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# PREMATING ISOLATION IS DETERMINED BY LARVAL REARING SUBSTRATES IN CACTOPHILIC *Drosophila mojavensis*. III. EPICUTICULAR HYDROCARBON VARIATION IS DETERMINED BY USE OF DIFFERENT HOST PLANTS IN *Drosophila mojavensis* AND *Drosophila arizonae*

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**Abstract**—Adult epicuticular hydrocarbon profiles of male and female *Drosophila mojavensis* have been implicated as determinants of mate choice leading to premating isolation between geographically isolated populations. Hydrocarbon profiles of a Baja California and a mainland Sonora population of *Drosophila mojavensis*, a *yellow* body mutant strain of *D. mojavensis*, and a population of *D. arizonae* were compared among flies that had been reared on two cactus substrates and a synthetic laboratory growth medium in order to assess the degree to which natural rearing substrates influence adult hydrocarbon composition. Twenty epicuticular hydrocarbon components, ranging from $C_{29}$ to $C_{41}$, were recovered by gas chromatography that represented major classes of alkanes, alkenes, and alkadienes. We found differences in relative amounts of epicuticular hydrocarbons among Baja and mainland *D. mojavensis*, and the *yellow* body mutants. There were few differences between *D. mojavensis* and *D. arizonae*. The effects of rearing substrates were remarkable: most of the differences were due to the effects of lab food vs. cactus, but there were significant rearing substrate effects due to differences in the two cacti used. Eleven hydrocarbon components differed in abundance between males and females or showed significant sex × rearing substrate interactions from ANOVA. The effects of rearing substrates on epicuticular hydrocarbon composition in *D. mojavensis* are concordant with changes in the intensity of premating isolation between populations, implicating host ecology as a major determinant in patterns of mate choice in this species.

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**Key Words**—Incipient speciation, sexual selection, mating behavior, cuticular hydrocarbons, cactus, Sonoran Desert, *Drosophila*.

## INTRODUCTION

The origins of reproductive isolation in organisms that share a common fertilization system remain an unresolved issue in the study of speciation. In outcrossing species, variation in mating systems has been hypothesized to be a potent factor in the reduction of gene flow among demes, particularly when success in mating depends upon the interaction of multiple cues exchanged between the sexes. These may include chemical, behavioral, tactile, and acoustic signals exchanged prior to successful fertilization. Paterson (1978) argued that the evolution of a specific mate recognition system, composed of multiple "coadapted stages," is essential to intersexual signaling and should be maintained by strong stabilizing selection. This, however, was described as a species-specific recognition system (Carson, 1995) and describes Paterson's definition of a species (Paterson, 1985).

Variation in mate recognition systems among isolated demes within species may be promoted by local ecological conditions, leading to the evolution of new equilibrium signaling systems (Butlin, 1995). The effects of local conditions leading to divergence in mating systems may lead to premating isolation should such demes ever come into secondary contact. These causes for divergence are essential to the understanding of species formation.

Carson (1987, 1995) has pointed out that the evolution of common mate recognition systems via coadaptation of male–female signaling systems and sexual selection may be a major cause of speciation in animals. These interactions between potential mates within demes must be the driving force of sexual selection with only secondary consequences for reproductive isolation, consistent with Paterson's recognition model (Paterson, 1985). However, most analyses of premating isolation among presumed species "in statu nascendi" (Dobzhansky and Spassky, 1959; Dobzhansky and Pavlovsky, 1967) require assessment of the kinds of changes in mate choice between groups (Stalker, 1942; Malagolowkin-Cohen et al., 1965; Wasserman and Koepfer, 1977; Zouros and D'Entremont, 1980; Ehrman and Wasserman, 1987; Wu et al., 1995). There is clearly a lack of data addressing the connections between intrademic and interdemic facets of sexual selection (but see Jamart et al., 1993) and patterns of mate choice, i.e., are the mechanisms driving mating success within demes, sensu Carson, related to patterns of premating isolation between demes?

Before we can approach the relationships between intra- and interdemic mating behavior, cases of consistent premating isolation between populations

judged to be incipient species must be identified. Within-deme mating success can then be directly compared to mating success between individuals of different demes. As a first step towards this goal, premating isolation in *Drosophila mojavensis* has been intensively analyzed (Zouros and D'Entremont, 1974, 1980; Wasserman and Koepfer, 1977; Markow, 1981, 1991; Markow et al., 1983; Koepfer, 1987a,b; Etges, 1992; Brazner and Etges, 1993). The present study concerns variation in premating isolation and the epicuticular hydrocarbon profiles as presumed contact pheromones of adult flies (Markow and Toolson, 1990; Toolson et al., 1990).

Allopatric populations of *D. mojavensis* have been described as incipient species because of low, but significant levels of premating isolation between Baja California and mainland Sonora, Mexico populations. Behavioral isolation has been characterized as "one-way" premating isolation because mainland females tend to discriminate against Baja males in multiple choice tests, but Baja California females tend not to discriminate between Baja and mainland males. Courtship attempts are somewhat reduced in Baja males, but the major decrease in copulation frequency is due to a lack of mainland female receptivity once courtship has been initiated (Krebs and Markow, 1989). So far, little is known about the intensity of sexual selection within demes of *D. mojavensis*.

Intersexual signaling includes tactile stimulation by males and exchange of chemical cues through sensory reaction to the epicuticular hydrocarbons (Markow and Toolson, 1990; Toolson et al., 1990). Cuticular hydrocarbon variation has been implicated as a critical factor influencing mate choice in *D. melanogaster* group species (Antony and Jallon, 1982; Scott and Richmond, 1988; Ferveur, 1991; Coyne et al., 1994), *D. pseudoobscura* and *D. persimilis* (Noor and Coyne, 1996), *D. virilis* (Bartelt et al., 1986; Oguma et al., 1992), *D. pallidosa* (Nemoto et al., 1994), several Hawaiian *Drosophila* (Thompkins et al., 1993), house flies, *Musca domestica* (Reed et al., 1996), and tetranychid mites, *Tetranychus urticae* and *Oligonychus pratensis* (Margolies and Collins, 1994).

Because premating isolation in *D. mojavensis* is influenced by larval rearing substrates (Etges, 1992; Brazner and Etges, 1993) variation in epicuticular hydrocarbon profiles of flies reared on two natural hosts was compared with hydrocarbons from flies reared on laboratory medium. In this study, we tested the hypothesis that larval rearing substrates determine the quantity and variety of cuticular hydrocarbons in a mainland and Baja population of *D. mojavensis*, *yellow* body *D. mojavensis* (Etges, 1993) which are characterized by reduced male mating success with nonmutant females (Etges, 1997), and *D. arizonae*, a sibling species that sometimes shares host plants with *D. mojavensis* in nature (Ruiz and Heed, 1988).

