Influences of Atmospheric Ethanol on Adult *Drosophila mojavensis:* Altered Metabolic Rates and Increases in Fitness among Populations

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Accepted 6/2/88

Abstract

Adult Drosophila mojavensis *are exposed to low-molecular-weight volatiles produced by fermenting tissues, "rots," of several columnar cacti endemic to deserts of Mexico and Arizona. Because these cactus tissues lack carbohydrates required for adult survival, volatiles have been hypothesized to be sources of energy. Extension of adult longevity in 4% ethanol versus water vapor under starvation conditions and in the presence of fermenting cacti used in nature for feeding was determined in a system designed to avoid hypercapnic side effects common to many studies. Adults from a mainland population survived longer in 4% ethanol vapor than adults from a Baja population, with females always living longer than males under starvation conditions. No carryover of stored metabolites derived from ethanol vapor was detected under starvation conditions. Metabolic rates, estimated by study of oxygen consumption in a closed system, were higher in ethanol vapor than in water vapor. Significant amounts of* ^{14}*C-ethanol metabolites were recovered in body tissues and as respired carbon dioxide in radioisotope studies. Ethanol vapor also increased lifetime fecundity but had no effect on age at first reproduction. Ability to survive and reproduce in low-molecular-weight volatile-rich, carbohydrate-poor cactus environments has allowed* D. mojavensis *to colonize extensive desert regions now within their species range.*

Introduction

Attempts to relate physiological consequences of enzyme polymorphisms with the ecological circumstances implicated in their maintenance are ex-

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Physiological Zoology 62(1):170–193. 1989. 0031-935X/89/6201-87115$02.00

emplified in studies of ethanol metabolism in *Drosophila*. Alcohol dehydrogenase (ADH; EC 1.1.1.1) is primarily responsible for oxidation of ethanol to acetaldehyde which is converted to acetate, ultimately entering the citric acid cycle or lipid biosynthetic pathways. Because a majority of *Drosophila* species worldwide feed and breed in fermenting substrates, ADH has been intensively studied and thought to play a role in energy metabolism (Daly and Clarke 1981) in both larvae (David et al. 1976; Deltombe-Lietaert et al. 1979; McKechnie and Geer 1984; Geer, McKechnie, and Langevin 1986) and adults (Van Herrewege and David 1974, 1980, 1984; Starmer, Heed, and Rockwood-Sluss 1977; Parsons, Stanley, and Spence 1979). In *D. melanogaster,* allelic variants at the Adh locus exhibit different specific activities (V_{max}; Day, Hillier, and Clark 1974) and different tolerances to ethanol stress, heat, and pH optima (Vigue and Johnson 1973; Briscoe, Malpica, and Robertson 1975; van Delden 1982). DNA sequence conservation in the coding regions of different ADH alleles, particularly third-position substitutions, suggests a strong role of purifying selection acting on nucleotide polymorphisms among ADH variants (Kreitman 1983).

Exposure to low concentrations of atmospheric ethanol and some low-molecular-weight volatiles (LMWV) has been shown to increase adult longevity in several species of *Drosophila* in laboratory starvation experiments (Starmer et al. 1977; Van Herrewege and David 1978; Parsons 1981; Batterham et al. 1982; Brazner, Aberdeen, and Starmer 1984). Most adult *Drosophila* require only simple carbohydrates, such as fructose or glucose, for survival (Sang and King 1961; Sang 1978). Atmospheric ethanol and other LMWV produced in the fermentation process may thus provide a major energy source, via the ADH pathway into the citric acid cycle and lipid biosynthesis, for adults in carbohydrate-poor environments. However, knowledge of breeding and feeding site ecology of most *Drosophila* species, particularly concentrations of LMWV, is incomplete. Therefore, the importance of ethanol and LMWV utilization in adult *Drosophila* involving the ADH pathway is unclear.

Several columnar cactus-breeding *Drosophila* of the Sonoran Desert in northwestern Mexico and Arizona are exposed to LMWV as a consequence of their dependence on fermenting cactus tissues, "rots," throughout their life cycle (Heed 1978; Heed and Mangan 1986). Of particular interest, *D. mojavensis* is polyphagic (Fellows and Heed 1972; Etges and Heed 1987), using two cactus species—organ pipe cactus (*Stenocereus thurberi*) and pitaya agria (*S. gummosus*) (fig. 1)—for feeding and breeding over most of its species range. Rots of both cacti are replete with by-products of microbial metabolism, particularly low-molecular-weight alcohols, fatty acids, and esters. Many of these are volatile (Fogleman and Heed 1989). Tissues of both

