JOURNAL OF Evolutionary Biology

doi: 10.1111/jeb.12073

# Premating isolation is determined by larval rearing substrates in cactophilic *Drosophila mojavensis*. IX. Host plant and population specific epicuticular hydrocarbon expression influences mate choice and sexual selection

J. A. HAVENS[1] & W. J. ETGES

*Program in Ecology and Evolutionary Biology, Department of Biological Sciences, University of Arkansas, Fayetteville, AR, USA*

*Keywords:*

cactus;
desert;
G × E interaction;
pheromone;
sexual isolation;
sexual selection.

## Abstract

Sexual signals in cactophilic *Drosophila mojavensis* include cuticular hydrocarbons (CHCs), contact pheromones that mediate female discrimination of males during courtship. CHCs, along with male courtship songs, cause premating isolation between diverged populations, and are influenced by genotype × environment interactions caused by different host cacti. CHC profiles of mated and unmated adult flies from a Baja California and a mainland Mexico population of *D. mojavensis* reared on two host cacti were assayed to test the hypothesis that male CHCs mediate within-population female discrimination of males. In multiple choice courtship trials, mated and unmated males differed in CHC profiles, indicating that females prefer males with particular blends of CHCs. Mated and unmated females significantly differed in CHC profiles as well. Adults in the choice trials had CHC profiles that were significantly different from those in pair-mated adults from no-choice trials revealing an influence of sexual selection. Females preferred different male CHC blends in each population, but the influence of host cactus on CHC variation was significant only in the mainland population indicating population-specific plasticity in CHCs. Different groups of CHCs mediated female choice-based sexual selection in each population suggesting that geographical and ecological divergence has the potential to promote divergence in mate communication systems.

## Introduction

When mate communication systems vary between ecologically divergent populations, natural and sexual selection can interact to influence reproductive isolation. Because sexual selection can drive changes in mate recognition traits, it can be a force in speciation (Panhuis *et al.*, 2001), but it is unlikely that sexual selection alone can cause reproductive isolation in the absence of natural selection (Ritchie, 2007). One source of divergent selection is spatial variation in natural selection on mate communication systems (Ryan & Rand, 1993; Boughman, 2002), which occurs when the intensity and direction of selection on sexual signals or preferences varies between local environments. Divergence in sexual signals can result from adaptation to different environments (Schluter & Price, 1993; Marchetti *et al.*, 1998; Sattman & Cocroft, 2003; Petfield *et al.*, 2005), and the fitness consequences of female mate choice resulting from sexual selection may also vary among environments (Jia & Greenfield, 1997; Lesna & Sabelis, 1999; Welch, 2003; Danielson-Francois *et al.*, 2009). Thus, environmental adaptation can lead to divergence in sexual signals, preferences and/or consequences of mate choice through pleiotropy, epistatic interactions or linkage disequilibrium with traits diverging due to natural selection (Rundle *et al.*, 2005).

Natural and sexual selection may interact during the evolution of mate recognition (Blows, 2002), and often

*Correspondence:* Julie A. Havens, Program in Ecology and Evolutionary Biology, Department of Biological Sciences, University of Arkansas, Fayetteville, AR, USA. Tel.: +1 479 575 6358; fax: +1 479 575 4010; e-mail: julie.havens@armstrong.edu
[1]Present address: Department of Biology, Armstrong Atlantic State University, Savannah, GA 31419-1997

oppose one another in direction on the same trait (Sharma *et al.*, 2012). When sexual signals or preferences become genetically linked to traits under natural selection, the indirect influences of natural selection on mate communication can result in the evolution of nonrandom mating (Maan & Seehausen, 2011). If spatial variation in natural selection on mate communication systems leads to divergence between populations, the resulting linkage disequilibrium between naturally and sexually selected traits within divergent populations can be maintained when there is no gene flow (Maan & Seehausen, 2011).

Genotype × environment interactions (GEI) for sexual signals and/or fitness consequences of female choice have been observed in many species, whereas GEI in mate preferences are far less common (Ingleby *et al.*, 2010). Interest in the influence of GEI on sexual selection has focused on the maintenance of genetic variation for sexual signals (Jia *et al.*, 2000; Greenfield & Rodriguez, 2004), and the potential disruption of sexual signal reliability (Kokko & Heubel, 2008; Higginson & Reader, 2009). When populations are diverging in allopatry, the reliability of sexual signals, and thus female preference should be maintained (Kokko & Heubel, 2008), allowing sexual selection to contribute to the formation of isolating barriers initiated by natural selection.

Investigating the link between sexual selection and population divergence is best attempted by studying diverging populations of species with a well-known biogeography and ecology. We seek an understanding of the role of male sexual signals, including epicuticular hydrocarbons and courtship songs, in intrademic mate choice in divergent populations exposed to contrasting ecological conditions to reveal the degree of plasticity in signals that may influence the strength of sexual selection. Epicuticular hydrocarbons are involved in chemical communication between the sexes in *Drosophila* mediated by the exchange of compounds that are either sensed from short distances via olfactory organs, or perceived by direct contact with taste organs, usually located on the tarsae or proboscis (Ferveur, 1997, 2005). Chemical compounds, usually fatty-acid derived nonvolatile hydrocarbons deposited on the cuticle, have been identified using gas chromatography/mass spectrometry (GC-MS) and other techniques (Blomquist *et al.*, 1987; Yew *et al.*, 2011). In *Drosophila*, most compounds range in chain length from 20 to 40 carbons, but the genus exhibits a remarkable variety with regard to molecular structure, sexual dimorphism and inter- and intraspecific variation. The types of epicuticular hydrocarbons (CHCs) include *n*-alkanes, branched alkanes, monoenes, alkadienes, alkatrienes and alkatetraenes with a wider variety found in some species than in others (Ferveur, 2005; Oliveira *et al.*, 2011; Yew *et al.*, 2011).

Hexane soluble CHCs in the *Drosophila mojavensis* species cluster (*D. mojavensis*, *D. arizonae* and *D. navojoa*) range in chain length from 28 to 40 carbons, with compounds consisting of straight chain and branched alkanes and alkenes and alkadienes (Stennett & Etges, 1997; Etges & Jackson, 2001). CHCs serve as contact pheromones determining mate choice that causes sexual isolation between populations (Etges & Ahrens, 2001; Etges *et al.*, 2009, 2010), but the exact blends of CHC components that determine mating success are unknown. Analyses of CHC variation in males from between-population mating trials have repeatedly implicated $C_{29}$ and $C_{31}$ branched alkanes, $C_{34}$ alkadienes, $C_{37}$ alkenes and dienes and a number of others (Etges & Tripodi, 2008; Etges *et al.*, 2009).

*Drosophila mojavensis* is a cactophilic species endemic to the Sonoran and Mojave Deserts and adjacent arid lands (Heed, 1982). Throughout its range, *D. mojavensis* uses several different host cacti for feeding and breeding. Baja California populations use agria cactus, *Stenocereus gummosus*, with occasional use of *Myrtillocactus cochal*, and populations in mainland Sonora, Mexico and Arizona mostly use organ pipe cactus, *S. thurberi*, with occasional use of sina cactus, *S. alamosensis*. Mojave Desert populations use California barrel cactus, *Ferocactus cylindraceus* and a disjunct population on Santa Catalina Island, California is associated with several species of *Opuntia* (Heed, 1982; Etges *et al.*, 1999; Beckenbach *et al.*, 2008). Allopatric populations have diverged in life histories due in part to a host plant switch from agria cactus to organ pipe cactus after colonization of mainland Mexico from Baja California (Heed, 1978, 1982; Etges, 1990), and premating isolation has evolved along with life history divergence (Etges, 1998). Courtship songs and CHCs vary among geographically isolated populations that exhibit premating isolation (Etges & Ahrens, 2001; Etges & Jackson, 2001; Etges *et al.*, 2006, 2009), and are phenotypically plastic due to different host rearing environments (Etges, 1992; Etges *et al.*, 2007, 2010).

Here, we assessed CHC variation in adult flies from an experiment comparing mate choice treatments designed to manipulate the opportunity for female choice-based sexual selection in two divergent populations of *D. mojavensis* reared on two different host plants: these were the same flies used to uncover the indirect effects of sexual selection in Havens *et al.*, 2011. We tested three hypotheses; (i) CHCs do not differ between mated and unmated males in within-population mating trials (Etges & Tripodi, 2008; Etges *et al.*, 2009), as in between-population mating trials, and (ii) CHCs do not influence female discrimination of males differently between populations, especially when reared on different host cacti (Havens *et al.*, 2011) and (iii) adults allowed to choose mates have offspring with CHC profiles that are not different from offspring of adults allowed no choice of a mating partner.

## Materials and methods

### Stocks and husbandry

Populations of *D. mojavensis* were collected in nature by aspirating adults from cactus rots and over banana baits. A mainland population from Punta Onah, Sonora originated from 472 baited adults plus 80 adults aspirated from agria rots in November 2007. In January 2008, 465 adults were collected over banana baits in San Quintin, Baja California. All flies were returned to the lab and maintained on banana food (Brazner & Etges, 1993) in 8 dr vials at room temperature. Prior to the experiments, both populations were cultured on banana food at medium larval densities in 1/2 pint bottles for one generation in an incubator programmed for 27:17 °C on a 14:10 LD cycle in large numbers for approximately five generations to minimize maternal effects due to uncontrolled larval densities in vials. All bottle-reared adults from each population were separated by sex and aged in vials (ca. 50 flies/vial) containing banana food for 12–14 days prior to culturing their offspring in fermenting cactus tissues.

