Host Preference Among Populations of *Drosophila mojavensis* (Diptera: Drosophilidae) that Use Different Host Cacti

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Previous studies have suggested that all populations of cactophilic Drosophila mojavensis *prefer pitaya agria cactus,* Stenocereus gummosus, *over all other potential hosts for feeding and breeding, including populations that inhabit areas where no agria grows. We sampled five geographically isolated populations of* D. mojavensis *from nature to assess host choice within and between populations. Host choice tests were performed in a laboratory "olfactometer" by allowing adult* D. mojavensis *to choose between plumes of synthetic volatile cocktails of two widespread host cacti. Overall, each population showed significant preference for agria volatiles with one exception: a mainland Sonora population that uses organ pipe cactus in nature exhibited preference for organ pipe volatiles, suggesting a possible shift in host preference. The degree of preference for agria volatiles was greatest in a population from southern California that use California barrel cactus as a host. Since southern Californian populations of* D. mojavensis *are thought to be derived from those in Baja California, preference for agria volatiles is considered a retained ancestral trait. Three populations from Baja California and mainland Mexico that use agria in the wild expressed lower, but similar preferences for agria volatiles. Because populations of* D. mojavensis *are ancestral to those in mainland Mexico, Arizona, and California, the shift from agria to alternate hosts has not been accompanied by strong changes in host preference behavior.*

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INTRODUCTION

Olfactory stimuli are used by most insects in assaying potential hosts used for food or oviposition sites (Haskell *et al.*, 1962; Gatehouse and Lewis, 1973; Fuyama, 1976, 1978; Hoffman and Parsons, 1984; Hoffman *et al.*, 1984; Hoffman, 1985; Alcorta and Rubio, 1988; Aluja and Prokopy, 1993). Understanding the determinants of host preference, particularly when several seemingly abundant hosts are present within the range of a population or group of populations, can yield insight into the pathways leading to host specialization (Singer, 1983; Jaenike, 1985, 1990), coevolution (reviewed by Thompson, 1994), and reproductive isolation (Bush, 1975; Tauber and Tauber, 1989; Craig *et al.*, 1993). Most phytophagous insects are host specialists (Ehrlich and Murphy, 1988), and host plant relationships are in many cases the result of insect responses to plant chemistry (Price, 1980). Unraveling the ways in which plant chemicals or by-products serve as olfactory cues continues to be a focus for many studies of host preference (Strong *et al.*, 1984).

Patterns of host preference have been revealed in a wide variety of species in both natural and experimental populations including leafhoppers in the genus *Oncopis* (Claridge *et al.*, 1977), butterflies, *Euphydryas editha* (Singer, 1983), *Heliothis virescens* (Schneider and Roush, 1986; Waldvogel and Gould, 1990), *Paplio glaucus* (Scriber, 1993), and cowpea weevils (Wasserman, 1986). Uncovering genetic variation in host preference is also critical to the understanding of the evolution of host specialization, as shown in studies of *Dacus* (Fitt, 1986), and *Drosophila* species (Fuyama, 1976, 1978; Cavener, 1979; Shorrocks and Nigro, 1981; Jaenike, 1983; Alcorta and Rubio, 1988; Hoffman, 1988; Barker *et al.*, 1994).

Behavioral studies with *Drosophila* have clearly shown that certain volatiles produced during fermentation elicit positive chemotaxis mediated by the third or terminal antennal segments (Barrows, 1907). These volatiles include compounds such as ethanol, acetate, acetoin, and acetaldehyde. Antennaless mutants of *D. melanogaster* are not responsive to these attractive volatiles (Begg and Hogben, 1946). It is less clear how drosophilids find resources over long distances, yet movement over many kilometers in search of food in short time periods has been documented (Johnston and Heed, 1976; Coyne *et al.*, 1982). Feeding and breeding sites differ in many species of *Drosophila* (Heed, 1968; Carson, 1971), and so the behavioral mechanisms associated with choosing these resources may be under the control of different genetic systems. In fact, Jaenike (1986) demonstrated that long-distance movements toward potential hosts and oviposition choice behaviors at the host site are controlled by independent sets of genes in a population of *D. tripunctata*.

The focus of the present study is to evaluate patterns of host preference within and between geographically isolated populations of cactophilic *D. moja-*

vensis because previous studies of both natural and experimental populations (Fellows and Heed, 1972; Downing, 1985) have suggested that fermenting pitaya agria cactus, *Stenocereus gummosus*, is the preferred host in all populations, even those located in regions where agria is absent. Of the four endemic drosophilids found in the Sonoran Desert, *D. mojavensis* uses the greatest variety of host cacti throughout its range (Table I). Because feeding and breeding sites are usually the same (Heed and Mangan, 1986), the chemical cues used in long-range movement and oviposition choice by *D. mojavensis* are specific to each host cactus. In this study, populations of *D. mojavensis* that use different host cacti were compared for their host preference to test the hypothesis that preference for agria cactus is common to all populations of *D. mojavensis*.

Natural History of *Drosophila mojavensis*

Drosophila mojavensis uses four species of columnar cactus in and around the Sonoran Desert as hosts including agria, organ pipe (*Stenocereus thurberi*), cina (*S. alamosensis*), and California barrel cactus (*Ferocactus cylindraceous*) in southern California (Fellows and Heed, 1972; Heed and Mangan, 1986; Ruiz and Heed, 1988). These flies are saprophytic and utilize the fermenting necrotic stems, rot pockets, of these cacti for food and oviposition sites. Prickly pear cactus (*Opuntia* spp.) is also used occasionally in parts of the range and serves as the sole host on Santa Catalina Island, California (Heed and Mangan, 1986;

Table I. The Host Cactus Species of Endemic *Drosophila* Found in the Sonoran Desert