## METHODS AND MATERIALS

The two wild populations of *D. mojavensis* used in this study were collected in 1994. Flies from Mission San Borja, Baja California Norte, were descended from 43 wild-caught adults, and mainland flies originated from 44 wild-caught adults and 181 flies that emerged from an organ pipe cactus, *Stenocereus thurberi*, rot from Cerro Colorado, Sonora. The *D. mojavensis yellow* body (*y*) strain was derived from an outbred population from Punta Onah, Sonora, in which the X-linked mutation arose spontaneously in the lab (Etges, 1993). A stock of the sibling species, *D. arizonae*, was derived from 534 adults that emerged from a *S. standleyi* cactus rot collected in Tomatlan, Jalisco in 1982 by W. B. Heed.

All flies were reared on banana–malt–yeast–agar food (Brazner and Etges, 1993) in 8-dr shell vials at room temperature (ca. 22°C) until the controlled growth experiment began. Each stock was then reared on lab food and fermenting pitaya agria, *S. gummosus*, and organ pipe cactus tissues. The four stocks of flies were cultured in an incubator programmed for a 14L:10D photoperiod and a temperature cycle of 27°C during the day and 17°C at night. Laboratory-food-reared adults were produced by allowing several hundred adults to oviposit directly onto lab food for several days in ½-pint bottles. Cactus-reared flies were cultured using standard methods (Etges, 1989); eggs were collected from several hundred adults and washed in deionized water, 70% ethanol, and again in sterile deionized water. Eggs were counted out in groups of 200, transferred to a 1-$cm^2$ piece of filter paper, and placed on fermenting cactus. Cactus cultures were established in plugged ½-pint bottles with 75 g of aquarium gravel at the bottom covered with a 5.5-cm-diameter piece of filter paper. Bottles were then autoclaved, and after 60 g of either agria or organ pipe tissue were in place, autoclaved again for 10 min. After cooling to room temperature, each culture was inoculated with 0.1 cc of a pectolytic bacterium, *Erwinia cacticida* (Alcorn et al., 1991), and 0 .1 cc of a mixture of the following seven species of yeasts common in natural agria and organ pipe rots (Starmer, 1982; Fogleman and Starmer, 1985); *Pichia cactophila, P. mexicana, P. amethionina* var. *amethionina*, *Cryptococcus cereanus*, *Candida valida*, *C. ingens*, and *C. sonorensis*. Eight replicate cultures of each food type were started for each of the four stocks of flies.

All flies were collected every day from each culture bottle, separated by sex, and aged in the incubator described above on lab food in vials for at least 12 days because cuticular hydrocarbon profiles change until adults are 8–10 days old (Toolson et al., 1990). Epicuticular hydrocarbons were extracted from counted groups of aged females and males (usually 20–30) in Biosil mini-columns. Each column consisted of a Pasteur pipet that contained packed glass wool and Biosil (silica gel, Sigma S-4133) washed several times with HPLC-

grade hexane. Flies were then added, washed in 8 ml of hexane, and the hydrocarbons were collected in hexane-rinsed vials. After the hexane was evaporated with nitrogen, each sample was sealed and stored at room temperature. Each hydrocarbon sample was redissolved in hexane (2.5 $\mu$l/fly) containing 382 ng of docosane ($C_{22}$) per microliter as an internal standard. One microliter of each sample was analyzed by capillary gas–liquid chromatography using a Shimadzu G14 fitted with a 30-m DB-1 fused-silica column. Injector and detector temperatures were set at 345°C with the injector port in split mode. Running temperatures started at 200°C and increased to 345° at 10°/min with a hold at 345°C for 7 min.

Peak identity was confirmed and equivalent chain lengths (ECLs) were calculated for each hydrocarbon component by coinjection of sample hydrocarbons with paraffin wax consisting of $C_{19}$ to $C_{38}$ straight-chain alkanes and docosane ($C_{22}$), hexatriacontane ($C_{36}$), and tetracontane ($C_{40}$) standards. Retention times of alkanes used to calculate ECLs were linearly related to their chainlengths (slope = 0.676, $r = 0.997$, 14 $df$, $P < 0.001$). Equivalent chain lengths for all hydrocarbon components were calculated by linear interpolation between alkane peaks of known chainlengths, i.e.,

$$ECL_{\text{sample}} = HC_n + (RT_{\text{sample}} - RT_n)/(RT_{n+1} - RT_n)$$

where $HC_n$ is an alkane of known hydrocarbon length $n$ and $RT$ is the retention time of each component. Alkane, alkene, and alkadiene peaks were identified by sequential elution of a sample using silver nitrate-impregnated Biosil in a minicolumn with successive washes of hexane, 2% diethyl ether in hexane, and 25% ether in hexane (Jackson et al., 1974; Toolson et al., 1990). At least two to four replicates of each group of flies grown on each rearing substrate were analyzed.

Hydrocarbon amounts were estimated by analysis of peak integrations using EZCHROM software (ver. 2.1) provided by Shimadzu. All data were expressed as nanograms per fly of cuticular hydrocarbons and were analyzed with ANOVA using PROC GLM in SAS (SAS Institute, 1985) with population, rearing substrate, and sex as main effects and all interactions between main effects. All significant main effects were further assessed by Duncan's multiple-range tests.

## RESULTS

The 20 most abundant epicuticular hydrocarbons from *D. mojavensis* and *D. arizonae* ranged from $C_{29}$ to $C_{39}$, but hydrocarbons with chain lengths greater than $C_{37}$ were usually variable in abundance and rare, including $C_{41}$ chain lengths (Table 1, Figure 1). The most abundant hydrocarbons consisted of molecules with chain lengths of $C_{29}$, $C_{31}$, $C_{33}$, $C_{35}$, and $C_{37}$, with the $C_{35}$ group accounting

TABLE 1. EPICUTICULAR HYDROCARBONS OF *D. mojavensis* AND *D. arizonae* IN THIS STUDY[a]

| Number of carbons | Type | Equivalent chain length |
|---|---|---|
| 29 | Branched alkane | 28.65 |
| 31 | Branched alkane | 30.65 |
| | *n*-alkene | 30.78 |
| 33 | Branched alkane | 32.47 |
| | Alkadienes | 32.63–32.70 |
| | *n*-alkene | 32.40–32.79 |
| 34 | Alkadienes | 33.50 |
| | *n*-alkene | 33.60 |
| 35 | Alkadienes | 34.59–34.66 |
| | Branched alkene | 34.45 |
| | *n*-alkene | 34.73 |
| 36 | Branched alkene | 35.60 |
| 37 | Alkadiene | 36.50 |
| | Branched alkene | 36.40 |
| | *n*-alkene | 36.60 |
| 38 | Alkenes | 37.40 |
| 39 | Alkenes | 38.50 |

[a]The types of alkanes, alkenes, and alkadienes are described in Toolson et al. (1990).

for 30–50% of total epicuticular hydrocarbons. Identification of alkanes, alkenes, and alkadienes and calculated equivalent chain lengths confirmed the molecular compositions and relative retention times of these hydrocarbons given in Toolson et al. (1990) with few exceptions. Because of the strong influence of rearing substrates on variation in epicuticular hydrocarbons, abundance of $C_{33}$, $C_{35}$, and $C_{37}$ branched alkenes could not always be reliably scored, so we have not included them in this analysis. They account for a small proportion ($< 10\%$) of total adult cuticular hydrocarbons in lab food reared flies (Toolson et al., 1990), and were occasionally absent in cactus reared flies.