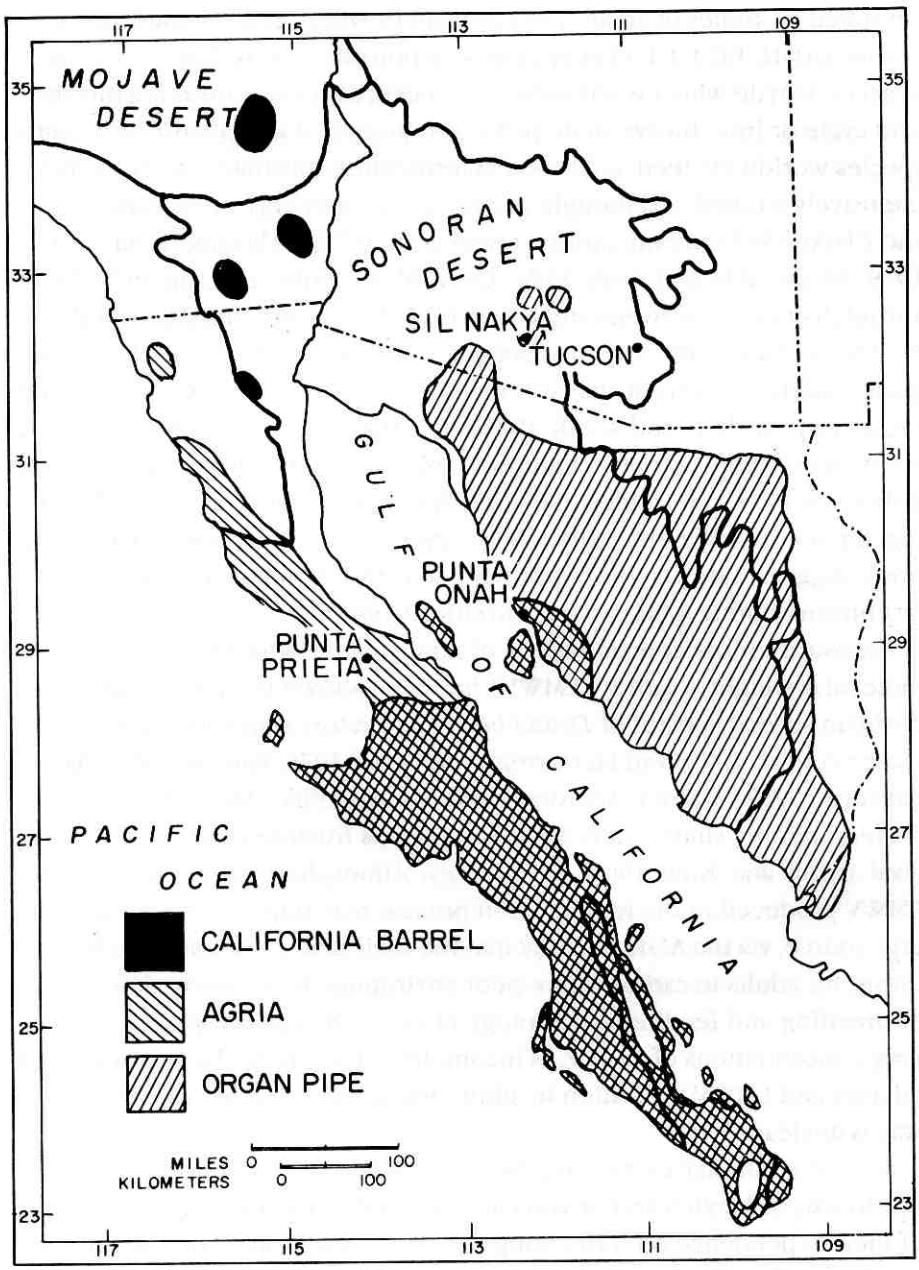

Fig. 1. Map of the Sonoran Desert, showing the origin of experimental populations and distribution of major host plants used by Drosophila mojavensis.

cactus species also contain 20%–36% (dry weight) triterpene glycosides which are hydrolyzed into aglycones and simple sugars, the latter being selectively consumed during fermentation (Kircher 1982; Fogleman and Heed

1989). Larvae of *D. mojavensis* may feed on those sugars early in the process of necrosis, but adults are prevented from using them, as sugars are quickly metabolized *within* the tissue-microbe matrix and the adults feed on rot surfaces (Vacek, Starmer, and Heed 1979). Thus, adult *D. mojavensis* inhabit carbohydrate-poor, LMWV-rich environments (Starmer 1982*b*; Fogleman 1982; Fogleman and Armstrong 1988; Fogleman and Heed 1989).

Geographic variation in host plant use is correlated with variation in ADH allele frequency (Zouros 1973; Starmer et al. 1977; C. Mahaffey, personal communication), and variation in ethanol increased adult longevity among populations of *D. mojavensis:* adults, particularly females, from Arizona and Sonora, Mexico, survived almost twice as long as Baja adults when exposed to low concentrations of atmospheric ethanol (Starmer et al. 1977). Populations from Baja California and the islands in the Gulf of California are usually monomorphic for the Adh-F allele, whereas mainland populations contain frequencies of 0.84 or greater of the Adh-S allele (Starmer et al. 1977). Heat stability, pH optima, and substrate specific activity differences of these Adh alleles differ and correspond to several temperature-chemical differences among host cacti, suggesting mechanisms for maintenance of the polymorphism (Starmer et al. 1977).

Therefore, the aims of this study were to (1) demonstrate ethanol vapor–induced increases in adult longevity in populations of *D. mojavensis* in a system designed to eliminate potential hypercapnic side effects, (2) assess the effects of feeding on natural fermenting substrates on longevity in ethanol atmospheres, (3) determine rates of incorporation of ^{14}C-ethanol and recovery of ^{14}C-carbon dioxide derived from metabolism of atmospheric ethanol, (4) measure metabolic rates in ethanol atmospheres, and (5) assess effects of ethanol vapor on other components of fitness, i.e., fecundity and maturation rates.

Material and Methods

Origin of Flies and Laboratory Rearing

Populations of *Drosophila mojavensis* were sampled by aspirating adults from fermenting cactus tissues in nature and by rearing adults from cactus rots returned to the lab. About 300 adults from Sil Nakya, Arizona (collection A900), were reared from two organ pipe rots, and over 10,000 adults were aspirated and reared from 17 agria rots originating from Punta Prieta, Baja California (A896; fig. 1). These flies were reared in large numbers on banana-yeast-malt-Karo-agar food in shell vials until needed.

For the longevity experiments, flies were cultured by placing about 50

mature virgin pairs in each of 20 ½-pint milk bottles and allowing oviposition for 4–5 days. File cards, inserted into the food medium for collection of pupae, were transferred to empty bottles to minimize posteclosion feeding. These bottles were cleared each day of adults, and all individuals were sexed and counted using CO_2 anesthesia.