Host species	Common name	Resident *Drosophila* species
Stenocereus gummosus	Pitaya agria	*D. mojavensis*
S. thurberi	Organ pipe	*D. mojavensis*
S. alamosensis	Cina	*D. arizonae*
		D. mojavensis
Ferocactus cylindraceous	California barrel[a]	*D. mojavensis*
Opuntia spp.	Prickly pear[a]	*D. mojavensis*
Lophocereus schottii	Senita	*D. pachea*
Carnegiea gigantea	Saguaro	*D. nigrospiracula*
	Saguaro soil[b]	*D. mettleri*
Pachycereus pringlei	Cardon	*D. nigrospiracula*
	Cardon soil[b]	*D. mettleri*

[a]California barrel cactus and *Opuntia* are also used by *D. mojavensis* in areas outside the Sonoran Desert. *Opuntia* spp. are also used by other related *Drosophila* species such as *D. navojoa* and *D. aldrichi*. The ecology of all Sonoran Desert *Drosophila* is discussed by Heed and Mangan (1986).

[b]*D. mettleri* is an obligate soil breeder, using the rot exudate-soaked soils beneath columnar cacti to carry out its life cycle.

Lofdahl, 1986). *D. mojavensis* occasionally uses cina along with organ pipe cactus in the more mesic areas of southern Sonora and northern Sinaloa where the Sonoran Desert borders the desert thornscrub biotic province (Ruiz and Heed, 1988). Each of these cacti, except cina, is found in Baja California (Fig. 1).

Populations of *D. mojavensis* are considered oligophagic because they use primarily only a single host in a region. If agria is present, *D. mojavensis* seem to ignore the presence of any other potential hosts, even those that are used exclusively in other areas where agria is absent (Fellows and Heed, 1972). Agria is inferred to be the ancestral host for *D. mojavensis* for the following reasons. (1) It is widespread in peninsular Baja California, where *D. mojavensis* presumably originated. Present-day Baja California has moved northwestward on the Pacific plate from western Mexico (Gastil *et al.*, 1975; Johnson, 1980), isolating *D. mojavensis* from its closest mainland relatives, *D. arizonae* and *D. navojoa*. Of these sibling species, *D. arizonae* has occasionally been collected in southern Baja California (W. B. Heed, personnal communication), but *D. navojoa* is restricted to mainland Mexico (Heed, 1982). (2) Peninsular Baja California populations of *D. mojavensis* harbor significant chromosomal inversion polymorphism, including a unique ancestral gene arrangement not found elsewhere (Ruiz *et al.*, 1990). (3) Use of any cactus other than agria is thought to be a derived characteristic, as *D. mojavensis* subsequently invaded southern California and mainland Mexico across the Gulf of California through host shifts from agria to organ pipe, barrel cactus, or *Opuntia* (Lofdahl, 1986; Etges, 1990).

Role of Volatile Variation in Host Preference

Downing (1985) investigated the use of low molecular weight cactus volatiles by *D. mojavensis* in host choice tests. Volatiles such as 1-propyl acetate are important in attracting flies to rots along with ethanol and 1-propanol (Downing, 1985; Fogleman and Abril, 1990). Propyl acetate is thought to be most important, as it occurs in all substrates of choice, and without this volatile, attraction response decreases (Fogleman and Abril, 1990). Host preference is thought to be based upon a combination of volatiles from fermenting cactus tissues rather than single volatiles (Hutner *et al.*, 1937; Fogleman and Abril, 1990).

Downing (1985) also proposed a cactus preference hierarchy for *D. mojavensis* using choice tests with the volatiles for all of the major host cacti in the Sonoran Desert (Table I). He hypothesized that adults should prefer rots with the greatest quantity of desirable low weight volatile molecules. Therefore, adult flies from all populations may prefer agria over organ pipe, regardless of geographic location, simply because agria rots produce more low molecular weight volatiles during the process of fermentation (Downing, 1985). His laboratory

results based on volatile abundance of naturally occurring rots showed that *D. mojavensis* adults preferred agria > organ pipe > saguaro (*Carnegiea gigantea*) > senita (*Lophocereus schottii*).

Thus, the main objective for this study was to determine whether populations of *D. mojavensis* from throughout their geographic range uniformly preferred agria cactus or whether host preference was population specific. Because preference for agria cactus has been extrapolated to the entire species range, including populations that do not use agria as a host (Fellows and Heed, 1972; Heed and Mangan, 1986), experiments were designed to assess volatile preference in *D. mojavensis* populations that use agria, organ pipe, or barrel cactus in the wild. A follow-up study was included to assess the observed variation between females and males in their response to different volatile cocktails designed to mimic the difference between agria and organ pipe rots. This sex ratio analysis was done with the aim of providing insight into host preference behavior, oviposition behavior, and sex-based differences in resource use.

MATERIALS AND METHODS

Five populations of *D. mojavensis* were collected from throughout the species range (Fig. 1). In March 1994, 1253 adults were collected from agria rots in Punta Onah, Sonora, Mexico (PO94), and 225 flies were collected from an organ pipe rot in Cerro Colorado, Sonora, Mexico (CC94). In March and April 1994, flies were collected from two Baja California populations. Forty-three adults were collected from baits near Mission San Borja (MSB94), and an isofemale line was established from Mission San Fernando (MSF94). From Anza-Borrego Desert State Park in southern California, USA, 46 adult *D. mojavensis* were collected from baits in March 1995 (AB95). All populations were cultured on banana–yeast–karo–malt–agar laboratory media (Brazner and Etges, 1993) in 8-dram shell vials or 0.5-pt milk bottles until sufficient numbers of flies were available for the host choice experiments. Laboratory cultures were maintained in mass cultures of thousands of individuals for several generations to avoid inbreeding and to minimize any host plant carryover effects from wild-caught flies. We did not include a cina-using population because cina is a secondary host sympatric with organ pipe cactus.