The predominant classes of hydrocarbons were $C_{35}$ positional isomers of *n*-pentatriacontadiene (Toolson et al., 1990). We recovered two different peaks of these $C_{35}$ alkadienes with equivalent chainlengths of $C_{34.59}$ and $C_{34.66}$, depending on the group of *D. mojavensis* being analyzed. There was a striking correlation between the presence of $C_{34.59}$ and one of the two $C_{33}$ alkadiene peaks: if $C_{34.59}$ alkadiene was abundant, so was the $C_{32.63}$ alkadiene peak (Figures 2 and 3). Such correlations in the abundance of hydrocarbon components within different groups of *D. mojavensis* strongly suggest a simple chain-length-

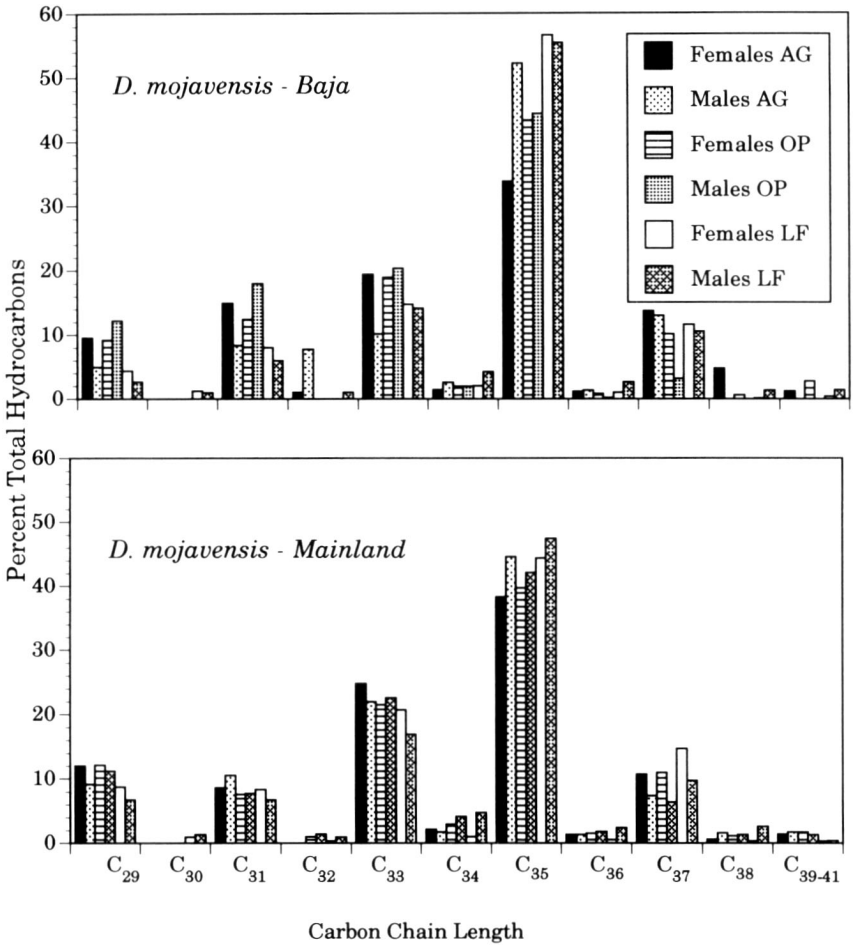


FIG. 1. The percent of total hydrocarbons (per fly) of each of the epicuticular hydrocarbon components observed in a population of *D. mojavensis* from Baja California, mainland Sonora, the *yellow* body (*y*) mutant strain, and a population of *D. arizonae*. The bars represent the average percent of each chain length for two to four replicates separated by sex and rearing substrate. The three rearing substrates are agria cactus (AG), organ pipe cactus (OP), and laboratory food (LF).

ening biosynthetic pathway for production of the variety of epicuticular hydrocarbons in this species (Toolson et al., 1990).

*Variation Due to Rearing Substrates.* Rearing *D. mojavensis* and *D. arizonae* on fermenting agria or organ pipe cactus tissues and laboratory food

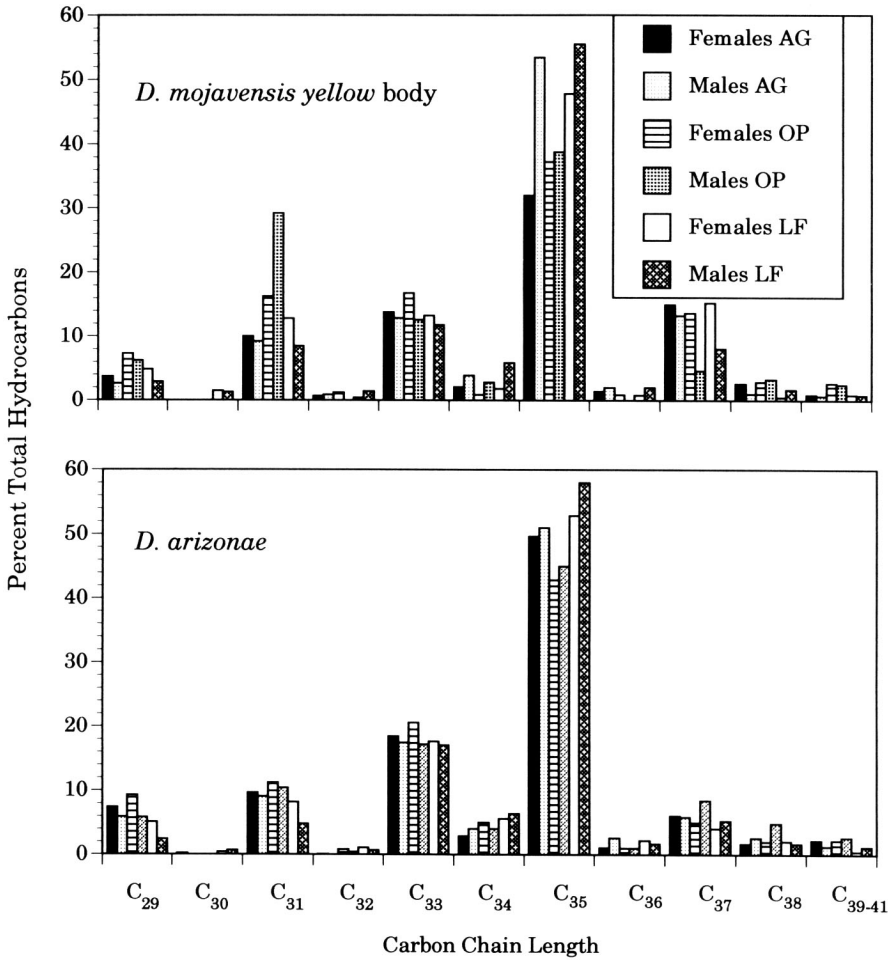


FIG. 1. Continued.