### Cactus rearing and manipulation of sexual selection

Half-pint bottles containing 75 g of aquarium gravel covered with a 5.5 cm diameter piece of filter paper (Etges, 1998) were autoclaved. To this, 60 g of either agria or organ pipe tissue was added and then the bottle was autoclaved again for 10 min. After cooling to room temperature, each culture was inoculated with 0.5 mL of a pectolytic bacterium, *Erwinia cacticida* and 1.0 mL of a mixture of seven yeast species common in natural agria and organ pipe rots: *Dipodascus starmeri*, *Candida sonorensis*, *Starmera amethionina*, *Candida valida*, *Pichia cactophila*, *Pichia mexicana* and *Sporopachydermia cereana*. Eggs were collected for 10 h from each group of aged adults and washed in deionized water, in 70% ethanol and again in sterilized deionized water. Eggs were counted out in groups of 200, transferred to a 1 $cm^2$ piece of sterilized filter paper and placed on fermenting cactus in the incubator. Upon eclosion, flies were separated by sex and aged until sexually mature, and then randomly assigned to two treatments designed to manipulate female-choice based sexual selection over two generations (Havens *et al.*, 2011).

Four bottles containing agria and four bottles containing organ pipe cactus were established for each population, in each generation. Flies emerging from two bottles of each population/cactus combination were randomly assigned to mating treatments: one allowing free interaction among adults ('choice') and the other in which males and females were paired by us and allowed to individually mate ('no choice'). Eight mating trials were conducted each generation. In the 'choice' treatment, 50 females and 50 males were allowed to choose mates for 30 min, and all copulating pairs were removed and housed individually for 48 h in 8 dr shell vials containing banana food (Fig. 1). Unmated flies of both sexes were immediately removed and frozen individually in 300 μL glass vial inserts (MicroLiter Analytical Supplies, Suwanee, GA, USA) for later hydrocarbon analysis. After 48 h, all mated pairs were separated by sex, and males were removed and frozen individually in vial inserts. Mated females were pooled in oviposition chambers and allowed to oviposit in small (60 × 15 mm) petri plates containing a mixture of fermenting cactus juice and agar. Eggs were collected and counted in groups of 200 onto cactus as described above to begin the next generation (Havens *et al.*, 2011). Mated females were frozen individually immediately after oviposition.

In the 'no choice' treatment, randomly chosen pairs were housed individually for 48 h, after which the males were removed and frozen. Females were pooled and allowed to oviposit; and then immediately frozen (Fig. 1). Unfortunately, all 'no choice' flies in generation 1 were accidentally lost prior to CHC extraction and thus were not available for analysis.

### Gas chromatography of CHCs

Whole fly CHC extractions were performed by immersing each fly in hexane for 10 min in a 300 μL glass vial insert, removing the fly, and then evaporating the hexane at 40 °C in a heating block. All sample extracts were stored at −10 °C prior to gas chromatography. Each extract was redissolved in 5 $\mu$L of a heptane solution containing 385 ng of docosane ($C_{22}$) $\mu L^{-1}$ as an internal standard. One microliter of this solution was analysed by capillary gas-liquid chromatography using a Shimadzu GC-17A (Shimadzu Scientific, Columbia, MD, USA) fitted with a 15 m (ID = 0.22 mm) Rtx-5 fused-silica column (Restek Corporation, Bellefont, PA, USA). Injector and detector temperatures were set at 290 and 345 °C with the injector port in split mode (ratio = 3 : 1). Running temperatures started at 200 °C and increased to 345 °C at 15 °C min, holding at 345 °C for 4 min.

### Statistical analysis

Cuticular hydrocarbons amounts were estimated by analysis of peak integrations using CLASS VP 4.2 software provided by Shimadzu, and all data were expressed as nanograms per fly of CHCs. CHC variation was assessed using ANOVA and MANOVA in SAS. All models included 32 CHC components (Etges & Jackson, 2001) with population, cactus, mating status, sexual selection treatment, generation and sex as main effects with all interactions. Comparing CHC amounts across treatments from the two generation experiment (Fig. 1) allowed direct assessment of CHC variation; (i) between mated and

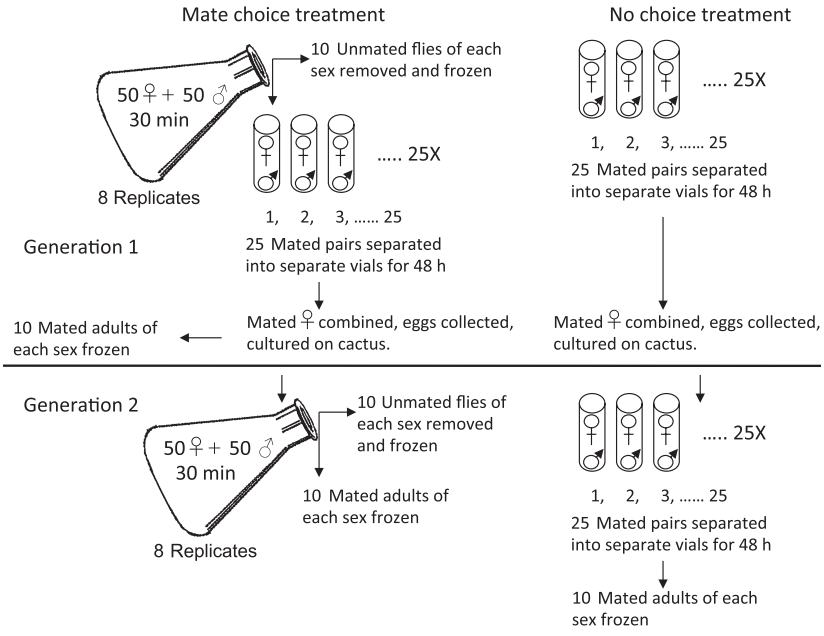


**Fig. 1** Experimental design of the 'choice' and 'no choice' mating experiments. Mated and unmated flies from both generations of the 'choice' treatment were saved for CHC analysis. Flies from the 'no choice' treatment were saved from Generation 2 only. CHC, cuticular hydrocarbons.

unmated males and females, (ii) between mated males and females from the 'choice' and 'no choice' treatments and (iii) the extent to which cactus rearing substrates influenced patterns of CHC determined mate choice. Thus, we tested the hypothesis that mated males that were 'chosen' by females had different CHC profiles than mated males that were randomly paired with individual females. Although sexual selection in *D. mojavensis* occurs primarily via female discrimination among males (Havens *et al.*, 2011), these analyses were performed on flies of both sexes. A total of 596 flies were assayed for CHC variation.

Canonical discriminant function analysis (CDFA) was conducted to further inspect the overall causes of covariation in CHC profiles and assess treatment differences using PROC CANDISC (SAS-Institute, 2004). Because CHCs are sexually dimorphic, separate analyses were conducted for each sex. Also, step-wise discriminant function analysis was performed separately for each population, sex and generation to determine which CHC components best discriminated between mated and unmated adults, and mated adults from the 'choice' and 'no choice' treatments.

## Results

### CHC variation in flies from the 'choice' trials

MANOVA for overall CHC variation over both generations for adults in the choice trials revealed significance of all main effects as well as all interaction terms (Table 1). MANOVA of the entire data set including adults from the no-choice trials also revealed significance of all main effects and interactions, despite the loss of generation 1 'no choice' samples (results not shown). Significant CHC differences due to population and sex were consistent with previous studies, and ANOVAs revealed significant Sex × Population interactions as observed for a broader sampling of populations in Baja California and mainland Mexico (Stennett & Etges, 1997; Etges & Ahrens, 2001). However, the magnitude of these population-specific sex differences was not always consistent with the previously observed regional patterns (Fig. S1). The Population × Cactus interaction (Table 1) revealed that the influence of larval rearing environment on CHC variation differs between populations, again consistent with earlier studies (Stennett & Etges, 1997; Etges & Ahrens, 2001; Etges *et al.*, 2009).