Adult Longevity Experiments

Adults of each sex were counted into groups of 10–12 and placed into empty 1-dram shell vials that were closed with several layers of sterile cotton gauze held in place with a rubber band. This insured free gaseous diffusion into and out of the vials and minimized potential hypercapnia suspected in lengthening longevity in other studies (Starmer et al. 1977). Each day that adults were collected, these vials were randomly assigned to sealed desiccators containing 1 L of distilled water or 4% ethanol which were replaced daily. Desiccators were incubated at 25 C in a 14L:10D photoperiod. Number of dead flies in each vial was counted every 12 h until all were dead. Desiccator air was replaced at these times. Median longevity per vial was calculated by linear interpolation between successive time intervals. Longevity differences due to ethanol treatment, population, and sex were assayed by analysis of variance.

Experiment 1. Longevities of males and females from Baja and Arizona populations were compared in water versus ethanol vapor to determine whether results obtained by Starmer et al. (1977) using sealed vials could be repeated in a more open atmospheric system. Fifty vials of adults of each sex in each treatment from each population were assayed.

Experiment 2. The design of experiment 1 was repeated, except that halfway through the experiment both water vapor and ethanol vapor groups were split evenly and half the vials were switched to the other treatment to measure carryover effects. Flies that were started in water vapor and transferred to ethanol vapor were expected to live longer than water vapor flies. Flies that were started in ethanol vapor and transferred to water vapor were expected to live nearly as long as those in the ethanol vapor group if ethanol metabolites are stored by adults. About 40 vials for each population and sex were assigned to four treatment groups: water vapor control, ethanol vapor, water vapor–ethanol vapor transfer, and ethanol vapor–water vapor transfer.

Experiment 3. Fermenting agria cactus was added as a food source to determine the effect of feeding on longevity in the presence of ethanol or water

vapor. Shell vials were sterilized, 10 g of fresh agria tissue were pressed into the bottom of each vial, which was closed with a cotton ball and sterilized again. After cooling, cactus tissues were inoculated with an aqueous suspension of a pectolytic bacterium and seven species of yeasts common to natural agria rots (Starmer 1982*a*; Fogleman and Starmer 1985): *Pichia cactophila, P. mexicana, P. amethionina* var. *amethionina, Cryptococcus cereanus, Candida valida, Candida ingens,* and *Candida sonorensis.* Control vials in both vapor treatments contained flies but no cactus.

Lifetime Fecundity Experiments

The influence of atmospheric ethanol and cactus substrate on fecundity, longevity, and age at first reproduction was measured for both Baja and Arizona populations. Females were collected on the day of eclosion and placed into 1-dram vials containing 5–10-m sections of either agria or organ pipe cactus that had been inoculated 4–5 days before use as in experiment 3. Vials were randomly assigned to either sealed desiccators at 25 C containing water or 4% ethanol vapor as before. Two mature males from the same population were kept with each female throughout her lifetime. Age at first reproduction was measured by changing vials daily and observing when the first eggs hatched. Forty females from each population were cultured on each cactus in ethanol or water vapor.

Measurement of Standard Metabolic Rates (SMRs). Oxygen consumption was measured; by using groups of 20 adults in sealed 60-cm^3 syringes, over 20–24 h with an S-3A oxygen analyzer (Applied Electrochemistry, Sunnyvale, Calif.). Each syringe contained a moistened Kimwipe and was flushed with atmospheric air before sealing. Empty control syringes also were flushed, sealed, and held overnight with the fly-containing syringes in an incubator at 25 C. All gas volumes were corrected to standard temperature and pressure. Mass of flies per syringe was recorded immediately after gas analysis. Gas samples were injected into the analyzer with a syringe pump through small in-line filters containing Ascarite (NaOH-coated silicate) and Drierite (anhydrous calcium sulfate) to remove carbon dioxide and water vapor, respectively. Reference oxygen levels were checked after each sample by sampling control syringes. Average volume of oxygen consumed, $\dot{V}_{O_2}$, was calculated using

$$\dot{V}_{O_2} = \frac{(V_I - V_{I-H_2O})(F_I - F_E)}{1 - F_E},$$

where V_I is the volume of gas in the syringe at the start of the experiment, V_{I-H_2O} is the initial volume of water or ethanol vapor in the chamber, F_I is the initial fractional oxygen concentration, and F_E is the fractional oxygen concentration at the end of the exposure period (Vleck 1987). Data were expressed as $\dot{V}O_2$ fly^{-1} h^{-1}.

Two SMR experiments were performed. First, influence of body size on SMR was determined for males and females from both populations cultured at high (small-size) and low (large-size) densities. Groups of 10 males and 10 females were placed in shell vials with lab food. Adults were allowed to oviposit for 2 or 7 days. Ten replicates were started for each treatment and cultured at 25 C. Offspring from these crosses were separated by sex and aged for 10 days on lab food. All syringes were randomly assigned to one of two blocks before oxygen consumption was measured. Live weights of adults in each syringe were measured to the nearest 0.001 g immediately after gas samples were analyzed.

Second, oxygen consumption was measured on freshly eclosed adults in both control and 4%-ethanol-vapor atmospheres. Many control runs confirmed that in-line filters removed atmospheric ethanol as well as water vapor. Tissue paper in the syringes was dampened with a few drops of 4% ethanol. Adults were enclosed in small glass vials covered with cheesecloth inside the syringes, preventing contact between liquid ethanol and adults. A randomized block design was used so that estimation of SMR in ethanol atmospheres could be directly compared with results of the longevity experiments.

In Vivo Metabolism of Ethanol. Groups of 50 1–3-day-old adult Arizona *D. mojavensis* of each sex were held in 10% agar vials to prevent feeding prior to exposure to radioactive ethanol. A solution of 1-^{14}C ethanol (New England Nuclear, Boston) was prepared from a 24-mCi/mmol stock and brought to an activity of approximately 10^6cpm/5 μL. Groups of flies were placed into sealed 125-mL conical flasks along with a screen-covered 1-dram shell vial containing 3 mL of 20% KOH attached to the inside of the flask for collection of labeled CO_2. Two-centimeter lengths of 1.5-cm stiff plastic tubing were stuffed with about 0.2 g of paper tissue, enclosed with nylon netting secured with a rubber band at each end, and suspended in the flasks with thread after the tissue was injected with 1.25 mL of 4% ethanol containing 5 μL of the labeled ethanol solution. Control flies were exposed to unlabeled 4% ethanol.