caused both qualitative and quantitative differences in the amounts of epicuticular hydrocarbons (Figures 1 and 2). These differences were apparent even though the flies had been fed lab food as young adults. Amounts of $C_{29}$ branched alkanes ($C_{29br}$, ECL = 28.65) were significantly greater in flies reared on organ pipe than agria or lab food (Figure 4; $F = 15.64$, $P < 0.0001$), and there was a significant food × sex interaction ($F = 6.19$, $P = 0.006$) due in part to the large increase in $C_{29br}$ in females reared on organ pipe (Figure 5). The small amounts of $C_{30}$ *n*-alkane were only observed in flies reared on lab food (Figure

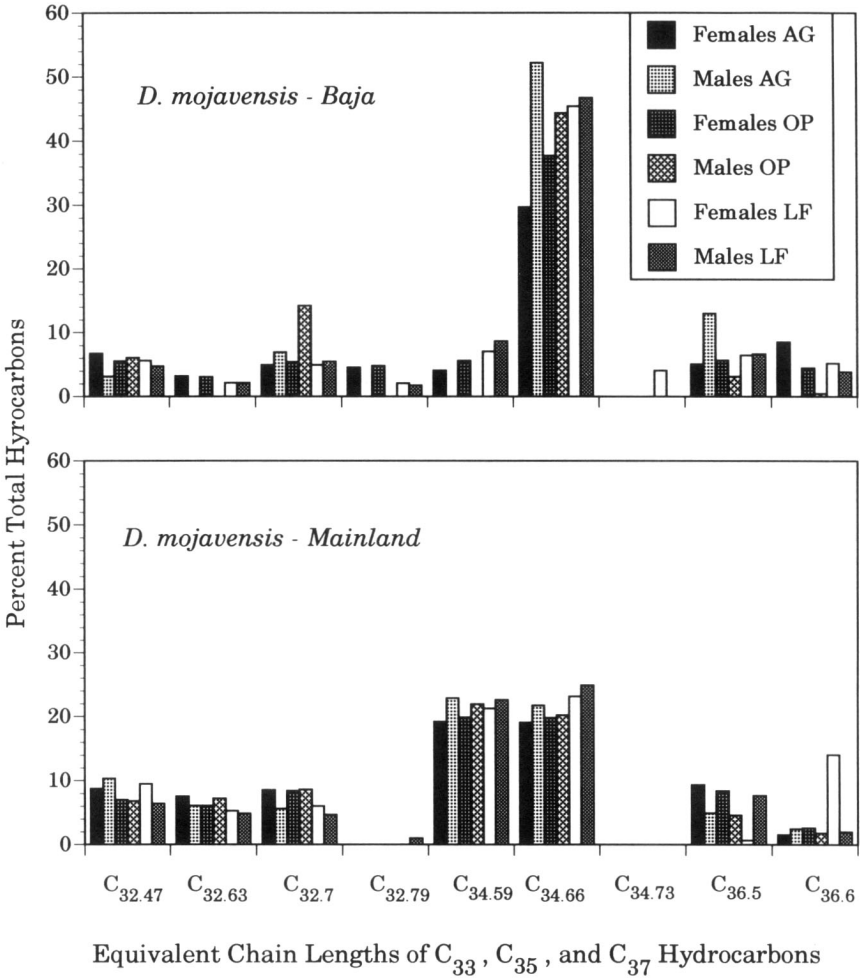


FIG. 2. The percent of total hydrocarbons (per fly) of each of the $C_{33}$, $C_{35}$, and $C_{37}$ epicuticular hydrocarbon components observed in the four groups of *D. mojavensis* and *D. arizonae* in this study designated by equivalent chain lengths. The labels are the same as in Figure 1.

1), thus some hydrocarbons in *D. mojavensis* and *D. arizonae* are manufactured only under laboratory conditions. The most abundant alkane, $C_{31br}$ (ECL = 30.65), was generally more abundant in flies reared on organ pipe tissues than either agria or lab food (Figure 4; $F = 8.25$, $P < 0.002$). There were no differences in amounts of any of the $C_{33}$ components due to rearing substrates.

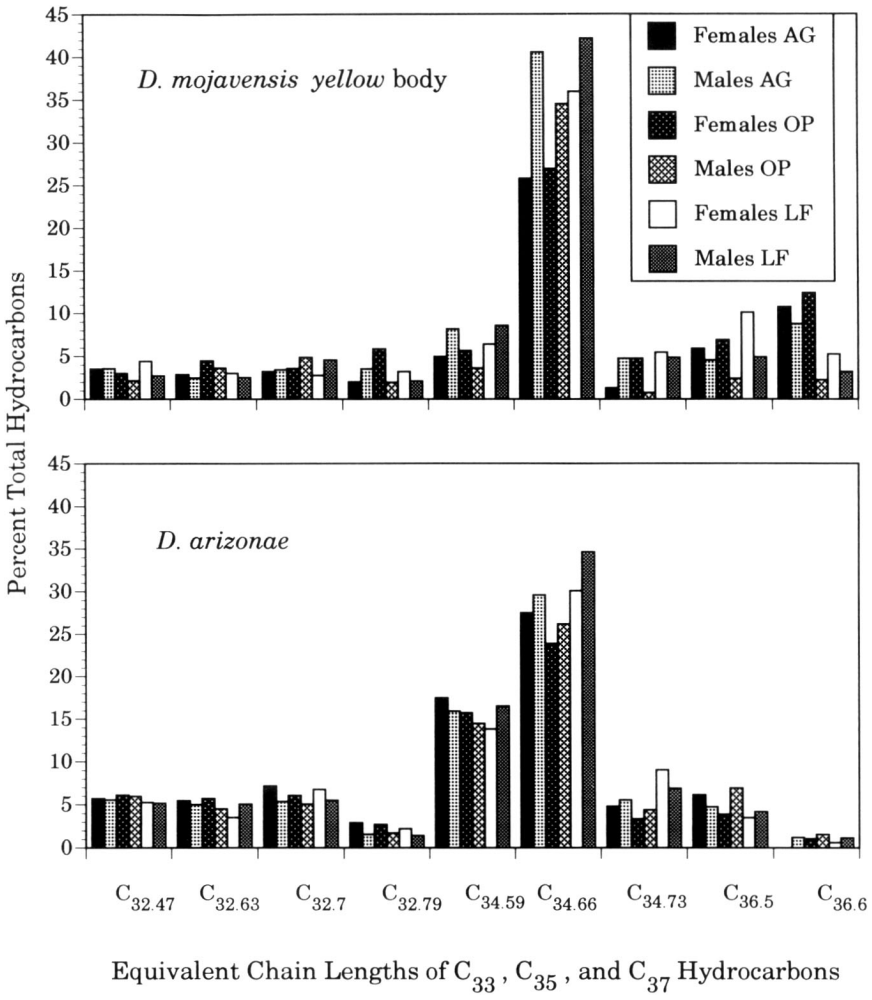


FIG. 2. Continued.