### CHC differences between mated and unmated males and females

MANOVA of CHCs from the 'choice' trials revealed significant differences between populations, rearing cactus, mating status, generation and all interaction terms (Table 2). For males, a Population × Mate interaction showed that CHC differences between mated and unmated flies were not consistent across populations (Table 2) due to the somewhat greater CHC differences between mainland mated and unmated males than those from Baja (Fig. 2). CDFA clearly grouped Baja and mainland CHCs along CV1, whereas mated and unmated male and female CHCs showed partially overlapping clusters along CV2 (Fig. 2). Cactus and mating effects were seen in the structure of CV3 where both effects influenced CHC differences in males much more than in females (all Euclidean distances between groups $P < 0.0001$; Fig. 2). CHCs of mated vs. unmated mainland

**Table 1** MANOVA results for overall cuticular hydrocarbon (CHC) variation for adult *Drosophila mojavensis* from the mate 'choice' treatment only ($n$ = 459). 'Mate' refers to differences in CHCs between mated and unmated adults over two generations. $F$ ratio d.f. for all effects and interactions = 32,396.

| Source of variation | Wilk's $\lambda$ | $F$ | Pr > $F$ |
|---|---|---|---|
| Population | 0.0895 | 125.86 | < 0.0001 |
| Cactus | 0.7641 | 3.82 | < 0.0001 |
| Mate | 0.4637 | 14.31 | < 0.0001 |
| Sex | 0.1371 | 77.89 | < 0.0001 |
| Generation | 0.3247 | 25.74 | < 0.0001 |
| Population × mate | 0.8863 | 1.59 | 0.025 |
| Population × cactus | 0.8036 | 3.02 | < 0.0001 |
| Population × sex | 0.5600 | 9.72 | < 0.0001 |
| Population × generation | 0.7626 | 3.85 | < 0.0001 |
| Cactus × mate | 0.8451 | 2.27 | < 0.0001 |
| Cactus × sex | 0.8387 | 2.38 | < 0.0001 |
| Cactus × generation | 0.8519 | 2.15 | 0.0004 |
| Mate × sex | 0.6269 | 7.36 | < 0.0001 |
| Mate × generation | 0.7579 | 3.95 | < 0.0001 |
| Population × cactus × mate | 0.8163 | 2.79 | < 0.0001 |
| Population × cactus × sex | 0.8356 | 2.43 | < 0.0001 |
| Population × cactus × generation | 0.8830 | 1.64 | 0.018 |
| Population × mate × generation | 0.8419 | 2.25 | 0.0002 |
| Cactus × mate × sex | 0.8110 | 2.88 | < 0.0001 |
| Cactus × mate × generation | 0.8918 | 1.50 | 0.042 |
| Cactus × sex × generation | 0.9111 | 1.21 | 0.207 |
| Sex × mate × generation | 0.7966 | 3.16 | < 0.0001 |
| Population × cactus × mate × sex | 0.8127 | 2.85 | < 0.0001 |
| Population × mate × sex × generation | 0.9121 | 1.19 | 0.222 |
| Population × cactus × mate × generation | 0.8817 | 1.66 | 0.015 |
| Cactus × mate × sex × generation | 0.8341 | 2.46 | < 0.0001 |
| Cactus × population × sex × generation | 0.8718 | 1.82 | 0.005 |
| Population × cactus × mate × sex × generation | 0.8403 | 2.35 | < 0.0001 |

**Table 2** MANOVA results for cuticular hydrocarbon variation in mated and unmated males ($n$ = 223) and females ($n$ = 236) from the 'choice' treatment. $F$ ratio d.f. for all effects and interactions for males = 32,176 and females = 32,189.

| | Males | | | Females | | |
|---|---|---|---|---|---|---|
| Source of variation | Wilk's $\lambda$ | $F$ | Pr > $F$ | Wilk's $\lambda$ | $F$ | Pr > $F$ |
| Population | 0.0758 | 67.04 | < 0.0001 | 0.0761 | 71.75 | < 0.0001 |
| Cactus | 0.6085 | 3.54 | < 0.0001 | 0.6643 | 2.99 | < 0.0001 |
| Mate | 0.2079 | 20.95 | < 0.0001 | 0.4929 | 6.08 | < 0.0001 |
| Generation | 0.2738 | 14.59 | < 0.0001 | 0.2397 | 18.74 | < 0.0001 |
| Population × mate | 0.7483 | 1.85 | 0.007 | 0.7653 | 1.81 | < 0.008 |
| Population × cactus | 0.6850 | 2.53 | < 0.0001 | 0.7023 | 2.50 | < 0.0001 |
| Population × generation | 0.6385 | 3.11 | < 0.0001 | 0.6302 | 3.47 | < 0.0001 |
| Cactus × mate | 0.7025 | 2.33 | 0.0003 | 0.6786 | 2.80 | < 0.0001 |
| Cactus × generation | 0.6916 | 2.45 | 0.0001 | 0.7997 | 1.48 | 0.0575 |
| Mate × generation | 0.5776 | 4.02 | < 0.0001 | 0.6166 | 3.67 | < 0.0001 |
| Population × cactus × mate | 0.6727 | 2.68 | < 0.0001 | 0.7551 | 1.92 | 0.0041 |
| Population × cactus × generation | 0.7734 | 1.61 | 0.028 | 0.7855 | 1.61 | 0.027 |
| Population × mate × generation | 0.8331 | 1.10 | 0.337 | 0.7216 | 2.28 | 0.0003 |
| Cactus × mate × generation | 0.7427 | 1.91 | 0.005 | 0.8101 | 1.38 | 0.0953 |
| Population × cactus × mate × generation | 0.7700 | 1.64 | 0.023 | 0.7503 | 1.97 | 0.0029 |

females appeared to overlap considerably (Fig. 2) yet were significantly different ($F_{32,420} = 4.6$, $P < 0.0001$). A Population × Cactus interaction (Table 2), caused by the significant influence of cactus in the mainland population (Table 3), indicated that CHCs of this mainland population were more plastic in response to rearing

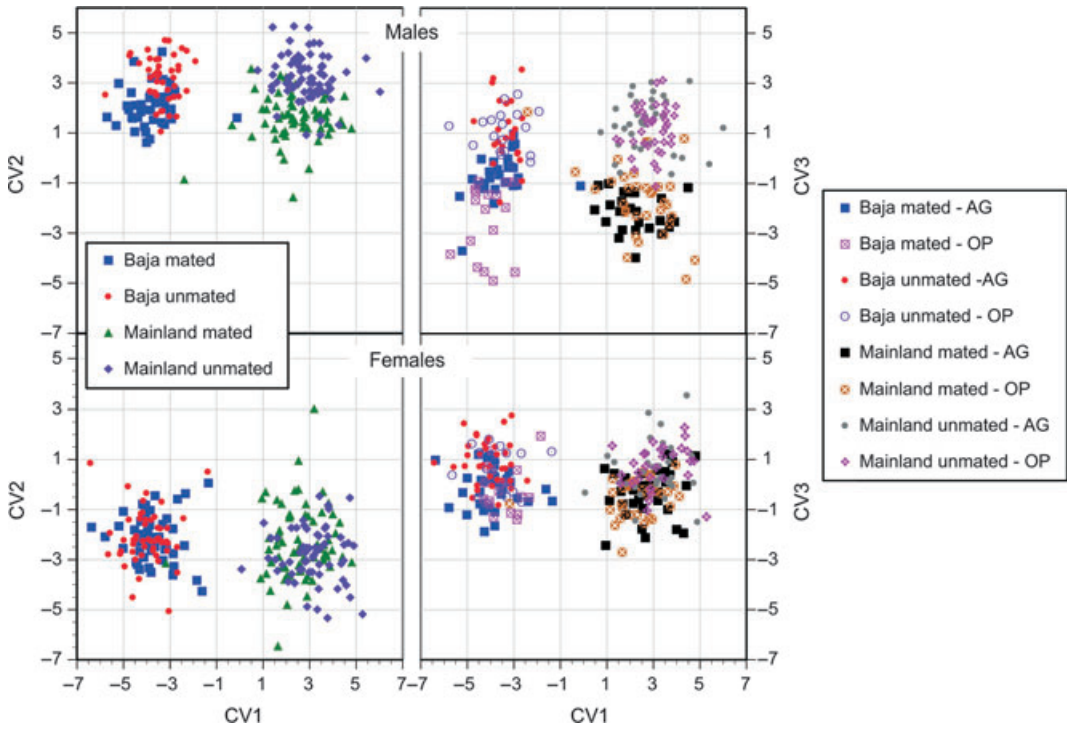


**Fig. 2** Canonical discriminant function (CDF) plots of *Drosophila mojavensis* CHC scores for the first three canonical variates for mated and unmated males and females reared on either host cactus for both generations. Leftward two panels show data pooled by cactus where CV1 separated Baja and mainland populations, CV2 separated unmated and mated males from females and CV3 showed the effect of cactus and also discriminated between mated and unmated adults. Significance of the pairwise squared Mahalanobis distance each group was $P < 0.001$. AG, agria cactus; OP, organ pipe cactus; CHC, cuticular hydrocarbons; CDF, canonical discriminant function.

substrates than the Baja California population. The Population × Cactus × Mate interaction (Table 2) suggested that regional and host-cactus specific influences on CHC mediated male mating success made simple predictions about the determinants of male mating success more difficult and certainly dependent on local patterns of host plant use. Pairwise, *post hoc* multivariate contrasts for each treatment combination revealed significant differences in CHCs between mated and unmated males for each contrast (Table 4), suggesting that female discrimination among males was mediated by male CHCs in both populations reared on both host cacti.

We also performed CDFA separately for each population, cactus and generation because of the significant effect of generation in the MANOVAS (Tables 1 and 2). CV1 discriminated between mated and unmated males in each trial (Generation 1, Baja $P = 0.0051$; Mainland $P < 0.0001$; Generation 2, Baja $P < 0.0001$; Mainland $P < 0.0001$). For males and females, we further assessed the total canonical structure coefficients of CV1 revealing that most CHC components were correlated with this canonical variate (Table 5). Analysis of the least square means from the ANOVAS for these CHC components revealed that in both populations, and for each generation, mated males had significantly lower amounts of the $C_{34}$ alkadienes and alkenes (8,26-tetratricontadiene, 6,24- and 6,26-tetracontadiene, 10-, 12- and 14 tetretricontene) than unmated males, whereas mated males had significantly higher amounts of the $C_{37}$ alkadienes and alkenes (8,28-heptatricontadiene and 14-, 16- and 12-hexatricontene; Table 5), consistent with within-population mating trials in Etges & Tripodi (2008). Further discriminant function analysis revealed a handful of CHC components that differentiated mated and unmated males within each population in each generation (Table S1), also consistent with previous studies (Etges & Tripodi, 2008; Etges *et al.*, 2009). In particular, the $C_{37}$ alkadienes best discriminated among mated and unmated males in the Baja California population, whereas the $C_{34}$ alkadienes best discriminated among mated and unmated males in the mainland population, indicating diverging patterns of female preference for male CHCs.