Determination of volatile cocktail preference for *D. mojavensis* was made using an olfactometer (Wright, 1966) with slight modifications (Hoffman et al., 1984; Hoffman, 1985) (Fig. 2). This design was chosen after comparison of several olfactometer designs (Haskell et al., 1962; Fuyama, 1978; Downing, 1985) because it allowed the flies more room than simple Y-tube olfactometers (McIndoo, 1926; Alcorta and Rubio, 1988). Mixing of volatile odors can interfere with discrimination between odors and Y-tube style olfactometers provide
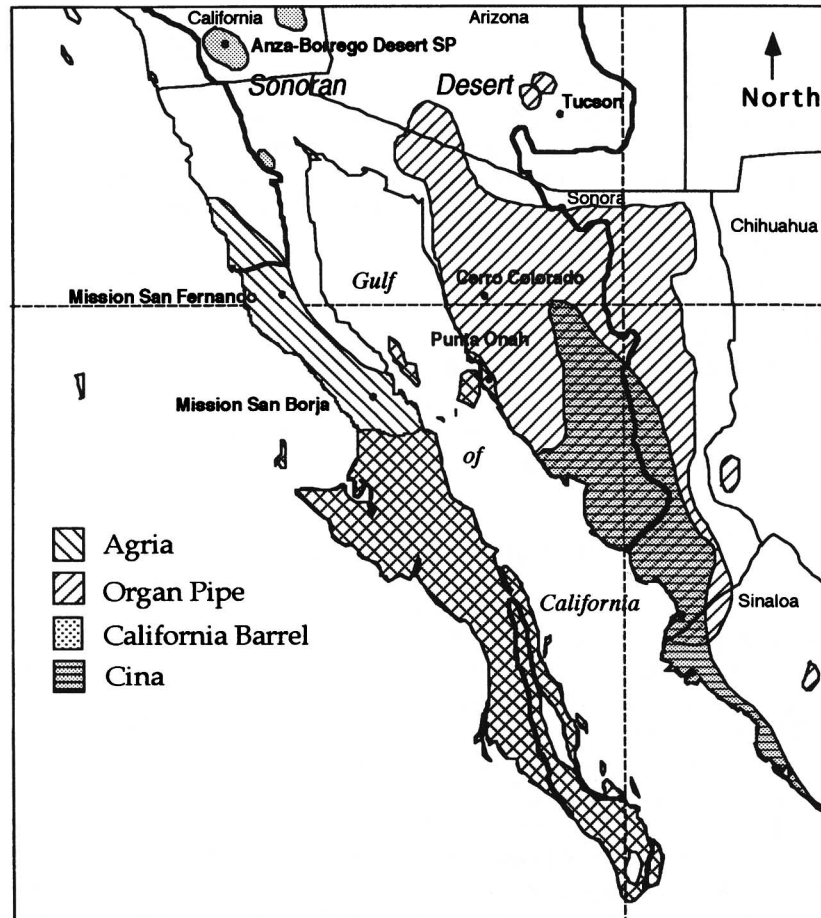
Fig. 1. A map of the geographical boundaries of the Sonoran Desert and the locations of populations of *D. mojavensis* used in this study. The species distributions of the four major cactus hosts are based on Heed and Mangan (1986) and Turner *et al.* (1995).

little room for sampling of both volatile cocktail patterns. Also, Y-tube olfac- tometers can provide misleading results due to the mixing of the two air streams at the juncture (Visser, 1976). Unlike other investigations (Wright, 1966; Hoff- man, 1985), the olfactometer used in this study had no exhaust fan, but instead the volatile plume was propagated by the inflow of air bubbling through the volatile cocktail. A fan was found to cause too much wind disturbance causing the flies to remain stationary. The olfactometer closely approximated Downing's
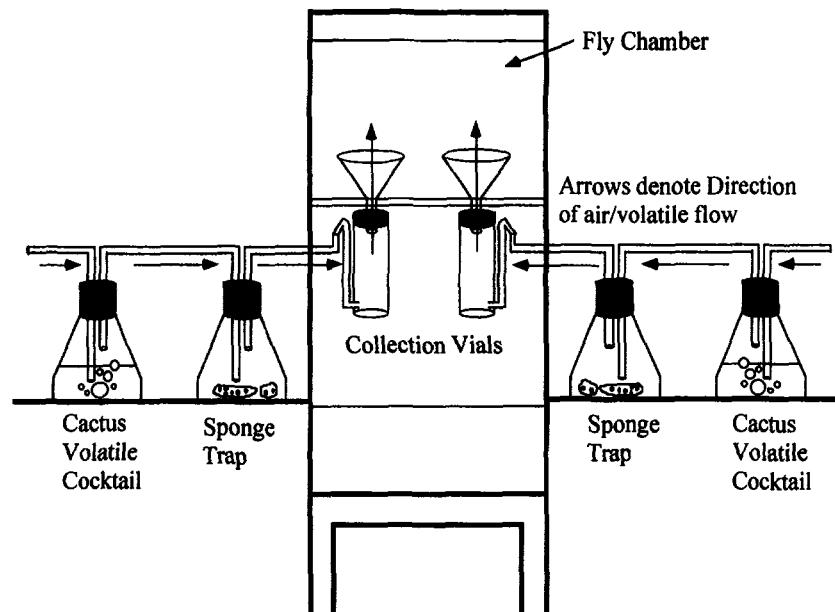
Fig. 2. The olfactometer used in this study, which consisted of a 4389-cm^3 observation chamber and two traps connected to volatile cocktails. The air flow originated from a single spigot. The traps were located in opposite corners of the observation chamber.

(1985) simple design of a box containing volatile traps. Placement of the two traps was randomized within the olfactometer before each trial. The olfactometer was disassembled, rinsed thoroughly with distilled water, and allowed to air-dry after each trial in order to remove as much residual scent as possible left by previous trials.