Both $C_{34}$ alkenes and alkadienes were present in significantly greater amounts in lab food-reared flies than those reared on either cactus substrate ($F = 4.43$, $P = 0.022$ and $F = 10.55$, $P < 0.001$, respectively, Figure 5). All three major $C_{35}$ components, both $C_{34.59}$ and $C_{34.66}$ dienes and $C_{34.73}$ *n*-alkene, were also significantly more abundant in lab food-reared flies (Figures 2, and 5; $F = 10.21$, $P = 0.0005$; $F = 5.64$, $P < 0.01$; and $F = 16.73$, $P < 0.0001$,

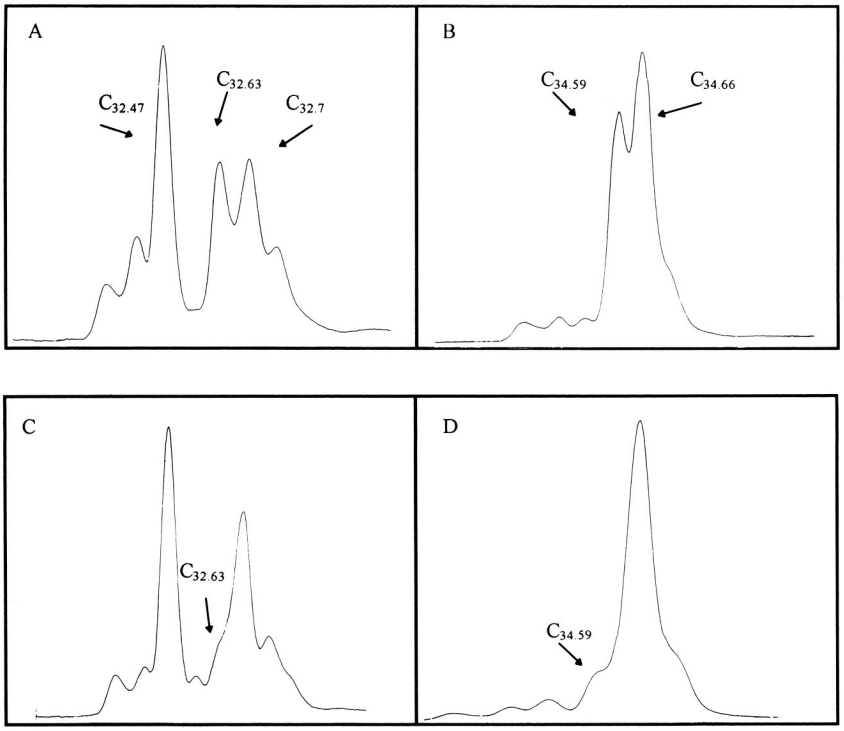


FIG. 3. Gas chromatograms of the $C_{33}$ and $C_{35}$ groups of epicuticular hydrocarbons showing the difference between mainland (A) and Baja (C) $C_{33}$ profiles, and the difference between mainland (B) and Baja (D) $C_{35}$ profiles. The equivalent chainlengths of each component designated in Table 1 are shown.

respectively). We routinely recovered a single $C_{36}$ alkene peak, which Toolson et al. (1990) identified as a straight-chain *n*-alkene, $C_{36:1}$. This hydrocarbon was also in greater abundance in flies reared on lab food ($F = 10.42$, $P = 0.0004$). Amounts of the $C_{36.5}$ alkadiene component did not differ between food types but amounts of $C_{36.6}$ *n*-alkene were present in higher quantities in lab food-reared flies ($F = 16.82$, $P < 0.0001$) than agria- or organ pipe-reared flies. There were no significant food effects on any of the hydrocarbons longer than $C_{37}$. Total epicuticular hydrocarbon amounts were greater in lab food-reared flies, 1443.6 ng/fly, than either organ pipe- or agria-reared flies, 1061.9 and 1013.1 ng/fly, respectively ($F = 6.13$, $P = 0.0064$).

In order to assess the degree to which variation in host use in nature may cause differences in epicuticular hydrocarbon profiles, these data were reana-

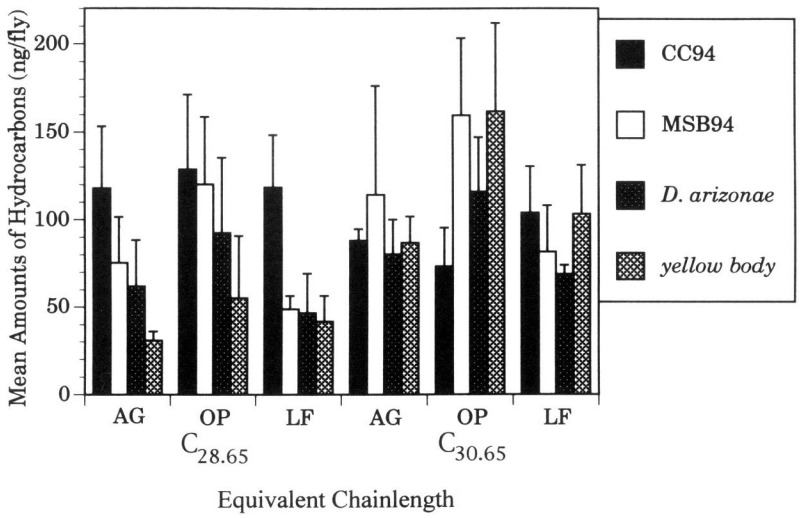


FIG. 4. Mean (+ 1 SD) amounts of the two major alkanes of the four groups of *D. mojavensis* and *D. arizonae* in this study showing the effects of rearing substrates on their relative abundances. Labels for the three rearing substrates are explained in Figure 1.

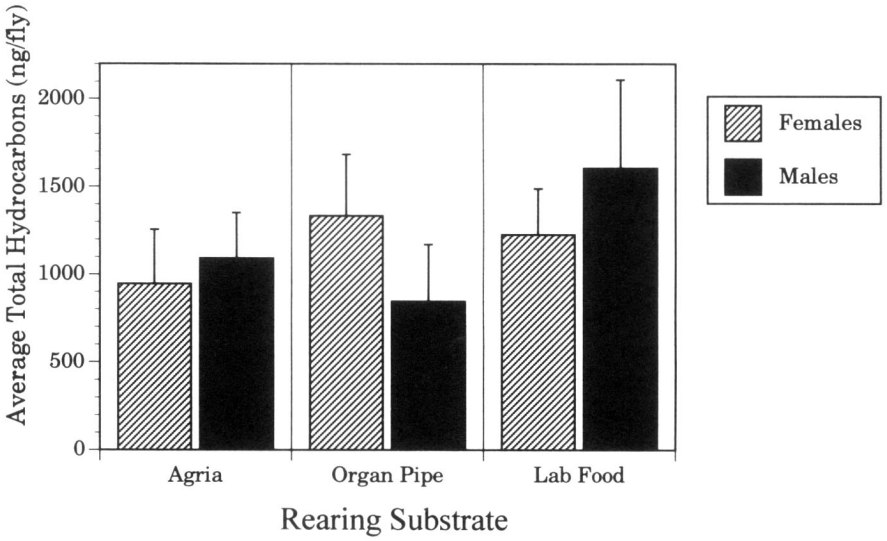


FIG. 5. The influence of rearing substrates (both cacti vs. lab food) on differences between males and females in average total amounts (+ 1 SD) of epicuticular hydrocarbons in this study.