For females, MANOVA for overall CHC variation between mated and unmated adults from both generations revealed significance of all main effects ($P < 0.0001$) as well as a significant Population × Cactus × Mate interaction (Table 2). A male/female Wilk's $\lambda$ ratio of 2.4 for the mate treatment suggested that the

**Table 3** MANOVA results for cuticular hydrocarbon variation of mated and unmated males and females from the 'choice' treatment, separately by population and generation. Sample sizes and d.f. for each sex are included and significant effects are shown in bold.

| | Baja California, Gen. 1 (♂ $n$ = 40) (♀ $n$ = 54) | | | | Mainland, Gen. 1 (♂ $n$ = 62) (♀ $n$ = 76) | | | |
|---|---|---|---|---|---|---|---|---|
| | d.f. | Wilk's $\lambda$ | $F$ | Pr > $F$ | d.f. | Wilk's $\lambda$ | $F$ | Pr > $F$ |
| Cactus | | | | | | | | |
| ♂ | 32,5 | 0.0812 | 1.77 | 0.275 | 32,27 | 0.2134 | 3.10 | **0.002** |
| ♀ | 32,19 | 0.2174 | 2.14 | **0.042** | 32,41 | 0.2647 | 3.56 | **< 0.0001** |
| Mate | | | | | | | | |
| ♂ | 32,5 | 0.0109 | 14.15 | **0.004** | 32,27 | 0.0868 | 8.88 | **< 0.0001** |
| ♀ | 32,19 | 0.2043 | 2.31 | **0.029** | 32,41 | 0.2935 | 3.08 | **0.0004** |
| Cactus × mate | | | | | | | | |
| ♂ | 32,5 | 0.0098 | 15.72 | **0.003** | 32,27 | 0.3796 | 1.38 | 0.199 |
| ♀ | 32,19 | 0.2833 | 1.50 | 0.177 | 32,41 | 0.3029 | 2.95 | **0.0006** |
| | Gen. 2 (♂ $n$ = 49) (♀ $n$ = 51) | | | | Gen. 2 (♂ $n$ = 72) (♀ $n$ = 55) | | | |
| | d.f. | Wilk's $\lambda$ | $F$ | Pr > $F$ | d.f. | Wilk's $\lambda$ | $F$ | Pr > $F$ |
| Cactus | | | | | | | | |
| ♂ | 32,14 | 0.2372 | 1.41 | 0.252 | 32,37 | 0.3068 | 2.61 | **0.003** |
| ♀ | 32,16 | 0.2606 | 1.42 | 0.232 | 32,20 | 0.2850 | 1.57 | 0.147 |
| Mate | | | | | | | | |
| ♂ | 32,14 | 0.0443 | 9.45 | **< 0.0001** | 32,37 | 0.0548 | 19.93 | **< 0.0001** |
| ♀ | 32,16 | 0.0798 | 5.77 | **0.0003** | 32,20 | 0.1098 | 5.07 | **0.0002** |
| Cactus × mate | | | | | | | | |
| ♂ | 32,14 | 0.2676 | 1.20 | 0.371 | 32,37 | 0.4072 | 1.68 | 0.064 |
| ♀ | 32,16 | 0.1549 | 2.73 | **0.018** | 32,20 | 0.3728 | 1.05 | 0.463 |

**Table 4** Pairwise, *post hoc* multivariate contrasts between cuticular hydrocarbons (CHCs) for mated vs. unmated males and females in each of eight mating trials in the 'choice' treatment based on all CHCs in mainland and Baja California populations of *Drosophila mojavensis* reared on both host cacti. Significance of all $F$ tests was $P < 0.0001$.

| | | Contrast | | | |
|---|---|---|---|---|---|
| | | Mated vs. Unmated males | | Mated vs. Unmated females | |
| Generation | Population and cactus | Wilk's $\lambda$ | $F$ | Wilk's $\lambda$ | $F$ |
| 1 | Baja – agria | 0.3891 | 8.64 | 0.2147 | 21.61 |
| | Baja – organ pipe | 0.3969 | 8.36 | 0.2787 | 15.29 |
| | Mainland – agria | 0.2866 | 11.69 | 0.3674 | 10.17 |
| | Mainland – organ pipe | 0.2864 | 13.70 | 0.4057 | 8.65 |
| 2 | Baja – agria | 0.2426 | 17.17 | 0.1925 | 24.77 |
| | Baja – organ pipe | 0.3667 | 9.50 | 0.2647 | 16.40 |
| | Mainland – agria | 0.3384 | 10.75 | 0.3193 | 12.59 |
| | Mainland – organ pipe | 0.3133 | 12.05 | 0.3819 | 9.56 |

difference in CHC profiles of mated and unmated males was more than twice that of females (Fig. 2). Although the effect of cactus on female CHCs was significant (Table 2), there were less obvious differences among female mate-cactus groups than for males along CV3 (Fig. 2). Pairwise, *post hoc* multivariate contrasts between mated and unmated females for each treatment combination revealedsignificance of mate status for each contrast ($P < 0.0001$) as for males (Table 4). Discriminant function analysis revealed that some of the same CHC components were associated with mate success in females as in males including 2-methyloctacosane and 2-methyltricontane, 8,26-tetratricontadiene and 10-, 12-, 14 tetretricontene, 9,27-heptatricontadiene, 8,28-heptatricontadiene and 14-,

**Table 5** Total canonical structure coefficients of the first canonical variate (CV1) contrasting mated and unmated male *Drosophila mojavensis* for two generations. Population and cactus treatments were pooled. Significant differences in LSMEANS of cuticular hydrocarbon amounts between mated and unmated males for each component are indicated: MA = mated males; UN = unmated males.

| | | | Baja males | | Mainland males | |
|---|---|---|---|---|---|---|
| Hydrocarbon | ECL | CV1 loading | Generation 1 | Generation 2 | Generation 1 | Generation 2 |
| 2-methyloctacosane | $C_{28.65}$ | −0.372**** | ns | MA > UN | MA > UN | MA > UN |
| 2-methyltricontane | $C_{30.65}$ | −0.368**** | ns | MA > UN | MA > UN | MA > UN |
| 7- and 9- hentricontene | $C_{30.78}$ | −0.200*** | ns | MA > UN | ns | MA > UN |
| Unknown | $C_{32a}$ | 0.807**** | MA < UN | MA < UN | MA < UN | MA < UN |
| Unknown | $C_{32b}$ | 0.348**** | ns | MA < UN | MA < UN | MA < UN |
| Unknown | $C_{32c}$ | 0.219*** | MA < UN | ns | ns | ns |
| Unknown | $C_{32d}$ | 0.506**** | MA < UN | MA < UN | ns | MA < UN |
| 11- and 13- methyldotricontane | $C_{33br2}$ | −0.107 | ns | MA > UN | MA > UN | MA > UN |
| Unknown alkene | $C_{33br3}$ | −0.030 | ns | MA > UN | ns | ns |
| 31-methyldotricont-8-ene | $C_{32.47}$ | −0.177** | ns | MA > UN | ns | MA > UN |
| 31-methyldotricont-6-ene | $C_{32.56}$ | 0.234*** | ns | ns | ns | ns |
| 8,24-tritricontadiene | $C_{32.63}$ | −0.070 | ns | MA > UN | ns | MA > UN |
| 7,25-tritricontadiene | $C_{32.70}$ | −0.037 | ns | MA > UN | ns | MA > UN |
| 10-, 12-, and 14-tritricontene | $C_{32.79}$ | −0.395*** | ns | MA > UN | ns | MA > UN |
| 8,26-tetratricontadiene | $C_{34diene}$ | 0.530**** | MA < UN | ns | MA < UN | MA < UN |
| 6,24- and 6,26-tetracontadiene | $C_{34diene}$ | 0.157* | MA < UN | MA < UN | ns | MA < UN |
| 10-, 12-, and 14tetretricontene | $C_{34ene}$ | 0.328**** | ns | ns | MA < UN | MA < UN |
| 33-methlytetratricont-10-ene | $C_{35alk1}$ | −0.074 | ns | ns | ns | ns |
| 33-methlytetratricont-8-ene | $C_{35alk2}$ | 0.007 | ns | ns | ns | ns |
| Unknown alkene | $C_{35alk3}$ | 0.263**** | ns | ns | ns | MA < UN |
| 9,25-pentatricontadiene | $C_{34.59}$ | 0.171* | MA < UN | ns | ns | MA < UN |
| 8,26- & 7,27-pentatricontadiene | $C_{34.66}$ | 0.013 | ns | ns | ns | ns |
| Unknown | $C_{34.73}$ | 0.076 | ns | MA > UN | | MA < UN |
| Unknown alkene | $C_{36a}$ | −0.048 | ns | MA > UN | ns | ns |
| Unknown alkene | $C_{36b}$ | 0.326**** | ns | ns | MA < UN | MA < UN |
| 35-methylhexatricont-10-ene | $C_{37br}$ | −0.147* | ns | MA > UN | ns | MA > UN |
| 9,27-heptatricontadiene | $C_{36.5}$ | 0.087 | MA < UN | ns | ns | ns |
| 8,28-heptatricontadiene | $C_{36.6}$ | −0.147* | ns | MA > UN | ns | MA > UN |
| 14-, 16-, and 12-hexatricontene | $C_{36.7}$ | −0.184** | ns | MA > UN | ns | MA > UN |
| Unknown alkene | $C_{38}$ | −0.198** | ns | MA > UN | ns | ns |
| Unknown alkene | $C_{39}$ | −0.029 | MA < UN | ns | ns | ns |
| Unknown alkene | $C_{40}$ | −0.347**** | ns | MA > UN | ns | MA > UN |

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$.