Groups of flies were exposed to ethanol for 46 or 120 h at room temperature and then frozen. Adults from each flask were counted into a glass scintillation vial and macerated with 2 mL of Scintigest tissue solubilizer (Fisher

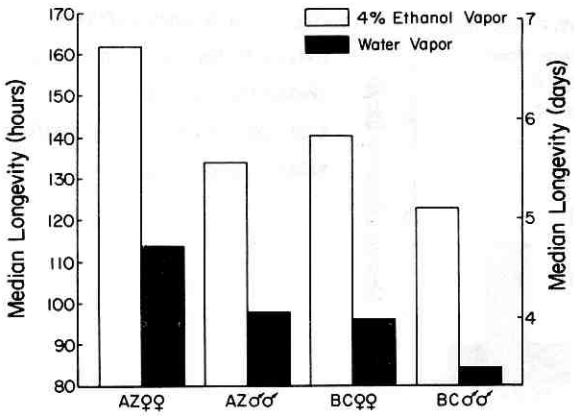

Fig. 2. Adult longevity differences from experiment 1. AZ *refers to the Arizona population, and* BC *refers to the Baja population. Sample size = 399 vials.*

Scientific) and 1–2 mL water. Vials were incubated at 50 C until all tissues were clarified and filled with Scinti Verse II scintillation fluid (Fisher Scientific).

The KOH solution in each flask was transferred to a scintillation vial and completely dried at 60 C to insure that all counts were due to $^{14}CO_2$ as carbonate and not to any residual labeled ethanol. After drying, the KOH was redissolved in 1 mL of distilled water and 0.5 mL of this solution was transferred to a full 10-mL scintillation vial.

Results

1. Effect of Atmospheric Ethanol on Adult Longevity

Adult longevity was significantly increased by 4% ethanol vapor (fig. 2). A preliminary study showed that, relative to water vapor, 6% ethanol vapor shortened longevity so toxicity probably arises at about 5% (see Starmer et al. 1977). The Arizona population exhibited greater longevities than the Baja population in both water and ethanol vapor treatments ($P < .0001$), and females lived longer than males ($P < .0001$). Relative longevity increases (RLI) were calculated by dividing median longevity in ethanol vapor by median longevity in water vapor for each population and sex (Starmer et al. 1977). Here relative increases were 1.43 and 1.37 for Arizona females and males, respectively, and 1.46 for both Baja females and males. Absolute increases in longevity cannot be compared with those reported by Starmer et al. (1977) because Starmer et al. allowed adult feeding on sugar-containing laboratory food for 4 days and used sealed vials. However, they used populations from the same sites as those used in the present study, so RLI can be compared. They recorded RLI of 1.89 and 1.96 for Arizona females and

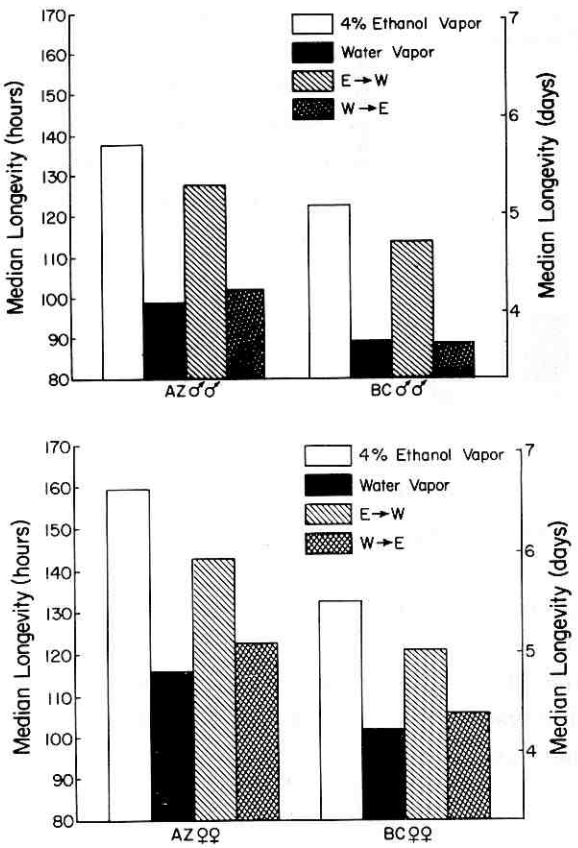

Fig. 3. Longevity differences from experiment 2, showing the absence of carryover effects. Sample size = 638 vials.

males, respectively, and of 1.75 and 1.55 for Baja females and males, respectively. These RLI are significantly greater than those from the present experiment (one-tailed Mann-Whitney *U*-test, $Z = 2.32$, $P = .01$), suggesting that in the study of Starmer et al. (1977) adult feeding and/or hypercapnia may have influenced the estimates of adult longevity.