lyzed after eliminating the lab food-reared flies. This way, differences between the effects of agria and organ pipe substrates on adult hydrocarbons were directly assessed. Amounts of $C_{29}$ branched alkanes, $C_{31br}$ alkanes, and $C_{34}$ alkenes were greater in organ pipe-reared flies than those reared on agria ($F = 15.68$, $P = 0.001$; $F = 7.83$, $P = 0.012$; and $F = 5.85$, $P = 0.027$, respectively). Thus, changes in the composition of epicuticular hydrocarbons in adult *D. mojavensis* due to different rearing substrates can be caused by their host cacti.

*Variation Among Populations and Species.* Although most of the hydrocarbon components differed in abundance among Baja and mainland *D. mojavensis*, *y* body *D. mojavensis*, and *D. arizonae* (Figures 1 and 2), the differences between mainland and Baja populations are of most interest due to the presumed role of epicuticular hydrocarbons as contact pheromones (Markow and Toolson, 1990). Levels of $C_{29}$ and $C_{33}$ branched alkanes and $C_{32.63}$ and $C_{34.59}$ dienes (Figure 3) were greater in mainland adults than Baja adults ($F = 11.48$, $P < 0.005$; $F = 6.79$, $P = 0.021$; $F = 55.0$, $P < 0.0001$; and $F = 91.26$, $P < 0.0001$, respectively), and $C_{31br}$ alkanes, $C_{33}$ *n*-alkenes, $C_{34}$ and $C_{34.66}$ dienes, and $C_{34.73}$ *n*-alkenes were greater in Baja adults than mainland adults ($F = 5.60$, $P < 0.05$; $F = 20.12$, $P = 0.0005$; $F = 22.34$, $P = 0.0003$; $F = 26.45$, $P < 0.0001$; and $F = 18.68$, $P < 0.001$, respectively). Total hydrocarbon amounts were not different between these two populations.

Since the *y* body mutation affects the formation of black melanin and its placement in the cuticle in *D. melanogaster* (Walter et al., 1991), we hypothesized that there would be little difference in amounts of epicuticular hydrocarbons between *y D. mojavensis* and normally pigmented flies from the mainland. The *y* body adults produced the smaller quantities of $C_{29}$ branched alkanes than any of the other groups (Figure 1): 121.5 ng/fly, *D. mojavensis* mainland; >86.8 ng/fly, *D. mojavensis* Baja; >66.8 ng/fly, *D. arizonae*; >42.4 ng/fly *y D. mojavensis* (Duncan's multiple-range test, $P < 0.05$). Amounts of $C_{31br}$ alkanes were intermediate when compared to the other groups, but there was a significant population × food interaction ($F = 2.81$, $P < 0.03$; Figure 4). Thus, amounts of $C_{31br}$ alkanes differed among groups, but these differences were not predictable across rearing substrates. Of the other most abundant hydrocarbon components, *y* adults had comparatively less $C_{33}$ branched alkanes, $C_{32.63}$ and $C_{32.7}$ dienes, and $C_{34}$ alkenes, but larger amounts of $C_{32.79}$ *n*-alkenes than the other populations (Duncan's multiple-range tests, all $P < 0.05$). Average total hydrocarbon amounts of *y* adults were not significantly lower than those of the other groups (941 ng/fly vs an average of 1243 ng/fly of the other three groups).

Overall, differences in hydrocarbon components between *D. mojavensis* and *D. arizonae* were unremarkable (Figures 1 and 2). In most cases, when there was significant variation among groups, *D. arizonae* were intermediate in amounts of cuticular hydrocarbons, particularly for the most abundant classes.

*D. arizonae* had the highest amounts of $C_{34}$ and $C_{34.73}$ *n*-alkenes, and the lowest amounts of $C_{36.6}$ *n*-alkenes than the other groups (Duncan's multiple range tests, all $P < 0.05$). For hydrocarbons greater in size than $C_{37}$, *D. arizonae* possessed greater amounts of $C_{38}$ and $C_{40}$ chain lengths than the other groups.

*Hydrocarbon Variation Among Males and Females.* The potential utility of any one or a suite of epicuticular hydrocarbons to serve as contact pheromones requires that these compounds alter intersexual recognition. If epicuticular hydrocarbon variation caused by rearing substrates is involved with decreased premating isolation between Baja and mainland adults (Etges, 1992; Brazner and Etges, 1993), then the cactus-reared flies should show alterations in hydrocarbon composition that break down behavioral isolation relative to lab food-reared flies. Thus, any sex × food interactions in the ANOVAs are of considerable interest. Eleven of the 20 major hydrocarbon components differed between sexes and/or showed sexual dimorphism that changed with rearing substrate (Table 2). Based only upon body size, total epicuticular hydrocarbons should be greater in females than males because female *D. mojavensis* are larger (Etges, 1990) and thus have a greater surface area. However, total hydrocarbons amounts did not differ among males and females (Table 2), so differences in amount of component hydrocarbons were likely due to sex and not body size. Adult males

TABLE 2. DIFFERENCES BETWEEN MALES AND FEMALES FOR AMOUNTS OF EPICUTICULAR HYDROCARBONS IN THIS STUDY[a]

| | Amount (ng/fly) | | $F^b$ | |
|---|---|---|---|---|
| Hydrocarbon | Male (mean ± SD) | Female (mean ± SD) | Sex | Sex × rearing |
| $C_{29Br}$ alkane | 63.48 ± 31.56 | 93.81 ± 48.87 | 28.66*** | 6.19** |
| $C_{C31Br}$ alkane | 96.90 ± 36.95 | 113.56 ± 47.00 | 4.77* | 1.20 ns |
| $C_{32.63}$ diene | 36.88 ± 33.57 | 53.55 ± 28.86 | 5.47* | 8.15** |
| $C_{32.79}$ *n*-alkene | 15.90 ± 16.67 | 28.93 ± 29.73 | 7.79** | 7.51** |
| $C_{34}$ diene | 14.20 ± 17.28 | 6.78 ± 8.28 | 11.99** | 6.17** |
| $C_{34}$ *n*-alkene | 34.47 ± 32.70 | 23.16 ± 26.64 | 3.09 ns | 4.17* |
| $C_{34.59}$ diene | 129.95 ± 120.34 | 149.63 ± 110.56 | 0.02 ns | 6.60** |
| $C_{34.66}$ diene | 435.62 ± 242.75 | 347.77 ± 161.06 | 3.04 ns | 3.54* |
| $C_{36Br}$ alkene | 19.07 ± 15.55 | 12.82 ± 8.25 | 8.30** | 13.69*** |
| $C_{36.5}$ diene | 65.76 ± 35.51 | 66.50 ± 54.03 | 0.06 ns | 8.16** |
| $C_{36.6}$ *n*-alkene | 66.85 ± 62.30 | 27.97 ± 30.36 | 58.64*** | 6.75** |
| Total hydrocarbons | 1157.20 ± 488.49 | 1172.79 ± 427.06 | 0.00 ns | 5.56** |

[a] All means are based on 26 observations.
[b] *F* statistics for the main effect of sex and significant sex × rearing substrate interaction terms from ANOVAs are given. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ns: not significant.

that were reared on agria tended to have more total epicuticular hydrocarbons then females, but this was reversed when the flies were reared on organ pipe (Figure 6). Total hydrocarbons were greater in lab food-reared flies (see above) with the smaller males having greater amounts of total hydrocarbons than females.