16-, 12-hexatricontene. Overall, a large proportion of all CHCs differed between mated and unmated males and females from our choice tests (Table S1).

### CHC variation between generation 1 and generation 2 adults from the 'choice' trials

We predicted that the consequences of female choice should cause observable shifts in male CHC profiles between generations. MANOVA of mated male and female CHCs over both generations of the 'choice' trials revealed significance for all effects and most interactions (Table 6). Because there were significant CHC differences between mated and unmated adults in generation 1 and 2 (Tables 3 and 4, Fig. 2), differences in overall CHC profiles between generations of mated adults of both sexes indicated that mating success due to female choice was a significant cause of heritable shifts in CHCs. Female CHCs shifted in parallel with those of males as seen in the decreases in CV2 scores (Figs 3 and 4). However, significant Population × Generation interactions suggested that this generational effect was not uniform across these two populations (Table 6).

MANOVA of unmated adults over both generations of the 'choice' trials also revealed significance for the same treatments effects as for mated adults (Table S2). Thus, unmated adults also differed with respect to CHC profile between generations (Figs 3 and 4).

### CHC variation between adults from 'choice' and 'no choice' trials

There were significant differences in CHCs between mated adults in the 'choice' trials and those from the 'no choice' trials, i.e. single mated pairs, documenting the consequences of allowing mate choice and sexual

**Table 6** MANOVA results for cuticular hydrocarbon variation in mated males ($n$ = 107) and females ($n$ = 118) from the 'choice' treatment over two generations. $F$ ratio d.f. for all effects and interactions for males = 32,68 and females = 32,79.

| | Males | | | Females | | |
|---|---|---|---|---|---|---|
| Source of variation | Wilk's $\lambda$ | $F$ | Pr > $F$ | Wilk's $\lambda$ | $F$ | Pr > $F$ |
| Population | 0.0633 | 31.47 | < 0.0001 | 0.0747 | 30.57 | < 0.0001 |
| Cactus | 0.3028 | 4.89 | < 0.0001 | 0.4022 | 3.67 | < 0.0001 |
| Generation | 0.1285 | 14.41 | < 0.0001 | 0.1856 | 10.84 | < 0.0001 |
| Population × cactus | 0.4366 | 2.74 | 0.0002 | 0.5524 | 2.00 | 0.007 |
| Population × generation | 0.5678 | 1.62 | 0.049 | 0.4086 | 3.57 | < 0.0001 |
| Cactus × generation | 0.4370 | 2.74 | 0.0002 | 0.6042 | 1.62 | 0.044 |
| Population × cactus × generation | 0.6007 | 1.41 | 0.117 | 0.5938 | 1.69 | 0.032 |

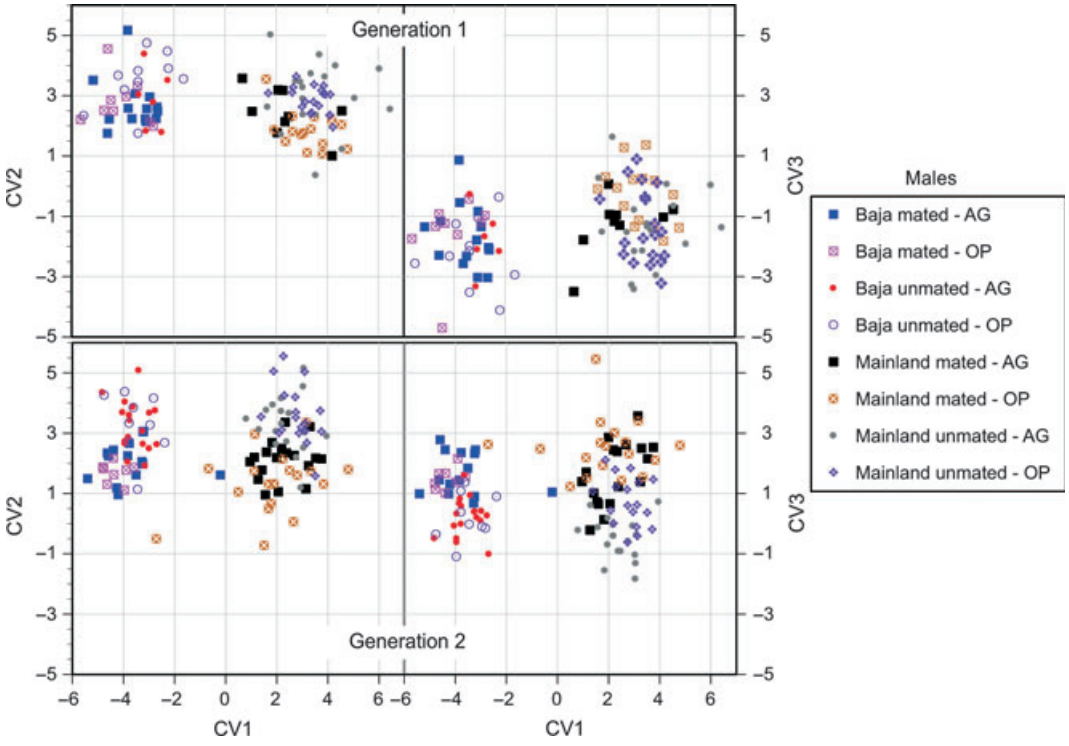


**Fig. 3** CDF plots of male *Drosophila mojavensis* CHC scores along the first three canonical variates for each of the 'choice' trials for mated or unmated males reared on either host cactus showing cross generation effects. For each generation, the squared Mahalanobis distances between of the eight groups were all significantly different, $P < 0.01$. AG, agria cactus; OP, organ pipe cactus; CHC, cuticular hydrocarbons; CDF, canonical discriminant function.

selection on CHC profiles in both males and females. MANOVA for overall CHC variation among mated males and females revealed significance of all main effects as well as all interactions (Table 7). As generation one 'no choice' adults were unfortunately lost prior to CHC extraction, we could only assess these effects in generation 2 flies and could not determine the extent to which these CHCs differences were heritable. Also, some of the 'choice' vs. 'no choice' differences in CHCs could have been due to differences in social effects because 'no choice' flies were exposed to only one fly of the opposite sex where 'choice' flies were exposed to groups of flies of both sexes. Social influences on CHCs so far uncovered in *D. mojavensis* were due to differences between virgin, same sex groups vs. flies in choice trials (Etges *et al.*, 2009).

Differences in CHCs of mated males from the 'choice' and 'no choice' treatments were greater than those for females (Fig. 5), although all group differences were significantly different (all $P < 0.0001$). Mated 'no choice' adults tended to have lower CV1 scores, particularly males, and Baja adults displayed