Preference tests were performed at room temperature (18–22°C). The flies were nutrient-deprived for 24–30 h prior to each experiment by holding them on 1% agar medium in shell vials. Air flow through each volatile cocktail was controlled by an air spigot and initiated at the same time the flies were introduced into the olfactometer. Flow rates were not measured but were kept the same for each trial to ensure a uniform rate of volatile flow into the chamber. We assumed that as each trial progressed, volatile concentrations were depleted. A minimum of several hundred flies (mean = 1392.5, SD = 798.3) aged 10–15 days was added to the observation chamber for each replicate. The test period ran continuously for 24 h to include both the morning and the afternoon activity periods. The olfactometer was covered with a sheet so the flies inside would not be disturbed by movement in the laboratory. Each trial was terminated by placing

the entire olfactometer in a freezer at approximately −20°C. The flies from each trap were collected in separate glass shell vials and then stored frozen until they were counted and sexed.

Each test employed separate volatile cocktails that represented the volatile patterns of agria and organ pipe host cacti, respectively. The cocktails were produced following the recipes of Downing (1985) and Fogleman and Abril (1990). The recipes were named Downing or Fogleman, respectively (Table II). One liter of each cactus volatile solution was prepared immediately before each of the choice tests. The concentrations of the volatiles used in these experiments were derived from gas chromatography of agria and organ pipe tissues fermented in the laboratory with yeast and bacteria extracted from cactus rots in the field (Downing, 1985) or fermenting cactus tissue collected from cactus rot pockets in nature (Fogleman and Abril, 1990). Both recipes were used because we wanted to precisely replicate the conditions in Downing's experiments, yet these volatile cocktails differed slightly from those that were derived from naturally occurring cactus rots (Fogleman and Abril, 1990). Unfortunately, it is unclear which recipe is "closer" to that which most flies in nature would be exposed to because of the known variation in tissue condition caused by changes in microbial flora over the duration of fermentation of cactus arms (Starmer, 1982).

Behavioral preference was estimated in all populations by calculating an olfactory index, IO, the number of flies caught in the agria trap divided by the

Table II. The Working Recipes for the Agria and Organ Pipe Cactus Volatile Cocktails Used in this Study Developed by Downing (1985) and Fogleman and Abril (1990)

	Downing recipe (μl)		Fogleman and Abril recipe (μl)	
	Agria	Organ pipe	Agria	Organ pipe
Acetic acid	1198	1486	1610	1884
Acetoin	0	0	62	80
Acetone	0	0	281	7
Butanediol	152	242	359	287
n-Butyric acid	282	247	229	64
Ethanol, 95%	94	82	267	129
Methanol	49	41	336	109
1-Propanol	135	68	210	68
2-Propanol	0	69	842	46
Propionic acid	257	115	880	604
1-Propyl acetate[a]	38	0	104	12
Total volatiles per liter of water	2205	2350	5180	3290

[a]Used instead of 1-propyl acetate and 2-propyl acetate.

total number of flies trapped (Fuyama, 1976; Alcorta and Rubio, 1988). IO values range from 0 to 1 with 0.5, indicating no preference. All IO data were arcsin transformed prior to ANOVA. Log-likelihood contingency or G tests were used to test for volatile preference. Both heterogeneity G_H tests, used to estimate homogeneity in numbers of flies across replicates for each population or group, and pooled G_P tests, used to test for goodness of fit to the hypothesized 50:50 ratio of flies in the traps indicating no preference, were used to test for preference for one type of volatile over another. Total $G_T = G_H + G_P$ was calculated to explore total heterogeneity in numbers of flies attracted to the traps (Sokal and Rohlf, 1981, p. 723).

Duncan's multiple-range tests were used to determine which populations or groups were different from each other when the ANOVA indicated a significant difference. This was performed after every two-way ANOVA and one-way ANOVA when a significant difference in IO value was found between populations or between recipes. Type III sums of squares were used throughout (SAS Institute, 1985).

Analysis 1: Preliminary Control Experiments

Initial choice tests were performed to determine whether the experimental conditions used allowed the flies to discriminate between the cactus volatile cocktails and water vapor. The null hypothesis was IO = 0.5. Each cactus cocktail was tested against distilled water vapor in the olfactometer. The placement of the cocktail and control traps were randomized before each trial. G tests were used to determine if each population was attracted to the volatile cocktails solution over water.

To determine if any population had an innate aversion to being trapped, the percentages of uncaught flies were compared using ANOVA (SAS Institute, 1985). The percentage of the total flies remaining in the observation chamber at the end of the experiment was compared among populations. Any population-specific aversion to being trapped would be apparent if there was an excessively large percentage of uncaught flies.

Analysis 2: Fogleman Versus Downing Choice Tests

A population from Baja California, MSB94, was used to determine whether there were differences in adult response to the Fogleman and Downing cocktail recipes. The flies were tested in the same way described above, except instead of two volatile cocktails representing different cacti, the two volatile cocktails were two different recipes for the same cacti. The IO value for these tests was the number of flies in the Fogleman recipe trap divided by the number of flies trapped. The null hypothesis was that there would be no difference in numbers of flies captured between the two agria or organ pipe recipes.

Analysis 3: Intrapopulation Variation

All five *D. mojavensis* populations (MSB94, MSF94, PO94, CC94, and AB95) were assessed to determine if the percentage of flies captured in the agria volatile trap of the olfactometer differed within populations. Since the MSF94 stock was derived from an isofemale line, it was of interest to observe whether this line would respond like the other populations from Baja California that use agria cactus in nature. The null hypothesis was that adults from each population should have no preference, IO = 0.5. *G* tests were performed to determine whether replicates of each population deviated from homogeneity and a 50:50 ratio of adults in volatile traps. Each replicate was assigned to a block based upon the time period the experiment was performed to assess any systematic variation associated with the time interval over which the experiments were performed. Any variation between blocks was determined by ANOVA. Blocks consisted of numbered 2-week intervals, where January 1–14 was interval 1, January 15–28 was interval 2, etc.