2. Carryover of Ethanol Vapor Derived Metabolites

Four treatments were compared for longevity differences: 4% ethanol vapor (*E*); water vapor (*W*); ethanol to water vapor, switched after 112 h (*E–W*); and water to ethanol vapor, switched after 86 h (*W–E*). These switch times were estimated from median longevities in each treatment from experiment I so as to maximize any possible carryover effects (fig. 3). In all cases, median longevity in the *E–W* treatment was reduced as compared with the *E* treatment, indicating small or negligible carryover of ethanol metabolites (Baja females: $t = 3.30$, $P = .002$, df = 77; Baja males: $t = 2.14$, $P = .037$, df = 62; Arizona females: $t = 5.38$, $P < 0.0001$, df = 78; Arizona males: $t = 3.32$,

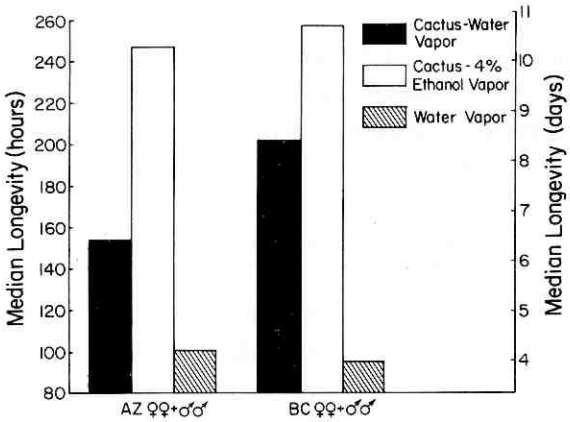

Fig. 4. Longevity differences resulting from the interaction of ethanol vapor and feeding on fermenting cactus, in experiment 3. Sample size = 333 vials.

$P = .001$, df = 67). No longevity increases were produced by switching adults from water vapor to ethanol vapor (all *C* vs. *W–E* comparisons, $P > 0.1$). Thus, 4% ethanol vapor did not extend longevity in prestarved adults.

These data were reanalyzed by calculating residual longevities after the switch from ethanol to water vapor and from water vapor to ethanol vapor in all four treatments. This corrected for intrinsic longevity differences among ethanol and water vapor treatments so that the critical comparison between *W* and *E–W* treatments could be made, i.e., if longevity extension were due to energy carryover derived from ethanol vapor, then, when placed into an ethanol vapor–free environment, adults exposed to ethanol vapor should live longer than adults that never experienced ethanol vapor. Residual longevity of the *E–W* adults was not different from that of the *W* adults (both sexes averaged, 13.5 h vs. 14.8 h, respectively, $t = 0.69$, $P = .488$, df = 320), indicating no significant carryover of ethanol-derived energy.

3. Effects of Adult Feeding on Longevity

When adults from both populations were exposed to fermenting cactus in addition to ethanol or water vapor, with no-cactus treatments included as controls, sex differences in longevity were eliminated, so data were pooled. Baja adults lived significantly longer than Arizona adults (fig. 4; $t = 2.54$, $P = .012$). A significant treatment × population interaction arose from the greater longevity of Arizona adults in the no-cactus treatment and from shorter longevities in the with-cactus treatment (fig. 4; table 1). Thus, this mainland population was more starvation-resistant than the Baja population yet did not live as long when fed fermenting agria cactus. These results suggest that mainland adults may be intrinsically more starvation resistant than Baja adults. To test this hypothesis, we used a two-way analysis of variance

TABLE 1

Analysis-of-variance results for experiment 3, involving adult feeding on fermenting agria tissues or with no cactus in water or ethanol vapor, showing the interaction between feeding and starvation on adult longevity among populations

Source of Variation	df	Mean Square	*F*
Population	1	35,385.802	20.969****
Sex	1	980.666	.446 (NS)
Treatment	2	576,671.518	341.729****
Population × treatment	2	19,629.528	11.627****
Sex × treatment	2	2,811.868	1.666 (NS)
Population × sex	1	1,701.949	.316 (NS)
Population × treatment × sex	2	3,421.739	.191 (NS)
Residual	321	1,687.514	

Note. Observations are median longevity per vial. See the text for details. NS = not significant.
**** $P < .0001$.

to reanalyze longevity differences in water vapor between the six Baja and seven mainland populations studied by Starmer et al. (1977). Differences among regions were not significant ($F = 1.00$, $P = .328$).

4. Influence of Atmospheric Ethanol on Standard Metabolic Rates

Oxygen consumption of aged adults, $\dot{V}O_2$ fly^{-1} h^{-1}, and live weight were homogeneous among blocks, so data were pooled. Sex, population, and culture density influenced oxygen consumption rates, but differences in live weights of adults were caused only by sex and culture density (table 2). Thus, differences in rates of oxygen consumption among populations were not due solely to differences in body mass. All oxygen consumption data were standardized by live weight for each group of adults and were reanalyzed. The main effect of culture density was thus removed (table 2), verifying the influence of body size on SMR. However, sex differences remained, and population differences were marginal ($P = .079$), suggesting intrinsic differences in metabolic rates because of sex and perhaps among populations.

In 1–2-day-old adults in both populations, 4% ethanol vapor increased metabolic rates from 2.25 to 2.59 cm^3 O_2 g^{-1} h^{-1} (table 2). Flies were han-

dled in the same way as those in the longevity studies: adults were not exposed to lab food. Thus, increased longevity caused by 4% ethanol vapor was not due to a metabolic slowdown. Mean live weights of adults in the ethanol treatment were greater than those in the water vapor treatment (0.71 vs. 0.61 mg, respectively, $F = 34.88$, $P < .0001$) after overnight exposure. Thus, SMR of adult *Drosophila mojavensis* in ethanol vapor increased because adults were metabolizing ethanol and presumably not using up larval fat reserves as fast as were flies in water vapor, thereby accounting for the differences in body weight.

Oxygen consumption is considered an allometric function of body mass (Kleiber 1961; Jolicoeur and Heusner 1971) and is expressed as the linear regression of log oxygen consumption on log body mass. All adults were pooled, and the regression coefficient in the first SMR experiment (aged, well-fed adults reared as larvae in low- and high-density conditions) was 0.379 (SE = 0.061); whereas, in the second experiment (1–2-day-old adults in water and ethanol vapor reared as larvae in low-density conditions), the slope increased to 0.804 (SE = 0.089). Thus, variation in metabolic rates was influenced by age and/or feeding, as shown by the change in slopes from the two SMR experiments. Since the well-fed flies in the first experiment were of considerably greater mass than the newly eclosed imagoes in the second experiment (1.12 vs. 0.66 mg), feeding on lab food may have masked individual differences in the first experiment and thus reduced the regression slope.