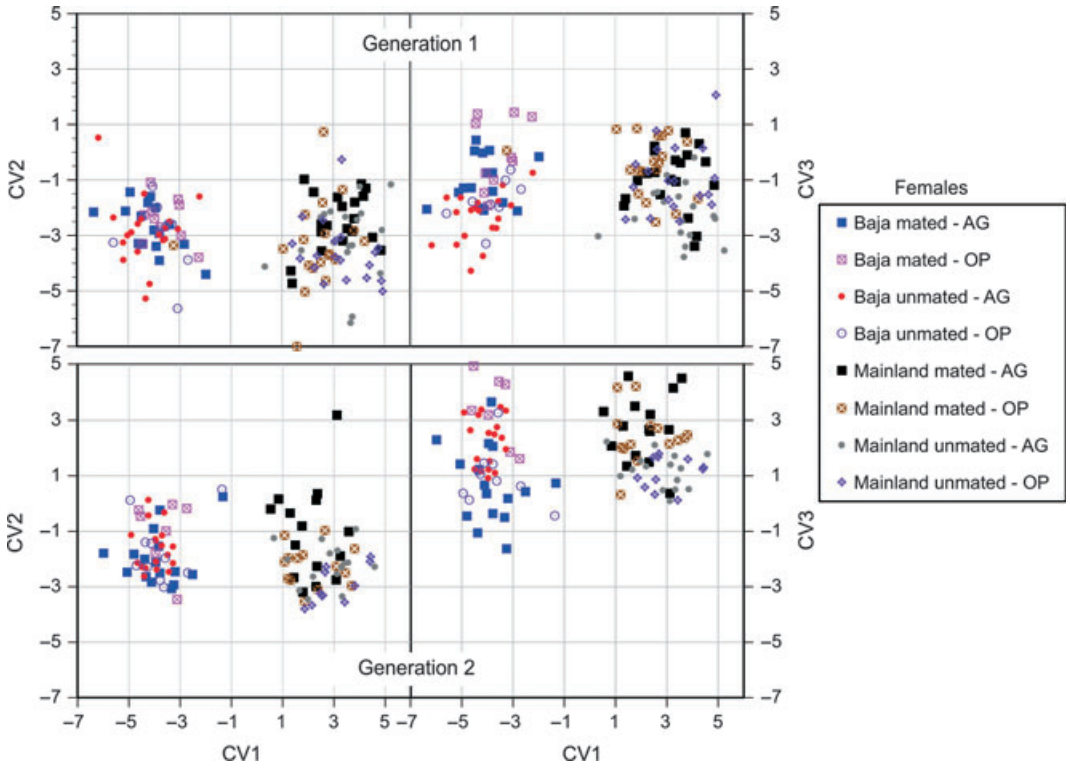


**Fig. 4** CDF Plots female *Drosophila mojavensis* CHC scores along the 1st, 2nd and 3rd canonical variates for the 'choice' trials for mated and unmated females reared on either host cactus. All squared Mahalanobis distances between groups were significant at $P < 0.01$. AG, agria cactus; OP, organ pipe cactus; CHC, cuticular hydrocarbons; CDF, canonical discriminant function.

**Table 7** MANOVA results for cuticular hydrocarbon variation of mated males and females from 'choice' vs. 'no choice' treatments. Results are from generation two. *F* test ratio d.f. for all main effects and interactions for males = 32,88 and females = 32,81.

| | Males | | | Females | | |
|---|---|---|---|---|---|---|
| Source of variation | Wilk's $\lambda$ | *F* | Pr > *F* | Wilk's $\lambda$ | *F* | Pr > *F* |
| Population | 0.0710 | 35.99 | < 0.0001 | 0.0501 | 47.97 | < 0.0001 |
| Cactus | 0.6271 | 1.63 | 0.038 | 0.5859 | 1.79 | 0.019 |
| Trial type | 0.2182 | 9.86 | < 0.0001 | 0.2960 | 6.02 | < 0.0001 |
| Population × cactus | 0.6270 | 1.64 | 0.037 | 0.6135 | 1.59 | 0.048 |
| Population × trial type | 0.5355 | 2.39 | 0.0007 | 0.4547 | 3.04 | < 0.0001 |
| Cactus × trial type | 0.5177 | 2.56 | 0.0003 | 0.5527 | 2.05 | 0.005 |
| Population × cactus × trial type | 0.6185 | 1.70 | 0.028 | 0.5547 | 2.03 | 0.006 |

greater CHC group differences than mainland adults, particularly along CV3, leading to a significant Population × Trial type interaction (Table 7). CV2 significantly discriminated between unmated and mated flies from both trial types particularly evident in males (Fig. 5), but not between mated flies from the 'choice' and 'no choice' trials. Unmated adults were also significantly separated along CV3 from mated adults with clearer discrimination between choice and no choice males, particular from Baja California. More negative male CV3 scores of these no choice flies were associated with decreased amounts of 2-methyloctacosane, all three $C_{34}$ components, $C_{36}$, $C_{38}$ and higher amounts of $C_{32d}$ (Table S3). Although there were fewer CHCs that differed between female groups, all were in the same direction as those for males (Table S4).

The significant population and cactus caused differences in CHCs, consistent with previous studies, were made clearer in MANOVAS for each population separately (Table 8). Cactus rearing substrates had significant effects on male and female CHCs in the mainland population, but not the Baja population (Table 8), suggesting greater cactus-induced plasticity for mainland adult CHCs in these comparisons. Significance of CHC

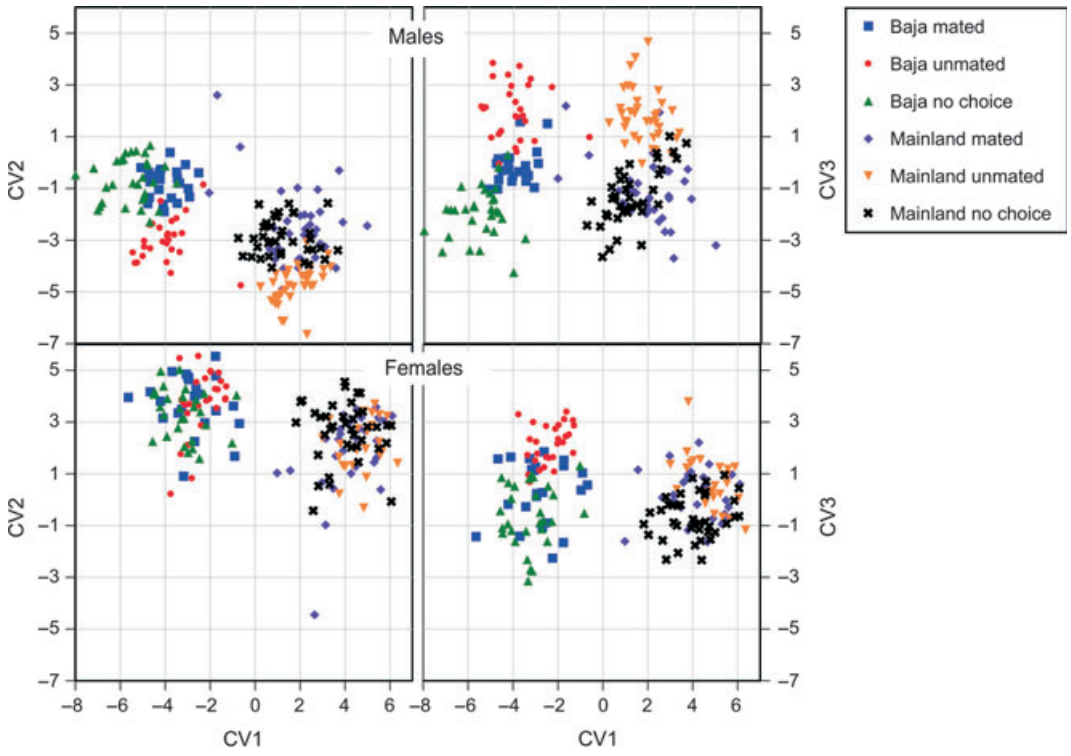


**Fig. 5** CDF plots of adult *Drosophila mojavensis* CHC scores along the first three canonical variates for the 'choice' and 'no choice' trials. CHC, cuticular hydrocarbons; CDF, canonical discriminant function.

**Table 8** MANOVA results for cuticular hydrocarbon variation of mated adults from the 'choice' treatment and 'no choice' treatment. Results are presented separately for each population. Sample sizes and d.f. for each sex are included and significant effects are shown in bold.

| Baja California Males ($n$ = 52) | d.f. | Wilk's $\lambda$ | $F$ | Pr > $F$ | Mainland Males ($n$ = 75) | d.f. | Wilk's $\lambda$ | $F$ | Pr > $F$ |
|---|---|---|---|---|---|---|---|---|---|
| Cactus | 32,17 | 0.3275 | 1.09 | 0.437 | Cactus | 32,40 | 0.3792 | 2.05 | **0.016** |
| Trial type | 32,17 | 0.0493 | 10.24 | **< 0.0001** | Trial type | 32,40 | 0.1627 | 6.44 | **< 0.0001** |
| Cactus × Trial type | 32,17 | 0.1664 | 2.66 | **0.018** | Cactus × Trial type | 32,40 | 0.3435 | 2.39 | **0.005** |
| Females ($n$ = 52) | d.f. | Wilk's $\lambda$ | $F$ | Pr > $F$ | Females ($n$ = 68) | d.f. | Wilk's $\lambda$ | $F$ | Pr > $F$ |
| Cactus | 32,17 | 0.2696 | 1.44 | 0.215 | Cactus | 32,33 | 0.2522 | 3.06 | **0.001** |
| Trial type | 32,17 | 0.1269 | 3.65 | **0.003** | Trial type | 32,33 | 0.1244 | 7.26 | **< 0.0001** |
| Cactus × Trial type | 32,17 | 0.1672 | 2.65 | **0.019** | Cactus × Trial type | 32,33 | 0.2578 | 2.97 | **0.001** |

differences between mated adults from the 'choice' and 'no choice' trials for males and females from each population, as well as significant Trial type × Cactus interactions (Table 8) underscored how preadult rearing environments altered outcomes of CHC mating success in *D. mojavensis*.

## Discussion

Female discrimination of males was mediated by different male CHC profiles in diverged populations of *D. mojavensis*. Consequences of CHC-mediated female discrimination varied between diverged populations in a cactus-specific manner, where indirect benefits of female choice, shorter offspring development times (DEVTs), were greater in the mainland population when reared on organ pipe cactus (Havens *et al.*, 2011). The results of this study highlight the inter-dependence of traits under natural selection, i.e. egg to adult DEVT, courtship signals i.e. CHCs, and consequences of female discrimination in ecologically diverged populations. When evaluated in a broader set of geographically isolated populations, these results have revealed links between host plant adaptation, sexual selection and

sexual isolation in the formation of reproductive isolation between populations in allopatry (Etges, 1992, 1998; Etges *et al.*, 2010).