Analysis 4: Interpopulation Variation for the Five Populations

Differences in volatile cocktail preference among the five populations tested were determined by use of a two-way ANOVA with population and recipe as fixed effects (SAS Institute, 1985). Model adequacy checks were performed to determine if the assumptions of the parametric test were violated. The null hypothesis tested was that all IO values should be the same for all populations.

Analysis 5: Sex Ratio Determination

G tests were performed to determine whether sex ratios deviated from parity in the traps or within the observation chamber. Only a small subset of the total number of choice trails was included in this analysis: all flies were sexed from two replicates of the AB95 population, three replicates of the PO94 population, and one replicate of the MSF94 population. The null hypothesis was that flies would distribute themselves randomly throughout all areas of the olfactometer regardless of sex. If one sex was overrepresented in the volatile cocktail traps, then there may be evidence for sex-specific movement toward volatile attractants.

RESULTS

Over 104,000 flies total were used in the control and cactus cocktail choice tests to determine the pattern of preference for agria and organ pipe volatile cocktails (Table III); 76,722 flies were used in cactus cocktail choice tests involving both agria and organ pipe cocktails.

Table III. Numbers of Adult *D. mojavensis* Used in All the Host Choice Experiments Versus Those Captured (A), Including Those Studied in the Control Tests (Cactus Volatiles vs Water Vapor); Numbers of Flies Used in the Agria vs Organ Pipe Choice Tests (B); and Numbers of Flies Used vs Those Captured for Each Population (C) Included in this Study

Population	Total flies	Mean	SD	Max	Min	N^a
A						
Overall experiments	104,437	1,392.5	798.30	3,398	318	75
Total captured	74,353	991.4	683.41	2,975	49	75
B						
Choice test experiments						
Total	76,722	1,394.9	775.62	3,283	318	55
Captured	55,326	1,005.9	651.93	2,673	201	55
C						
Baja California populations						
Mission San Borja (MSB94)						
Total	24,042	1,502.6	602.86	2,659	832	16
Captured	15,629	9,760.8	482.38	1,954	495	16
Mission San Fernando (MSF94)						
Total	16,445	1,644.5	901.39	3,087	448	10
Captured	12,805	1,280.5	831.31	2,673	283	10
Mainland Sonora populations						
Cerro Colorado (CC94)						
Total	11,787	1,178.7	679.98	2,809	318	10
Captured	8,791	879.1	620.90	2,444	201	10
Punta Onah (PO94)						
Total	16,643	1,664.3	1,049.05	3,283	407	10
Captured	12,595	1,259.5	804.15	2,570	282	10
Anza-Borrego (AB95)						
Total	7,805	867.2	378.84	1,615	442	9
Captured	5,506	611.8	350.21	1,398	259	9

^{a}Number of replicates.

Analysis 1: Preliminary Control Experiments

Adult *D. mojavensis* preferred the volatile cocktails when given a choice between a cocktail and water vapor with one exception. Only the CC94, PO94, and MSB94 populations were tested in these preliminary water vapor tests. The PO94 population was found to be more attracted to the organ pipe cocktail than water vapor ($G_T = 14.55$, $P < 0.005$, 2 df) and more attracted to the agria cocktail than water vapor ($G_T = 629.36$, $P \ll 0.005$, 3 df). Individuals from the other mainland Sonora population, CC94, were also found to be more attracted to the organ pipe cocktail than water vapor ($G_T = 286.18$, $P \ll 0.005$, 4 df) and more attracted to the agria cocktail than water vapor ($G_T =$

615.56, $P \ll 0.005$, 4 df). The only Baja population tested, MSB94, was more attracted to the agria cocktail than to water vapor ($G_T = 653.01$, $P <$ 0.005, 1 df), but in the organ pipe vs water vapor tests, more flies were found in the water vapor trap than in the organ pipe cocktail trap ($G_T = 14.99$, $P <$ 0.005, 2 df), suggesting that MSB94 adults may be repelled by organ pipe volatiles. The fact that the adult flies went into the water control trap instead of avoiding both traps altogether was not unexpected, as Downing (1985) demonstrated that the flies would be drawn to a moisture gradient caused by pure water. No population demonstrated an aversion to being trapped (two-way ANOVA; population effect, $P = 0.92$; recipe effect, $P = 0.099$; population × recipe interaction, $P = 0.23$).

Analysis 2: Fogleman Versus Downing Recipe Choice Tests

The Fogleman agria cocktails were found to be more attractive for the MSB94 population than the Downing cocktails ($G_T = 2393.29$, $P < 0.005$, df $= 1$), consisted with the hypothesis that adult *D. mojavensis* prefer volatile cocktails in high concentration. The Fogleman agria cocktail contained more than twice the concentration of volatiles than Downing's (Table II). No significant difference was found in attractiveness between the two organ pipe recipes ($G_T = 0.705$, $P > 0.1$, df $= 1$), despite the higher concentration of volatiles in the Fogleman recipe. Thus, these flies can discriminate between subtle quantitative and qualitative differences in agria volatile patterns, such as those used here that were based on slight differences in fermentation profiles. However, since only one population was tested, this result may not be general to all populations.

Analysis 3: Intrapopulation Variation

Significantly more adults were attracted to agria volatiles than organ pipe volatiles in all populations (Table IV). Across all replicates for each populations, the hypothesis of no preference was rejected. The results of the pooled G tests demonstrated that, in all cases, the 50:50 capture ratio could be rejected. The attraction to agria volatiles by the MSF94 adults was intermediate to the other two agria-using populations, showing that the MSF94 isofemale line was presumably a random sample of that population. Thus, preference for agria over organ pipe volatiles was shared by all five populations, even those that do not use agria in the wild. Results of one-way ANOVAs for each population with either recipe or time blocks as main effects showed that preference for agria was not influenced by recipe or blocks (all $P > 0.1$).