5. In Vivo Metabolism of Atmospheric Ethanol

After exposure to 4% ^{14}C-labeled ethanol vapor for 46 and 120 h, increasing amounts of label were detected in both adult tissue homogenates and in CO_2 bound to KOH as carbonate (tables 3, 4). Female tissues contained more label than did male tissues, consistent with the SMR data, but there were no sex differences in counts per minute released in CO_2, suggesting that higher female metabolic rates result in greater storage of ethanol metabolites, presumably as lipids, than do male metabolic rates. Even so, counts per minute, both in body tissues and released in CO_2 per adult, were positively correlated ($r = .74$, 32 df, $P < .001$).

6. Ethanol Vapor Effects on Fecundity, Age at First Reproduction, and Longevity with Adult Feeding

Differences in longevity among those same populations were not observed when individual females were reared on fermenting agria and organ pipe

TABLE 2

Analysis-of-variance results for $\dot{V}O_2$, body mass, and SMRs of aged, well-fed adult Drosophila mojavensis *and for newly eclosed adults in water vapor–4% ethanol vapor*

Source of Variation	df	Mean Square	*F*
$\dot{V}O_2$ ($cm^3\ O_2\ fly^{-1}\ h^{-1}$):			
Population	1	120.064	12.010****
Sex	1	107.024	10.875***
Density	1	117.738	11.964****
Population × sex	1	2.718	.285 (NS)
Population × density	1	2.805	.150 (NS)
Sex × density	1	4.009	.407 (NS)
Population × sex × density	1	9.760	.992 (NS)
Residual	76	9.841	
Fly mass: per fly (mg):			
Population	1	.040	1.603 (NS)
Sex	1	2.468	98.007****
Density	1	.217	8.604***
Population × sex	1	.007	.291 (NS)
Population × density	1	.012	.480 (NS)
Sex × density	1	.000	.000 (NS)
Population × sex × density	1	.001	.044 (NS)
Residual	69	.025	
SMR (log [$\dot{V}O_2$]/log [fly mass]):			
Population	1	3.833	3.171 (NS)
Sex	1	62.089	51.356****
Density	1	.074	.061 (NS)
Population × sex	1	2.201	1.821 (NS)
Population × density	1	.251	.207 (NS)
Sex × density	1	.720	.595 (NS)
Population × sex × density	1	2.273	1.880 (NS)
Residual	69	1.209	
SMR in ethanol vs. SMR in water vapor:			
Population	1	.017	.064 (NS)
Sex	1	.207	.795 (NS)
Treatment	1	3.163	12.138***
Population × sex	1	.209	.802 (NS)

TABLE 2—Continued

Source of Variation	df	Mean Square	*F*
SMR in ethanol vs. SMR in water vapor (continued):			
Population × treatment	1	.494	1.897 (NS)
Sex × treatment	1	.013	.049 (NS)
Population × sex × treatment	1	.527	.158 (NS)
Residual	103	.261	

Note. NS = not significant.
*** $P < .01$.
**** $P < .001$.

(table 5), but 4% ethanol vapor uniformly increased longevity in the presence of either cactus. In ethanol vapor treatments, lifetime fecundity was increased 30–75 times with agria and 6–7 times with organ pipe. Arizona females laid more eggs than Baja females, yet both populations laid more eggs on organ pipe, particularly Arizona females in ethanol vapor on organ pipe ($t = 2.21$, $P = .031$), accounting for a significant interaction between population and treatment (table 6).

Ethanol vapor had no significant effect on average age at first reproduction, but 73% of the females in the water vapor treatment never laid eggs (table 5). Thus, volatiles such as ethanol may be considered required nutrients for sexual maturation and egg production in cactus-rot environments. Arizona females matured faster than Baja females overall, suggesting geographical variation in this component of fitness as well.

Discussion

Longevity extension in *Drosophila mojavensis* adults that is due to low concentrations of atmospheric ethanol is due to energy derived from the metabolic breakdown of ethanol molecules. The mechanism underlying longevity extension must involve the ADH pathway and conversion of ethanol and other LMWV (Starmer et al. 1977; Heed 1978; Batterham et al. 1982; Heinstra et al. 1983) to acetate and then the common intermediate in both lipid and carbohydrate metabolic pathways—i.e., acetyl-CoA (Gilmour 1965)—as shown by the accumulation of labeled CO_2 derived from atmospheric etha-

TABLE 3

Mean ± SD amounts of ^{14}C label (in cpm) from atmospheric ethanol in groups of whole adult Drosophila mojavensis, *and their CO_2 recovered in 20% KOH after approximately 2 and 5 days*

	Exposure Period	
	46 h	120 h
Females:		
^{14}C 4% ethanol:		
cpm in adult tissue	381.74 ± 96.31	515.26 ± 160.24
cpm in KOH	21.05 ± 4.15	40.60 ± 13.13
N	7	8
Controls:[a]		
cpm in adult tissue	5.65 ± .0	.97 ± .09
cpm in KOH	.67 ± .0	.59 ± .01
N	1	2
Males:		
^{14}C 4% ethanol:		
cpm in adult tissue	301.32 ± 54.62	355.61 ± 95.40
cpm in KOH	10.63 ± 6.25	39.46 ± 16.32
N	5	5
Controls:		
cpm in adult tissue	2.14 ± 1.84	1.36 ± .0
cpm in KOH	.78 ± .11	.65 ± .0
N	3	1
Background[b] (*N*)	.44 ± .07 (6)	

Note. All cpm were corrected for the number of adults per flask and are thus expressed as cpm/fly. *N* = number of replicates.

[a] Unlabeled 4% ethanol vapor.