### Environmental effects on patterns of female discrimination

A previous study investigating the role of CHCs in mating success within populations of *D. mojavensis* suggested that CHCs were not strongly involved in intrademic mate choice as compared with sexual isolation between populations (Etges & Tripodi, 2008). However, this study employed only agria-reared flies: agria cactus tends to reduce sexual isolation between populations and outside of Baja California, is restricted to one relatively small area in coastal Sonora (Heed, 1978). Here, we found influences of CHCs on male mating success in both a mainland and a Baja population, using flies that were reared on both host cacti used in nature. Thus, female-choice based sexual selection was mediated by chemical signals when females were allowed to choose among males reared on both host cacti, consistent with GEI of CHCs caused by host cactus (Etges *et al.*, 2009, 2010). Mating success in both populations was associated with higher amounts of the $C_{29}$–$C_{31}$ alkanes and $C_{38}$–$C_{40}$ alkenes, and lower amounts of $C_{34}$ alkadienes, consistent over two generations. The $C_{37}$ alkadienes best discriminated between mated and unmated males in the Baja population, whereas the $C_{34}$ alkadienes discriminated between mated and unmated males in the mainland population (Table S1). Thus, there were subtle differences in female preferences of male CHCs between populations.

Cuticular hydrocarbons of mated males and females differed over two generations (Table 2), but the degree of CHC-based female discrimination was mostly consistent (Fig. 2). This suggested that female preference did not change significantly over time, but rather, that the available pool of male CHCs from which the females chose differed over generations. The difference between mated and unmated males was greatest in mainland flies reared on organ pipe cactus, consistent over two generations (Table 3, Fig. 3), illustrating the influence of larval rearing environment on sexual selection, especially in this derived population.

### CHCs and sexual selection

Cuticular hydrocarbons of mated males from the 'choice' and 'no choice' treatments were more similar to each other than to CHCs of unmated males with far less differentiation in females (Fig. 5). Thus, when given the opportunity for choosing among males, females were able to discriminate among males based on CHC profiles, but these males had significantly different CHCs than males in the 'no choice' trials. We do not know if female choice is based on preference for particular CHCs, discrimination against males with certain CHC profiles, or both. Taken together, the pattern of differences between mated and unmated males and females over both generations (Table 5; Figs 3 and 4), the similarity between successfully mated males from both 'choice' and 'no choice' treatments (Fig. 5), and the shift in CHCs in mated and unmated adults between generations suggest that sexual selection can rapidly shift male CHC profiles to a greater extent than in females. The greater difference between mated and unmated males in the mainland (derived) population reared on organ pipe cactus indicates that host cactus adaptation (Etges, 1990; Etges *et al.*, 2010) has led to shifts in patterns of CHC-mediated female discrimination.

Although this experimental design confounds the influence of female choice on CHC variation with exposure to multiple adults before mating, effects of the social environment on mating signals have been demonstrated in *D. mojavensis* and other species. In *Drosophila melanogaster*, flies exposed to social interaction with multiple genotypes expressed different CHC profiles than flies exposed to a single genotype (Kent *et al.*, 2008). Male assessment of females during sexual encounters can result in a rapid plastic response of male CHC profiles (Petfield *et al.*, 2005). In *D. mojavensis*, QTL analysis of CHC variation among males showed GEI for exposure to females, as well as Cactus × Exposure interactions (Etges *et al.*, 2009). Thus, social interaction can lead to GEIs for sexual traits and preferences. Further research should include comparisons between varying types and levels of social exposure (i.e. sight, smell) with individuals of the same and different genotypes to examine indirect genetic effects of the social environment on CHC variation.

### Consequences of CHC-mediated female discrimination

Detailed knowledge of the effects of environmental variables on female preferences, such as levels of discrimination and/or consequences to offspring fitness, is necessary to fully understand the influence of GEI on sexual selection (Lesna & Sabelis, 1999; Etges *et al.*, 2007). Under low gene-flow conditions, such as divergence in allopatry, GEI in mate preference and/or sexual signals can maintain selection for female preferences, further promoting divergence between populations (Etges, 2002; Sattman & Cocroft, 2003; Greenfield & Rodriguez, 2004; Kokko & Heubel, 2008; Rodriguez *et al.*, 2008; Danielson-Francois *et al.*, 2009; Ingleby *et al.*, 2010). Indirect genetic benefits of female choice, consistent with 'good genes' sexual selection in allopatric populations of *D. mojavensis* reared on two host cacti were revealed in offspring of females from the 'choice' trials that had significantly shorter egg to adult DEVTs (Havens *et al.*, 2011). Genetic variation in DEVT was revealed by artificial selection for longer and shorter

DEVT along with correlated responses of lower levels of sexual isolation between Baja and mainland populations (Etges, 1998). Thus, DEVT and sexual isolation are genetically correlated. Shorter egg to adult DEVTs and higher viabilities of Baja California vs. mainland populations have been attributed to differences in breeding site duration in the wild, and thus represent adaptations to the use of different host cacti, particularly the elongated rates of tissue fermentation in organ pipe cacti (Heed, 1978; Etges, 1990, 1992; Etges *et al.*, 2010). Thus, natural selection for longer DEVT and sexual selection (Havens *et al.*, 2011) can act in opposite directions on this key fitness component. Furthermore, a QTL-based genetic correlation between DEVT and CHC variation (Etges *et al.*, 2010) suggested that CHC differences associated with mating success were a significant determinant of mate choice differences. Further fine mapping of these QTLs should reveal insights into the genetic basis of this interaction between natural and sexual selection.

Estimating the effects of natural and sexual selection on mate recognition in *Drosophila* have shown that male CHCs evolve faster when the two forms of selection work together rather than in isolation (Blows, 2002; Rundle *et al.*, 2009), as do mate preferences. Using an approach that independently manipulated the opportunity for natural and sexual selection, (Rundle *et al.*, 2009) showed that female preferences for male CHCs diverged between populations when the two processes were allowed to operate simultaneously. Female preferences weakened in populations of *Drosophila serrata* evolving under natural selection alone, but changes in the direction of preferences tended to evolve when natural and sexual selection were unconstrained, suggesting both processes are likely keys to revealing the initial stages of ecological speciation. Further experiments suggest that sexual selection is unlikely to cause divergence among natural populations without a concomitant change in natural selection (Hine *et al.*, 2011). In *Drosophila simulans*, the effects of natural selection and sexual selection and CHCs differed across sexes, where male CHCs evolve in response to both forms of selection as well as through the interaction between the two, with some male CHC components only evolving in the direction of natural selection when sexual selection was relaxed (Sharma *et al.*, 2012).

We have shown that female choice is mediated by CHCs within ecologically diverging populations of *D. mojavensis*, and that these courtship signals have diverged between populations and display GEI caused by different host cacti (Etges *et al.*, 2009). Here, context (cactus) dependent female choice-based sexual selection indirectly opposed natural selection for DEVT due to the genetic correlation between CHCs and DEVT. Because divergence between female preferences as well as male signals is necessary for the evolution of assortative mating (Maan & Seehausen, 2011), future work should also address the genetic architecture of female preferences. Direct testing of hypotheses concerning the consequences of host plant adaptation to sexual selection will be aided by high resolution analysis of the genetic correlation between mating success, CHC variation and life history variation in *D. mojavensis*.

## Acknowledgments

We thank G. Almeida for technical assistance and C. C. de Oliveira and J. Jennings for discussion. Funding was provided by NSF DEB-02111025 and EF-0723930 to WJE.

## References

Beckenbach, A.T., Heed, W.B. & Etges, W.J. 2008. A mitochondrial DNA analysis of vicariant speciation in two lineages in the *Drosophila mulleri* subgroup. *Evol. Ecol. Res.* **10**: 475–492.

Blomquist, G.J., Dillworth, J.W. & Adams, T.S. 1987. Biosynthesis and endocrine regulation of sex pheromone production in Diptera. In: *Pheromone Biochemistry* (G.D. Prestwitch & G.J. Blomquist, eds), pp. 217–250. Academic Press, London.

Blows, M.W. 2002. Interaction between natural and sexual selection during the evolution of mate recognition. *Proc. R. Soc. Lond. B Biol. Sci.* **269**: 1113–1118.

Boughman, J.W. 2002. How sensory drive can promote speciation. *Trends Ecol. Evol.* **17**: 571–577.

Brazner, J.C. & Etges, W.J. 1993. Pre-mating isolation is determined by larval rearing substrates in cactophilic *Drosophila mojavensis*: II. Effects of larval substrates on time to copulation, mate choice and mating propensity. *Evol. Ecol.* **7**: 605–624.

Danielson-Francois, A.M., Zhou, Y. & Greenfield, M.D. 2009. Indirect genetic effects and the Lek paradox: inter-genotypic competition may strengthen genotype × environment interactions and conserve genetic variance. *Genetica* **136**: 27–36.

Etges, W.J. 1990. Direction of life history evolution in *Drosophila mojavensis*. In: *Ecological and Evolutionary Genetics of Drosophila* (S.F. Starmer & R.J. MacIntyre, eds), pp. 37–56. Plenum, New York.

Etges, W.J. 1992. Premating isolation is determined by larval rearing substrates in cactophilic *Drosophila mojavensis*. *Evolution* **46**: 1945–1950.