Table IV. Overall Capture Results in the Agria and Organ Pipe Volatile Traps for Each Population for All Host Choice Tests Showing the Preference for Agria Volatiles

|	Trap type			G statistics[a]					
Population	Organ pipe	Agria	N^b	G_H	df	G_P	df	G_T	df
MSB94	333.0[c]	643.8	16	1300.98	15	1610.21	1	2911.18	8
MSF94	467.8	812.7	10	693.05	9	940.55	1	1633.61	10
CC94	380.0	499.4	10	717.39	9	162.62	1	880.01	10
PO94	553.7	705.8	10	590.16	9	184.13	1	774.28	10
AB95	182.4	429.3	9	243.50	8	922.79	1	1166.29	9

[a]All G statistics are highly significant at the $P = 0.005$ level.
[b]Number of replicates per population.
[c]Numbers in the organ pipe and agria columns refer to the mean number of flies per replicate.

Analysis 4: Interpopulation Variation for the Five Populations

A significant difference existed in IO values among the five populations (Fig. 3, Table V). Differences in volatile recipes had a marginal effect ($P = 0.09$) on patterns of host preference across all five populations and no significant population × recipe interaction was found. The Downing recipe caused greater preference for agria volatiles for both the CC94 and AB95 populations. The CC94 population had the lowest average IO value, significantly different from both the MSB94 and the AB95 populations (Duncan's multiple-range test, $P = 0.05$). When the Fogleman recipe data were analyzed separately, the CC94 population exhibited a preference for organ pipe volatiles ($G_H = 209.62$, 6 df, $P \ll 0.001$; $G_P = 31.69$, 1 df, $P \ll 0.001$). Therefore, adults detected the differences in volatile recipes (Table II) in both the CC94 and the AB95 populations. Adult *D. mojavensis* from the Anza-Borrego Desert, AB95, exhibited significantly greater IO values than those two populations from mainland Sonora (CC94 and PO94). Thus, preference for agria cactus volatiles was greatest in the AB95 population that uses California barrel cactus as its host in the wild. The agria-using populations, MSB94, MSF94, and PO94, were not significantly different from each other in host preference.

Analysis 5: Sex Ratio Variation

Across the sample of six choice test trials including the AB95, PO94, and the MSF94 populations, the overall average sex ratio was 1 male: 1.25 females. This female-biased ratio was significantly different from parity ($G_T = 20.29$, P
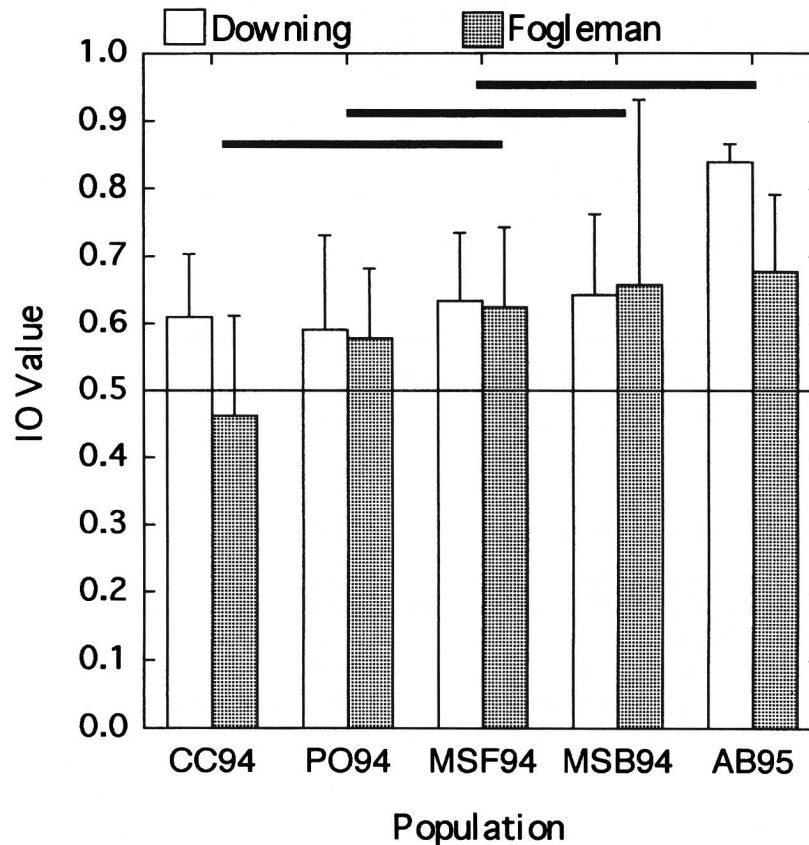
Fig. 3. The mean IO values (+1 SD) for each population by volatile recipe. Although no recipe differences were indicated by ANOVA, the data are shown for both the Downing and the Fogleman recipes. Each population had significantly more flies in the agria trap than in the organ pipe trap (G test; all $P < 0.005$) and the populations differed in agria preference ($P = 0.014$). Population bars connected by a horizontal line indicate that no significant difference existed between them (data pooled; Duncan's multiple-range test, $P < 0.05$).