[b] cpm in untreated scintillation fluid. To make this standard comparable with the rest of the data expressed as cpm/fly, here cpm for each vial was divided by the average number of flies/vial in the experiment, 45.7.

nol. Since no carryover effects were detected, insignificant amounts of metabolites are stored under starvation conditions, i.e., in starving flies acetate is probably shunted directly into the citric acid cycle. Since adult *D. mojavensis* are exposed to negligible amounts of free carbohydrates in naturally occurring cactus rots throughout Baja California, Sonora, and southern Ari-

TABLE 4

Analysis-of-variance results for ^{14}C incorporated into adult Drosophila mojavensis *tissues and recovered as CO_2 bound to KOH*

Source of Variation	df	Mean Square	*F*
Whole Body Tissues:			
Treatment	1	767,659.175	64.932****
Time	1	40,998.633	3.468 (NS)
Sex	1	59,437.455	5.028*
Treatment × time	1	10.578.978	.895 (NS)
Treatment × sex	1	15,588.881	1.319 (NS)
Time × sex	1	7,433.501	.629 (NS)
Treatment × time × sex	1	1,973.657	.167 (NS)
Residual	24	11,822.428	
CO_2 bound to KOH:			
Treatment	1	3,630.192	34.428****
Time	1	2,555.748	24.238****
Sex	1	61.103	.579 (NS)
Treatment × time	1	677.007	6.421**
Treatment × sex	1	42.099	.399 (NS)
Time × sex	1	103.957	.986 (NS)
Treatment × time × sex	1	24.863	.236 (NS)
Residual	24	105.444	

Note. Observations were untransformed cpm from table 3. NS = not significant.
* $P < .05$.
** $P < .025$.
**** $P < .0001$.

zona (Brazner et al. 1984; Kircher 1982), the ability to feed on atmospheric LMWV has probably allowed them to colonize desert areas where these volatile-rich host cactus rots occur (Brazner et al. 1984).

Effects of atmospheric ethanol on adults that were fed fermenting cactus included large increases in fecundity but no effect on age at first reproduction. Increases in fecundity were quantitative and qualitative because so many females failed to produce any eggs in the water vapor treatment. Well-fed adult *D. melanogaster* incorporate ethanol metabolites into lipids (Middleton and Kacser 1983). A majority of total ADH activity is located in *D. mojavensis* fat bodies (Batterham et al. 1983), suggesting that feeding adults

TABLE 5

Mean ± SD (N) *effects of atmospheric ethanol and adult feeding on natural substrates on longevity, age at first reproduction, and lifetime fecundity in two populations of* Drosophila mojavensis

	Treatment			
	Agria		Organ Pipe	
Population	Water Vapor	4% Ethanol	Water Vapor	4% Ethanol
Longevity (days):				
Santa Rosa Mountains (A900)	7.36 ± 2.66 (39)	12.44 ± 4.96 (32)	8.60 ± 3.28 (35)	13.56 ± 4.83 (39)
Punta Prieta, Baja California Norte (A896)	7.45 ± 3.55 (40)	13.70 ± 7.04 (37)	9.13 ± 4.50 (40)	12.67 ± 6.36 (33)
Lifetime fecundity:				
Santa Rosa Mountains (A900)	.87 ± 2.03 (39)	76.97 ± 80.34 (32)	17.26 ± 28.37 (35)	112.97 ± 97.43 (39)
Punta Prieta, Baja California Norte (A896)	1.63 ± 4.36 (40)	61.78 ± 71.01 (37)	9.53 ± 17.39 (40)	67.58 ± 72.49 (33)
Age at first reproduction (days):				
Santa Rosa Mountains (A900)	4.86 ± 1.35 (7)	5.16 ± 1.89 (25)	6.07 ± 2.27 (14)	5.29 ± 1.49 (31)
Punta Prieta, Baja California Norte (A896)	7.83 ± 3.13 (6)	7.27 ± 2.05 (22)	6.00 ± 2.29 (14)	5.75 ± 1.57 (24)

TABLE 6

Analysis-of-variance results for the influence of atmospheric ethanol on three components of fitness in two populations of Drosophila mojavensis *cultured on two kinds of cacti*

Source of Variation	df	Mean Square	*F*
Female longevity:			
Population	1	5.453	.235 (NS)
Cactus	1	46.419	2.004 (NS)
Treatment	1	1,837.398	79.344****
Population × cactus	1	11.887	.513 (NS)
Population × treatment	1	.266	.011 (NS)
Cactus × treatment	1	37.729	1.629 (NS)
Population × cactus × treatment	1	30.867	1.333 (NS)
Residual	287	23.157	
Lifetime fecundity:			
Population	1	20,329.644	6.138**
Cactus	1	21,225.185	6.408**
Treatment	1	390,998.794	118.045****
Population × cactus	1	6,521.602	1.969 (NS)
Population × treatment	1	13,125.778	3.963*
Cactus × treatment	1	1,344.850	.406 (NS)
Population × cactus × treatment	1	2,161.383	.653 (NS)
Residual	287	3,312.274	
Age at first reproduction:			
Population	1	45.692	12.557****
Cactus	1	9.344	2.568 (NS)
Treatment	1	3.255	.895 (NS)
Population × cactus	1	34.497	9.481***
Population × treatment	1	.010	.003 (NS)
Cactus × treatment	1	1.218	.335 (NS)
Population × cactus × treatment	1	3.179	.874 (NS)
Residual	135	3.639	

Note. NS = not significant.
* $P < .05$.
** $P < .025$.
*** $P < .01$.
**** $P < .0001$.

shunt acetyl-CoA in the lipid biosynthetic pathway, just as larvae do (Geer, Langevin, and McKechnie 1985).