Etges, W.J. 1998. Premating isolation is determined by larval rearing substrates in cactophilic *Drosophila mojavensis.* IV. Correlated responses in behavioral isolation to artificial selection on a life history trait. *Am. Nat.* **152**: 129–144.

Etges, W.J. 2002. Divergence in mate choice systems: does evolution play by rules? *Genetica* **116**: 151–166.

Etges, W.J. & Ahrens, M.A. 2001. Premating isolation is determined by larval rearing substrates in cactophilic *Drosophila mojavensis.* V. Deep geographic variation in epicuticular hydrocarbons in isolated populations. *Am. Nat.* **158**: 585–598.

Etges, W.J. & Jackson, L.L. 2001. Premating isolation is determined by larval rearing substrates in cactophilic *Drosophila mojavensis.* VI. Epicuticular hydrocarbon variation in *Drosophila mojavensis* cluster species. *J. Chem. Ecol.* **27**: 2125–2149.

Etges, W.J. & Tripodi, A.D. 2008. Premating isolation is determined by larval rearing substrates in cactophilic *Drosophila mojavensis.* VIII. Mating success mediated by epicuticular

hydrocarbons within and between isolated populations. *J. Evol. Biol.* **21**: 1641–1652.
Etges, W.J., Johnson, W.R., Duncan, G.A., Huckins, G. & Heed, W.B. 1999. Ecological genetics of cactophilic *Drosophila*. In: *Ecology of Sonoran Desert Plants and Plant Communities* (R. Robichaux, ed.), pp. 164–214. University of Arizona Press, Tucson.
Etges, W.J., Over, K.F., de Oliviera, C.C. & Ritchie, M.G. 2006. Inheritance of courtship song variation among geographically isolated populations of *Drosophila mojavensis*. *Anim. Behav.* **71**: 1205–1214.
Etges, W.J., de Oliviera, C.C., Ritchie, M.G. & Noor, M.A.F. 2007. Genetics of incipient speciation in *Drosophila mojavensis.* I. Male courtship song, mating success and genotype × environment interactions. *Evolution* **61**: 1106–1119.
Etges, W.J., de Oliviera, C.C. & Noor, M.A.F. 2009. Genetics of incipient speciation in *Drosophila mojavensis:* II. Host plants and mating status influence cuticular hydrocarbon QTL expression and G × E interactions. *Evolution* **63**: 1712–1730.
Etges, W.J., de Oliviera, C.C., Noor, M.A.F. & Ritchie, M.G. 2010. Genetics of incipient speciation in *Drosophila mojavensis.* III. Life history divergence and reproductive isolation. *Evolution* **64**: 3549–3569.
Ferveur, J.F. 1997. The pheromonal role of cuticular hydrocarbons in *Drosophila melanogaster*. *BioEssays* **19**: 353–358.
Ferveur, J.F. 2005. Cuticular hydrocarbons: their evolution and roles in *Drosophila* pheromonal communication. *Behav. Genet.* **35**: 279–295.
Greenfield, M.D. & Rodriguez, R.L. 2004. Genotype–environment interactions and the reliability of mate signals. *Anim. Behav.* **68**: 1461–1468.
Havens, J.A., Orzack, S.H. & Etges, W.J. 2011. Mate choice opportunity leads to shorter offspring development time in a desert insect. *J. Evol. Biol.* **24**: 1317–1324.
Heed, W.B. 1978. Ecology and genetics of Sonoran Desert *Drosophila*. In: *Ecological Genetics: The Interface* (P.F. Brussard, ed.), pp. 109–126. Springer-Verlag, New York.
Heed, W.B. 1982. The origin of *Drosophila* in the Sonoran Desert. In: *Ecological Genetics and Evolution: The Cactus-Yeast-Drosophila Model System* (J.S.F. Barker & W.T. Starmer, eds), pp. 65–80. Academic Press, Sydney.
Higginson, A.D. & Reader, T. 2009. Environmental heterogeneity, genotype-by-environment interactions, and the reliability of sexual traits as indicators of male quality. *Proc. R. Soc. Lond. B Biol. Sci.* **276**: 1153–1159.
Hine, E., McGuigan, K. & Blows, M.W. 2011. Natural selection stops the evolution of male attractiveness. *Proc. Natl Acad. Sci. USA* **108**: 3659–3664.
Ingleby, F.C., Hunt, J. & Hosken, D.J. 2010. The role of genotype- × -environment interaction in sexual selection. *J. Evol. Biol.* **23**: 2031–2045.
Jia, F. & Greenfield, M. 1997. When are good genes good? Variable outcomes of female choice in wax moths. *Proc. R. Soc. Lond. B Biol. Sci.* **264**: 1057–1063.
Jia, F.Y., Greenfield, M.D. & Collins, R.D. 2000. Genetic variance of sexually selected traits in wax moths: maintenance by genotype × environment interaction. *Evolution* **54**: 953–967.
Kent, C., Azanchi, R., Smith, B., Formosa, A. & Levine, J. 2008. Social context influences chemical communication in *D. melanogaster* males. *Curr. Biol.* **18**: 1384–1389.
Kokko, H. & Heubel, K. 2008. Condition-dependence, genotype-by-environment interactions and the lek paradox. *Genetica* **132**: 209–216.
Lesna, I. & Sabelis, W. 1999. Diet dependent female-choice for males with 'good genes' in a soil predatory mite. *Nature* **401**: 581–584.
Maan, M.E. & Seehausen, O. 2011. Ecology, sexual selection and speciation. *Ecol. Lett.* **14**: 591–602.
Marchetti, K., Nakamura, H. & Gibbs, H.L. 1998. Host-race formation in the common cuckoo. *Science* **282**: 471–472.
Oliveira, C., Manfrin, M., Sene, F., Jackson, L. & Etges, W. 2011. Variations on a theme: diversification of cuticular hydrocarbons in a clade of cactophilic *Drosophila*. *BMC Evol. Biol.* **11**: 179.
Panhuis, T.M., Butlin, R., Zuk, M. & Tregenza, T. 2001. Sexual selection and speciation. *Trends Ecol. Evol.* **16**: 364–371.
Petfield, D., Chenoweth, S.F., Rundle, H.D. & Blows, M.W. 2005. Genetic variance in female condition predicts indirect genetic variance in male display traits. *Proc. Natl Acad. Sci. USA* **102**: 6045–6050.
Ritchie, M. 2007. Sexual selection and speciation. *Annu. Rev. Ecol. Evol. Syst.* **38**: 79–102.
Rodriguez, R.L., Sullivan, L.M., Snyder, R.L. & Cocroft, R.B. 2008. Host shifts and the beginning of signal divergence. *Evolution* **62**: 12–20.
Rundle, H.D., Chenoweth, S.F., Doughty, P. & Blows, M.W. 2005. Divergent selection and the evolution of signal traits and mating preferences. *PLoS Biol.* **3**: 1988–1995.
Rundle, H.D., Chenoweth, S.F. & Blows, M.W. 2009. The diversification of mate preferences by natural and sexual selection. *J. Evol. Biol.* **22**: 1608–1615.
Ryan, M.J. & Rand, A.S. 1993. Species recognition and sexual selection as a unitary problem in animal communication. *Evolution* **47**: 647–657.
SAS-Institute 2004. *SAS/STAT 9.1.2*. SAS Institute Inc., Cary, NC.
Sattman, D.A. & Cocroft, R.B. 2003. Phenotypic plasticity and repeatability in the mating signals of *Enchenopa* treehoppers, with implications for reduced gene flow among host-shifted populations. *Ethology* **109**: 981–994.
Schluter, D. & Price, T. 1993. Honesty, perception and population divergence in sexually selected traits. *Proc. R. Soc. Lond. B Biol. Sci.* **253**: 117–122.
Sharma, M.D., Hunt, J. & Hosken, D.J. 2012. Antagonistic responses to natural and sexual selection and the sex-specific evolution of cuticular hydrocarbons in *Drosophila simulans*. *Evolution* **66**: 665–677.
Stennett, M.D. & Etges, W.J. 1997. 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*. *J. Chem. Ecol.* **23**: 2803–2824.
Welch, A. 2003. Genetic benefits of a female mating preference in gray tree frogs are context dependent. *Evolution* **57**: 883–893.
Yew, J.Y., Dreisewerd, K., de Oliviera, C.C. & Etges, W.J. 2011. Male-specific tranfer and fine-scale characterization of newly identified cuticular hydrocarbons and triacylglycerides in a *Drosophila* species pair. *PLoS ONE* **6**: e16898.

## Supporting information

Additional Supporting Information may be found in the online version of this article:

**Figure S1** Comparisons of the Sex × Region interactions for CHCs from the population survey (top three panels) in Etges & Ahrens (2001) and in the present study (bottom three panels).

**Table S1** Best discriminating CHCs for mated vs. unmated males and females from the 'choice' treatment in both generations, all $P < 0.05$.

**Table S2** MANOVA results for CHC variation in unmated males and females from the 'choice' treatment over two generations ($n = 116$).

**Table S3** Correlations of CHC amounts with the first canonical variate in the 'choice' and 'no choice' trials for males only.

**Table S4** Correlations of CHC amounts with the first canonical variate in the 'choice' and 'no choice' trials for females only.

*Received 29 September 2012; revised 6 November 2012; accepted 9 November 2012*