adults were dead. Mean longevity per vial was calculated by linear interpolation between successive time intervals. Dry weights of adults were recorded at the end of the experiment, after drying all vials containing the dead adults for two weeks at 60°C. Adults were recounted and weighed to the nearest 0.0001 g. Longevity and body-weight differences were assayed by analyses of variance.

RESULTS

Adult longevity was increased by 4% ethanol vapor in all cases, except for *D.* "Sp. N" females and *D. mulleri* males (Fig. 1, Table 2). Relative increases in longevity ([longevity in 4% ethanol vapor]/[longevity in water vapor]; Starmer et al., 1977) were much greater for *D. mojavensis* than for the other species: 1.1 for *D.* "Sp. N" and *D. mulleri,* 1.2 for *D. arizonensis,* and 1.6 for *D. mojavensis.* Thus, this population of *D. mojavensis* derived a 64% increase in adult longevity due to the assimilation of 4% ethanol vapor.

Under such starvation conditions, adults did not lose weight as rapidly in ethanol vapor as in water vapor. Because all eclosed adults used at the start of the ex-

TABLE 2. Results of analysis of variance for adult longevity and dry weight among four species of cactophilic *Drosophila.* Treatment refers to lifetime exposure to water vapor or 4% ethanol vapor. The error *d.f.* reflect the fact that 12 vials were lost during the experiment.

Source of variation	*d.f.*	Mean longevity (hr)		Body weight (mg/fly)	
		MS	*F*	MS	*F*
Species	3	23,391.8	89.99****	0.109	315.59****
Sex	1	26,206.6	100.82***	0.147	374.64****
Treatment	1	115,223.7	443.27***	0.135	345.27****
Species × sex	3	2,282.3	8.78***	0.002	5.87**
Species × treatment	3	33,192.0	127.69****	0.012	31.57***
Sex × treatment	1	1,073.5	4.13*	0.006	14.19***
Species × sex × treatment	3	2,044.4	7.87***	0.000	0.86
Error	484	259.9		0.00035	

* $P < 0.05$; ** $P < 0.025$; *** $P < 0.001$; **** $P < 0.0001$.

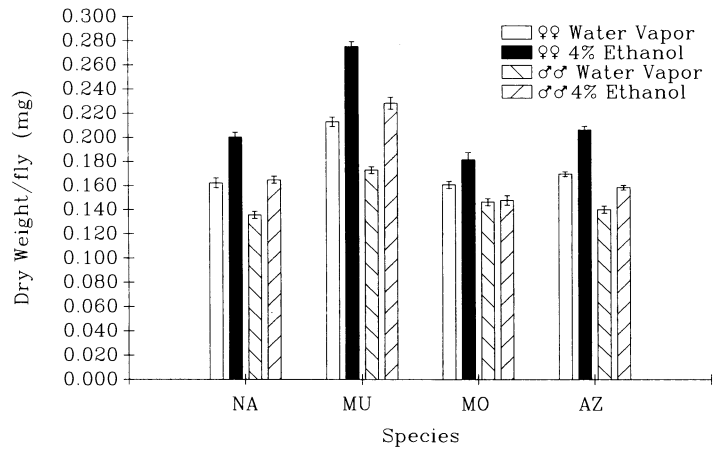

FIG. 2. Mean dry weight (±SE) of adult *Drosophila* held over water vapor or 4% ethanol vapor. Species designations: MO = *D. mojavensis,* AZ = *D. arizonensis,* NA = *D.* "Sp. N," and MU = *D. mulleri.*

periment were randomly assigned to each treatment each day, differences in dry weight at the end of the experiment due to ethanol vapor resulted from adult assimilation of ethanol into body tissues (Fig. 2). This confirms earlier observations of ^{14}C-ethanol-metabolite accumulation in *D. mojavensis* adults exposed to labelled 4% ethanol vapor (Etges and Klassen, 1989). All groups in 4% ethanol vapor except *D. mojavensis* males weighed more at the end of the experiment than did control flies exposed to water vapor.

Among species, the degree to which ethanol vapor-derived metabolites were assimilated into body tissues was negatively correlated with increases in longevity. Flies of a particular sex and species that lived longer due to ethanol vapor lost body weight more quickly ($r = -0.64$). Whereas both *Opuntia* breeders (*D. mulleri* and *D.* "Sp. N") derived slight increases in longevity compared to *D. mojavensis,* the former species were able to assimilate ethanol-metabolites into adult tissues at a higher rate than was *D. mojavensis.* This suggests that *D. mojavensis* and, to a lesser extent, *D. arizonensis* metabolize assimilated ethanol vapor engendering increased longevity at a higher rate than do *D. mulleri* and *D.* "Sp. N." The weight differences were not due to storage of ethanol, because the adult carcasses were dried for two weeks at 60°C prior to weighing, and any residual ethanol would have vaporized.

DISCUSSION

Since *D. mojavensis* can metabolize ethanol vapor (Etges and Klassen, 1989), the longevity increases (or lack of them) in its sibling species can be inferred to have resulted from the same metabolic pathway involving alcohol dehydrogenase, the enzyme responsible for oxidation of a number of primary alcohols that ultimately undergo lipid biosynthesis or are metabolized via the citric-acid cycle (Middleton and Kacser, 1983; Geer et al., 1986). It is not clear whether storage involves lipogenesis and the fat body (where 70% of total alcohol dehydrogenase [ADH] activity in *D. mojavensis* is located in males and where 30% of ADH activity is located in females), egg development in ovarian tissues (where 50% of total ADH activity is located in females [Batterham et al., 1983]), or accumulation in growing adult tissues, such as the inner muscle attachments in the thorax (Johnston and Ellison, 1982).

Longevity extension caused by exposure to atmospheric volatiles varies considerably in other *Drosophila* species (Van Herrewege and David, 1974; Parsons, 1981; Parsons et al., 1979; Holmes et al., 1980). Parsons (1981) suggested that ethanol tolerance is correlated with ambient volatile concentration in natural breeding sites among Australian *Drosophila.* While liquid-phase volatile concentrations may vary among natural breeding sites (Starmer et al., 1986; McKechnie and Morgan, 1982), concentrations of gaseous-phase volatiles that adults can use are unknown for any species. Ethanol and ethyl acetate vapor-induced increases in body weight in *Opuntia*-breeding *D. buzzatii* have been noted (Le, 1983).

Coinciding with the speciation of the *repleta* species-group and the *mulleri* subgroup of *Drosophila* was the North American radiation of columnar cacti, which evolved from lineages in central Mexico during the Oligocene or Miocene (Throckmorton, 1975; Gibson, 1982). The platyopuntias (*Opuntia*) are considered at least as old as the columnar cacti and represent the ancestral growth form; therefore, use of columnar cacti is considered to be a derived habit (Heed, 1982; Brazner et al., 1984; Ruiz and Heed, 1988). Divergence and speciation of these cactophilic *Drosophila* during colonization of columnar cacti from platyopuntias can reasonably be inferred to have been facilitated by evolution of those specialized adult physiological mechanisms that have permitted successful colonization of abundant resources, the volatile by-products of the fermenting tissues of columnar cacti.

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