< 0.005, df $= 6$; $n = 2942$). There was no a priori reason for expecting anything other than a 1:1 sex ratio in this species (W. B. Heed, personal communication), so all tests were performed using this biased sex ratio as a baseline. This bias toward higher numbers of females could have been due to chance or, more likely, to the systematic collection of the earliest eclosing adults used in the preference tests because the female egg-to-adult development time is shorter than that of males (Etges 1993). The data were divided into three

Table V. ANOVA Results for the Agria vs Organ Pipe Cactus Volatile Choice Analyses
Comparing the Five Populations of *D. mojavensis* Studied

Source	df	Type III SS	Mean square	F	P value
		Fogleman and Downing recipes			
Population	4	0.2534	0.6335	3.54	0.014
Recipe	1	0.0522	0.0522	2.92	0.09
Population * Recipe	4	0.0764	0.0191	1.07	ns[a]
Error	45	0.8064	0.0179		

[a]Not significant.

areas within the olfactometer: the two volatile traps and the remaining space in the chamber. Numbers of males and females were distributed nonrandomly throughout the olfactometer (G_T = 162.06, P < 0.005, df = 17). Organ pipe traps, regardless of population, attracted greater numbers of females than males (female mean = 57.19%, SD = 9.63%; G_T = 49.39, P < 0.005, df = 6). The agria traps also contained a higher proportion of females (female mean = 61.78%, SD = 2.99%) and a sex ratio significantly different from 1:1.25 (G_T = 25.93, P < 0.005, df = 5). This female-biased attraction to the volatile traps, of course, meant that relatively more males were counted in the observation chamber at the end of the experiments than would be expected given the initial sex ratio (female mean = 50.82%, SD = 14.49%; G = 85.15, P < 0.005, df = 5).

The average sex ratio of the combined PO94 population trials was heavily female biased, 1 male:1.41 females, significantly different from a 1:1 sex ratio (G_T = 17.19, P < 0.005, df = 3). Sex ratios of flies captured in the agria traps were significantly different from 1:1.41, with a lower percentage of females (female mean = 57.1%, SD = 12.87%; G_T = 10.91, P < 0.005, df = 1). Sex ratios of flies captured in organ pipe traps were not different from 1:1.41 (G_T = 0.71, P > 0.1, df = 1).

The average sex ratio of the flies used in AB95 population trials was 1:1.2, not significantly different from a 1:1 sex ratio (G_T = 3.02, P > 0.1, df = 2). The AB95 agria traps contained a higher proportion of females than 1:1.2 (female mean = 62.79%, SD = 0.42%; G_T = 17.2, P < 0.005, df = 1). Sex ratios in the organ pipe traps were also greater than 1:1.2 (female mean = 54.64%, SD = 11.997%; G_T = 5.78, P < 0.025, df = 1). Flies remaining in the observation chamber were male-biased, as expected (male mean = 61.42%, SD = 2.46%; G_T = 36.25, P < 0.005, df = 1).

These data were reanalyzed to detect potential bias in the host preference

trails due to female-based sex ratios. For all of the trials where males and females were counted, numbers of males were subjected to G tests. Male flies preferred agria over organ pipe volatiles ($G_T = 93.25$, $P < 0.005$, df = 6; $G_P = 36.26$, $P < 0.005$, df = 1), suggesting that the overall results of host preference for agria pertain to both sexes.

DISCUSSION

The results of this study generally support the hypothesis that adult *D. mojavensis* tend to prefer the volatile fermentation by-products of agria cactus vs those of organ pipe cactus consistent with both previous host choice studies (Fellows and Heed, 1972; Downing, 1985). Of the five geographically isolated populations studied using both volatile recipes, in all but one case (CC94 with Fogleman recipe) was agria preferred. Thus, Downing's conclusions, based on a single population, extend to populations using all three major hosts within the range of *D. mojavensis*, agria, organ pipe, and California barrel cactus. Since Fellows and Heed (1972) made similar conclusions from field conducted host choice tests, the results of these laboratory studies help to explain the patterns of host cactus use in the wild: if agria is present, *D. mojavensis* adults tend to ignore all other potential hosts. In regions where agria is absent (Fig. 1), organ pipe and other less-preferred hosts are used (Johnson, 1980; Heed, 1982). Thus, a host preference hierarchy exists for *D. mojavensis* as Fellows and Heed (1972) and Downing (1985) hypothesized.

For those cacti with which *D. mojavensis* are typically associated (excluding the hosts of other Sonoran Desert *Drosophila*), this hierarchy is much simpler in natural populations because only very rarely do more than one or two host cacti occur sympatrically in any location. No preference data exist for those populations inhabiting southern Sonora and Sinaloa, where cina and organ pipe are sympatric, other than the observation that cina rots can be an abundant resource and occasionally shared with *D. arizonae* (Ruiz and Heed, 1988). It would be informative to include cina volatiles in future host choice tests as well. Recent collections from southern Sonora and Sinaloa revealed little, if any, use of cina but widespread use of organ pipe tissues by *D. mojavensis* (W. J. Etges, unpublished data).

Despite the apparent uniformity in preference for agria, there were significant differences in host preference between populations in the degree to which agria was preferred over organ pipe volatiles, suggesting that there may be population-specific genetic differences in host preference. The three populations that use agria cactus in the wild exhibited similar degrees of preference for agria volatiles (Fig. 3) even though two, MSB94 and MSF94 (an isofemale line), were from central Baja California and the third, PO94, from coastal Sonora,

separated by the Gulf of California (Fig. 1). The strongest evidence for a shift toward a preference for an alternate host was the observed response of the other mainland Sonora population, CC94. Populations of *D. mojavensis* from this area are restricted to organ pipe cactus: the closest agria plants are over 120 km away. *Drosophila mojavensis* use organ pipe throughout the distribution of this cactus from southern Arizona to northern Sinaloa (Fig. 1) (Heed, 1982). The overall response of CC94 adults was preference for agria, yet this was the lowest IO value recorded for any of the populations. The choice test results using the Fogleman recipe clearly indicated that CC94 adults preferred organ pipe volatiles, yet they preferred agria in the tests using the Downing recipe. While this single exception to the general preference for agria volatiles deserves further study, it is the only case so far that suggests that *D. mojavensis* has evolved a preference for a secondary host.