Females had significantly greater amounts of both of the major alkanes, $C_{29Br}$ and $C_{31Br}$, than males and the pattern of expression was similar across rearing substrates (Figure 5). Females expressed greater amounts of $C_{32.63}$ dienes and $C_{32.79}$ *n*-alkenes than males consistent across cactus hosts, but reversed on lab food, leading to a significant sex × rearing substrate interaction (Table 2). A similar pattern was apparent for both $C_{34}$ components and $C_{34.59}$ and $C_{36.5}$ dienes, but not for $C_{34.66}$ dienes. Although overall amounts of $C_{34.66}$ were not different in males and females, males possessed more of this component than females when reared on agria and lab food, but not on organ pipe (Figure 5). Relative amounts of $C_{36.6}$ *n*-alkene were also dependent on substrate type. Thus, for many of the epicuticular hydrocarbons that differed in amounts between adult male and female *D. mojavensis*, lab food caused a reversal in the relative amounts expressed by males and females compared to cactus-reared flies.

## DISCUSSION

Epicuticular hydrocarbon profiles of adult *D. mojavensis* and *D. arizonae* are dependent on larval rearing substrates. Thus, the presumed chemical cues involved with mate recognition in these species are sensitive to the type of larval substrates used in nature that could cause local shifts in mate signaling (Butlin, 1995). The relevance of these results to patterns of reproductive isolation between *D. mojavensis* and *D. arizonae*, while not the focus of this study, are tied to the hypothesized cause for behavioral premating isolation between geographically isolated populations of *D. mojavensis*. The presence of *D. arizonae* on the mainland is thought to be responsible for causing reproductive character displacement in mainland *D. mojavensis* populations (Zouros and D'Entremont, 1974; Wasserman and Koepfer, 1977). Although the extent to which *D. arizonae* is fully sympatric with *D. mojavensis* is unclear (Brazner and Etges, 1993), these species have been documented to occasionally share both agria in coastal Sonora and cina, *S. alamosensis*, in southern Sonora (Markow et al., 1983; Ruiz and Heed, 1988; Etges and Heed, unpublished data).

In general, past studies have shown that low but significant premating isolation exists between mainland Sonora populations and Baja California populations of *D. mojavensis* (Zouros and D'Entremont, 1980; Markow, 1981; Koepfer, 1987a,b; Krebs and Markow, 1989). Unfortunately, most past studies have been conducted with lab food-reared flies or flies reared on lab food sup-

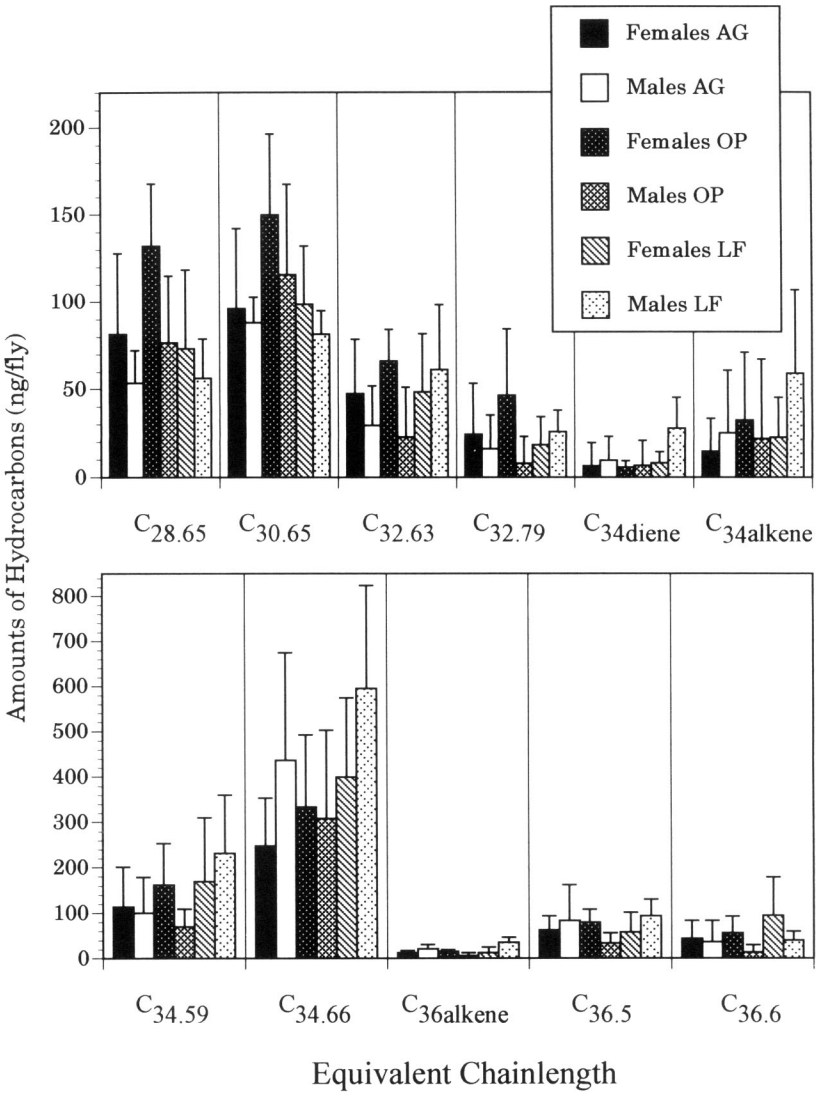


FIG. 6. The influence of rearing substrates on those hydrocarbon components that differed between males and females or showed sex × rearing substrate interactions in this study. Substrate labels are explained in Figure 1.

plemented with cactus (Markow et al., 1983), and these rearing conditions artificially induce significant premating isolation, increase mainland female-based assortative mating, and increase levels of mainland male mating propensity (Brazner, 1983; Brazner and Etges, 1993). Toolson et al. (1990, p. 1174) reported that flies reared on agria tissues were "only about half as large" and yielded only 33% as much total hydrocarbons as compared to flies reared on lab food. Unfortunately, they provided no information on culture conditions or any further information about these experiments, but pointed out that "compounds in *Stenocereus* tissue can directly affect synthesis and deposition of epicuticular hydrocarbons." We agree. Larval growth on artificial medium, "lab food," causes multiple significant differences in *D. mojavensis* and *D. arizonae* epicuticular hydrocarbons and premating isolation between peninsular and mainland populations of *D. mojavensis* as compared to flies reared on cactus substrates.