In 10-day-old adult *D. mojavensis* that have exhausted larval fat reserves, 70% of the total ADH activity in males and 30% in females are located in adult fat body. No ADH activity is detectable in male reproductive tissues, but about 50% of the total ADH activity in females is located in ovarian tissues (Batterham et al. 1983). Increased fecundity owing to atmospheric ethanol on natural substrates suggests *D. mojavensis* are dependent on LMWV for reproduction and are physiologically able to transfer ethanol metabolites directly into developing eggs. Recovery of ^{14}C label in eggs from females exposed to labeled atmospheric ethanol has recently been observed (C. S. Klassen and W. J. Etges, unpublished data).

Many previous experiments documenting increased longevity in volatile atmospheres have employed the "sealed-vial technique" originally used by Starmer et al. (1977), which was specifically avoided here. In those studies, one gauze-covered shell vial containing adult flies and one shell vial with an absorbant containing the test solution are placed end to end and sealed together, usually with Parafilm. Hypercapnia may influence longevity (Van Herrewege and David 1978) by altering metabolic rates in vials sealed for a week or longer (Van Herrewege and David 1974; Starmer et al. 1977; Parsons 1981) because adult *Drosophila* have high SMRs relative to other insects, in the range of 1–5 $cm^3\ O_2\ g^{-1}\ h^{-1}$ (Kucera 1934; Chadwick and Gilmour 1940; Hocking 1953; Kammer and Heinrich 1978; this study). Cohan and Hoffman (1986) showed that longevity in 5% ethanol could be increased 42% in *D. melanogaster* by using sealed vials instead of desiccators, and they pointed out that the two techniques may be measuring different traits.

Respiration in *Drosophila* depends on gaseous diffusion only (Wies-Fogh 1964). Elevated CO_2 concentrations cause opening of the spiracles (Miller 1966), so the presence of atmospheric ethanol, even in low "nutritive" concentrations, in hypercapnic conditions may induce either mild toxicity of atmospheric ethanol or physiological slowdown. Ethanol vapor becomes toxic to adult *Drosophila* at concentrations of 5%–10%, but this varies tremendously among species (Holmes, Moxon, and Parsons 1980; Van Herrewege and David 1980, 1984). Larval fat reserves are carried over into the adult (Rizki 1978) and are metabolized within 2–5 days in *D. melanogaster* (Wigglesworth 1949; Butterworth and Bodenstein 1968) and are gone by 10 days in *D. mojavensis* (Batterham et al. 1983). A mildly toxic effect of ethanol vapor may not only lower metabolic rates but also interfere with glycogen/fat metabolism under starvation conditions.

Unfortunately, only concentrations of liquid volatiles in rots used by *D. mojavensis* have been measured (Fogleman and Heed 1989). Concentra-

tions of atmospheric LMWV are not yet known. Natural populations of *D. melanogaster* and *D. simulans* inhabiting wineries (McKenzie and McKechnie 1978, 1979; McKenzie 1980) are exposed to concentrations of liquid ethanol in wine barrel seepages that vary up to 10%, much higher than in fermenting fruits used as breeding sites (Gibson, May, and Wilks 1981; McKechnie and Morgan 1982). However, it remains unclear how dependent winery populations are on available LMWV for adult survival, if any carbohydrate sources are present—and if available LMWV present in *natural* breeding/feeding sites worldwide are concentrated enough to engender increased adult longevity.

Among populations of *D. mojavensis,* longevity-extension variation due to atmospheric ethanol is dependent on physiological status of the flies (fig. 4). Longevities in natural populations will be determined mainly by nutrient stress because mainland *D. mojavensis* are larger than those from Baja (Heed and Mangan 1986; Etges and Heed 1987; W. B. Heed and W. J. Etges, unpublished data), although these two populations do not differ in SMR at 1–2 days or in posteclosion (10–12 days) (table 2). Only when adults are dispersing among cacti will intrinsic starvation resistance matter, since atmospheric ethanol increases SMR and high concentrations of LMWV are found only in rots. Population differences in longevity will become apparent only if adults are excluded from feeding on cactus tissues but can enter rot cavities in which LMWV vapor concentrations are in the range of approximately 0.5%–4.0% (Batterham et al. 1982).

Since *D. mojavensis* invaded mainland Sonora from Baja, changes in several components of fitness have occurred, including increased body size (Mangan 1982; Etges and Heed 1987). Since, in *Drosophila,* body size is correlated with flight ability (Roff 1977, 1981)—and hence with dispersal ability among rots—the larger size of mainland *D. mojavensis* may have evolved in response to the greater interrot distances in organ pipe populations. Agria plants are more dense than organ pipe plants, and agria rots are 40 times more abundant where they occur than are organ pipe rots (Mangan 1982). Thus, greater body size and increased metabolism in ethanol vapor, leading to higher incorporation of ethanol metabolites into fat body, may be directly related to the switch to organ pipe cactus.

The ability of *D. mojavensis* to metabolize volatiles such as ethanol has allowed invasion of carbohydrate-poor *Stenocereus* cactus rots throughout their geographical range. The LMWV not only attract adults to rots (Fogleman 1982) but provide a supply of energy and also influence several components of fitness: longevity, lifetime fecundity, and age at first reproduction.

Acknowledgments

Fred Cohan shared his expertise on the measurement of ethanol-increased longevity, and Dave Vleck provided use of his oxygen analyzer. Y. Matsuda provided unending advice, encouragement, and support. W. Van Voorhees, H. Alonso-Pimental, N. Baccus, and E. S. Etges provided much needed technical support without which we could not have completed this study. Thanks to W. Scharloo for pointing out the Middleton and Kacser paper. Tom Starmer, Phil Ganter, Bill Heed, Kim Smith, Dave Vleck, Fred Cohan, Tom Tobin, and Bill Geer improved the manuscript. We thank the Tohono O'odham Nation, formerly the Papago tribe of Arizona, for permission to collect on their reservation. This work was supported by NSF grant BSR-8503472 to W.J.E.

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