The higher degree of preference for agria volatiles expressed by AB95 adults (flies derived from a population in the Mojave Desert where the major host is California barrel cactus) compared to adults from any of the other populations tested may be slightly biased. The average proportion of adults that preferred agria in these trails was 71.4% (SD = 12.15%), yet there was a considerable disparity between trials using the different volatile recipes (Fig. 3). Just as in the CC94 choice tests, the degree of preference for agria volatiles was lower in the Fogleman recipe trials, but there were just two replicate trials involving the Downing recipe cocktails. Despite this limitation, these data suggest that the barrel cactus breeding flies have retained the ancestral preference for agria similar to the other north–central Baja California populations (Fig. 3).

The results of Analysis 2 demonstrated that Baja adults clearly preferred the Fogleman agria volatile recipe over the Downing agria recipe. Therefore, populations of *D. mojavensis* can discriminate between variation in volatile profiles of a given host, not a surprising finding given the range of breeding site conditions observed in nature, from freshly initiated rots to older tissues no longer able to sustain *Drosophila* development. Variation in volatile concentrations within rots (subsamples of the same rot), between rots in different locations, and across seasons have been documented (Fogleman and Abril, 1990). The Fogleman agria recipe contained over twice the concentration of volatiles than the Downing agria recipe, as well as relative differences in several volatiles between agria and organ pipe recipes (Table II). The agria volatiles in the Fogleman choice tests were preferred by Baja adults (MSB94), which may have also provided a greater signal than the Downing agria volatiles for CC94 adults, allowing them to discriminate better between organ pipe and agria volatiles. Unfortunately, both concentration differences and subtle differences in the composition of the cocktails may be responsible (Table II). While both recipes contained known *Drosophila* attractants such as ethanol and acetic acid (Bar-

rows, 1907; Hutner *et al.*, 1937), the Fogleman cocktails, based on rot profiles in nature, contained acetoin and acetone that were not present in the Downing recipe. Acetic acid is an attractant as well as a stimulus to oviposition (Fogleman, 1982). High concentrations of acetoin are avoided by adult *D. mojavensis*, while acetone is a general attractant (Fogleman and Abril, 1990) that can engender increased adult longevity (Starmer *et al.*, 1982). Several of the other volatiles in the Fogleman cocktails are also preferred at high concentrations by *D. mojavensis*; butanediol, proprionic acid, and 2-propyl acetate, while *n*-butyric acid, 1-propanol, and 2-propanol are repellents at high concentrations (Fogleman and Abril, 1990). Thus, the Fogleman recipe contained a mixture of volatiles that can serve as attractants and repellents depending on concentration.

The sex-specific differences in numbers of flies that were trapped is consistent with the natural history and behavior of *D. mojavensis* adults. During morning and evening activity periods in the wild, males evenly space themselves on the outside of the cactus near openings to the fermenting tissues inside waiting for females to arrive (Krebs and Bean, 1991). Females must have access to the tissues inside for oviposition and feeding. During the day, both sexes can be found inside rots protected from high temperatures and desiccation. Since females in the olfactometer were aged well past sexual maturity along with males and were briefly food-deprived before the trials began, the volatile traps also served as a stimulus for oviposition. At the end of the host choice trials, eggs were often found in the volatile traps but not in the observation chamber. Thus, the nonrandom location of males and females within the volatile traps and the olfactometer was due to a female-biased attraction to the volatiles. Since both sexes this study showed preference for agria volatiles, the observed female-based sex ratios should not have altered this general result.

For populations of *D. mojavensis* in the wild, preference for agria has immediate consequences for fitness in both adults and their progeny. Adult *D. mojavensis* are dependent on volatiles from fermenting cactus tissues for energy (Starmer *et al.*, 1977; Brazner *et al.*, 1984), as agria and organ pipe tissues possess few free sugars that are available for adult consumption (Kircher, 1982; Fogleman and Abril, 1990). Assimilation of volatiles such as ethanol, acetic acid, 2-propanol, and acetone extends adult longevity (Batterham *et al.*, 1982), and ethanol vapor increases metabolic rates, lowers age at first reproduction, and increases fecundity (Etges and Klassen, 1989). Agria tissues also ferment faster than organ pipe tissues (Etges, 1989), providing greater quantities of these fermentation by-products for adults and larvae. Increased rates of fermentation imply greater abundances of yeast populations, also vital to adult nutrition. Therefore, higher agria volatile production must be partly responsible for the host preference hierarchy suggested by Downing (1985), in accord with many predictions concerning optimal diet breadth: once preference is established for a particular host that enhances individual fitness over other "suboptimal"

hosts, host shifts will occur only if the preferred host is absent (Rausher, 1984; for a review see Jaenike, 1990).

Unfortunately, few data are available concerning genetic determinants of host selection or how host preference is genetically related to host performance. Agria cactus tissues contain lower percentages of lipids and higher amounts of sugars than organ pipe tissues. Also, agria, cina, and organ pipe lack large amounts of alkaloids but instead contain high quantities of triterpene glycosides and lipids that the flies must overcome in order to use these hosts (Kircher and Bird, 1982; Fogleman and Abril, 1990). Studies of larval performance in agria and organ pipe tissues have shown that these cacti are experienced as very different environments in a population-specific manner. Mainland organ pipe-using populations and peninsular agria-using populations are considered host races because they exhibit genetic differences in life history in a host-specific manner (Etges, 1990). Within populations from Baja California and mainland Sonora, genetic variability for development time and adult thorax size is also expressed in a host-specific manner, indicating that evolution of these performance-related traits is ongoing (Etges, 1993, 1998). Together these data suggest that performance-related traits have evolved faster than preference for hosts other than agria. Since the age of the current Sonoran Desert plant association is ca. 14,000 years, the divergence in life histories from Baja California to the mainland is thought to be relatively recent. Therefore, the evolution of host performance has preceded host preference in *D. mojavensis*. Further genetic analysis of mainland populations, particularly the barrel cactus-using populations from the Mojave Desert, will help resolve the extent to which preference for alternate hosts is evolving, reinforcing the genetic divergence from agria to other secondary hosts.

ACKNOWLEDGMENTS

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