Environmentally induced shifts in insect cuticular hydrocarbons have also been associated with temperature and humidity experienced by larvae or adults (Toolson and Kuper-Simbron, 1989; Markow and Toolson, 1990; Reidy et al., 1991). A shift towards increased proportions of longer-chain hydrocarbon components at higher temperatures has been shown to result in decreased cuticle permeability, a response to thermal stress (Toolson and Hadley, 1979; Toolson, 1982). Thus, epicuticular hydrocarbon profiles in wild populations of *D. arizonae* and *D. mojavensis* are likely to be influenced by both rearing substrates and ambient temperatures.

Markow and Toolson (1990) selected total $C_{35:2}$ and $C_{37:2}$ alkadienes as the most likely components to serve as contact pheromones because of their abundance and degree of sexual dimorphism. They found that the ratio of $C_{35:2}$ to $C_{37:2}$ alkadienes, "*R* values," increased in adults held at 17°C for eight days after eclosion and that males with higher *R* values experienced greater mating success than males with lower *R* values caused by holding them at 34°C for eight days (Table 3). Overall amounts of total $C_{35:2}$ and $C_{37:2}$ alkadienes did not differ among males and females in the present study, but because there were sex × rearing substrate interactions, $C_{35:2}$ and $C_{37:2}$ alkadiene differences between males and females were not predictable across substrates. Analyses of flies reared on agria and organ pipe substrates with the lab food data deleted revealed that amounts of $C_{34.59}$ alkadiene were 67% greater in females than males ($F = 9.91$, $P < 0.01$), but this was dependent on cactus type. Analysis of *R* values by ANOVA with just the mainland and Baja populations revealed several interesting interaction terms involving rearing substrates. *R* values ranged from 4 to 9, with two prominent outliers caused by very small amounts of $C_{37:2}$ alkadienes (Figure 7). This pattern of *R* values is inconsistent with the observations made by Markow and Toolson (1990), who suggested that lab food-

TABLE 3. ANALYSIS OF VARIANCE RESULTS FOR RATIOS OF $C_{35:2}$ TO $C_{37:2}$ ALKADIENES, *R* VALUES (TOOLSON ET AL., 1990), FOR MAINLAND AND BAJA CALIFORNIA POPULATIONS OF *D. mojavensis* REARED ON LAB FOOD AND BOTH AGRIA AND ORGAN PIPE CACTUS TISSUES IN THIS STUDY

| Source | *df* | Type IV SS | *F* | *P* |
|---|---|---|---|---|
| Model | 11 | 621.12 | 3.29 | 0.026 |
| Population | 1 | 3.37 | 0.20 | 0.666 |
| Food | 2 | 96.71 | 2.82 | 0.099 |
| Sex | 1 | 0.23 | 0.01 | 0.910 |
| Population × food | 2 | 137.97 | 4.02 | 0.046 |
| Population × sex | 1 | 33.59 | 1.96 | 0.187 |
| Food × sex | 2 | 224.78 | 6.55 | 0.012 |
| Population × food × sex | 2 | 121.78 | 3.55 | 0.062 |
| Error | 12 | 206.06 | | |

*reared males with higher R values (up to 8) were more readily accepted by* females during courtship. Cactus-reared mainland males generally had larger *R* values than females, similar to observations reported by Toolson et al. (1990) for wild caught adults from San Carlos, Sonora. *R* values for Baja adults reared on agria were not consistent with the greater mating activity of agria-reared Baja

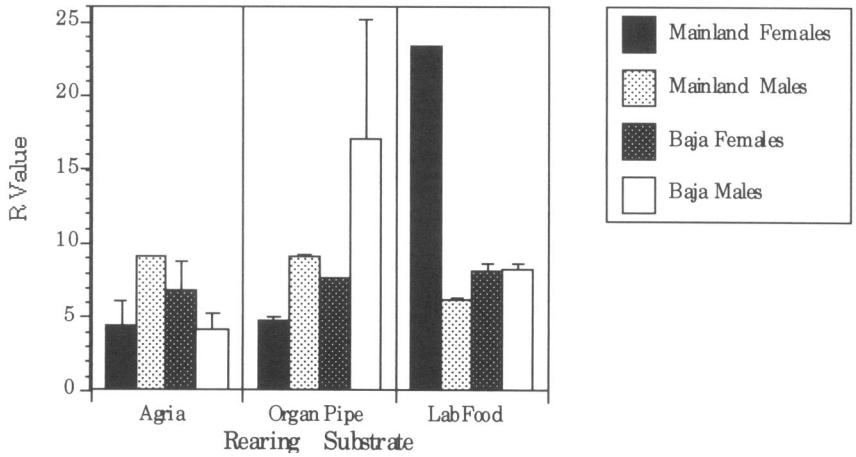


FIG. 7. Plot of $C_{35:2}/C_{37:2}$ alkadiene ratios, *R* values (+ 1 SD), of males and females reared on cactus and lab food in this study.

males and decreases in premating isolation with mainland adults (Brazner and Etges, 1993). More data for a larger number of natural populations are certainly required, but it is clear that if both $C_{34.59}$ and $C_{36.5}$ alkadienes are involved in mate recognition, their utility as cues will differ depending on the cactus used for larval growth and development.

The number of epicuticular hydrocarbon components that differed among sexes in a host-specific fashion suggests that the chemical basis of mate recognition in *D. mojavensis* involves a suite of contact pheromones as opposed to the relatively simple sexual differences in *D. melanogaster* (Jallon, 1984; Scott, 1994). Any interpretation of the contributions of particular hydrocarbons to mate discrimination, including female and male choice, must include the variation due to cactus rearing substrates in this species if we are to understand the role of epicuticular hydrocarbons in premating isolation and sexual selection outside of the laboratory. Further analysis of the role of particular hydrocarbon profiles will be required before we can conclude which components are responsible for the shifts in premating isolation. Also necessary will be analyses of cuticular hydrocarbon composition among wild-caught flies and the effects of cina cactus on epicuticular hydrocarbons of both *D. mojavensis* and *D. arizonae*. Since use of particular hosts varies across the range of *D. mojavensis* (Heed and Mangan, 1986; Etges et al., 1997) chemical cues important in mate discrimination will vary among populations that use different hosts.

Obviously, the results of our studies of premating isolation and the role of cuticular hydrocarbon variation in mating systems could not have been conducted in *D. mojavensis* without knowledge of their ecology. If these results are in any way general to other species, one conclusion from the present study concerning the role of cuticular hydrocarbons in premating studies carried out with flies reared on artificial substrates is: Proceed with caution.

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