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Inheritance of courtship song variation among geographically isolated populations of *Drosophila mojavensis*

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Courtship song variation in many *Drosophila* species evolves rapidly and is thought to contribute to species-specific mate recognition. Studies using song playback experiments have shown that courtship songs are part of this recognition system in the sibling species *D. mojavensis* and *D. arizonae*. We examined variation in components of courtship songs in *D. mojavensis* to evaluate further the influence of courtship song divergence on sexual isolation. We found significant geographical variation in mean interpulse interval and mean burst duration, and significant variation in mean burst duration among geographically isolated populations from Baja California and mainland Mexico and Arizona. Significant regional differences in these song components were detected, as well as variation among populations nested within regions. Residual maximum likelihood (REML) analysis of F_1 and F_2 generations from a cross between a Baja California and an Arizona population revealed additive autosomal influences on mean interpulse interval with a significant effect of dominance and significant effects of the Y chromosome on mean burst duration. Regional differences in song variation and the type of genetic architecture observed are consistent with patterns of geographical variation associated with the colonization of the mainland from Baja California and documented differences in sexual isolation between populations.

Revealing the genetic basis of traits that influence reproductive isolation among populations is ultimately necessary for understanding how speciation is achieved. Dobzhansky (1940) and Mayr (1963) realized that the formation of both premating and postmating isolating barriers caused by drift and/or selection in allopatry, could serve to separate gene pools, leading to a series of studies emphasizing the roles of these barriers. By identifying the number and effects of genes that contribute to sexual isolation between diverging populations of the same species, we can begin to understand the nature of the underlying genetic mechanisms responsible for incipient speciation (Carson & Lande 1984; Ritchie & Phillips 1998; Ting et al. 2001; Etges 2002; Feder et al. 2005).

Of great interest is understanding how evolution proceeds at the earliest stages of reproductive divergence. Isolating those phenotypes that influence initial stages of divergence and understanding their genetic basis should reveal how speciation proceeds. Comparative studies in clusters of closely related species with known evolutionary histories will help to resolve how predictable pathways of divergence are. In many *Drosophila* species, phenotypes associated with premating isolation tend to evolve earlier than those associated with postmating isolation in recently diverged sympatric populations (Coyne & Orr 1989, 1997). Identifying which components of sexual isolation systems are most likely to diverge first, whether multiple signalling elements are part of the recognition system, and the order in which these phenotypes diverge (Etges 2002; Coyne & Orr 2004) remain to be resolved.

Courtship songs in insects, and *Drosophila* in particular, are known to influence female receptivity during courtship and species recognition (Ewing & Bennet-Clark 1968; Liimatainen et al. 1992; Tomaru & Oguma 1994; Hoikkala et al. 2005). Studies of acoustic properties of male *Drosophila* wing vibration have typically focused on frequency characteristics of song trains or pulse songs, but courtship song characteristics can be remarkably diverse (Hoy et al. 1988). Species recognition is often based on variation in the interpulse interval (IPI) or pulse

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frequency (Ewing & Bennet-Clark 1968; Bennet-Clark & Ewing 1969; Ritchie et al. 1999). Ewing & Miyan (1986) evaluated the evolution of song types in the large *D. repleta* group in terms of IPI as well as loss/gain of particular song types, based on known phylogenies, and found that short- and long-pulse songs were largely species specific. However, song evolution in this group does not always show phylogenetic trends (Alonso-Pimentel et al. 1995; Etges 2002), suggesting that courtship songs in *Drosophila* species may often evolve too rapidly to discern clear patterns of evolution, as in the *D. willistoni* group (Gleason & Ritchie 1998). Therefore, studies of species' populations undergoing divergence are needed to identify patterns of song evolution in *Drosophila*.

Variation in courtship song is thought to contribute to reproductive isolation between cactophilic *D. mojavensis* and *D. arizonae*. Byrne (1999) verified Ewing & Miyan's (1986) observations that these songs consist of two pulse types with short and long IPIs. In playback experiments, she demonstrated that these courtship songs were part of the species recognition system. Analyses of these species' courtship songs also revealed intraspecific variation in song characteristics, extending the early studies of song evolution (Spieth 1974; Ewing & Miyan 1986). Long and short IPIs, as well as burst length, differed between species, and between populations of *D. mojavensis* in southern California, Baja California and mainland Mexico and Arizona (Byrne 1999).

We analysed courtship songs of geographically isolated populations of *D. mojavensis* from Baja California and mainland Mexico and Arizona that show low, but significant, sexual isolation in the laboratory, no postmating isolation, and are considered incipient species (Zouros & d'Entremont 1974; Markow 1991; Brazner & Etges 1993; Etges & Ahrens 2001) to evaluate the role of courtship song divergence on sexual isolation. Populations in mainland Mexico and Arizona are evolutionarily derived from those in Baja California based on studies of chromosome evolution and patterns of host cactus use (Heed 1978; Ruiz et al. 1990). Mate recognition in mainland Mexico and Arizona populations of *D. mojavensis* has been hypothesized to have been influenced by the presence of *D. arizonae*, resulting in reproductive character displacement (Wasserman & Koepfer 1977; Zouros & d'Entremont 1980) as well as adaptive divergence in host plant use (Etges 1998). The origins of southern California populations are less certain, but they may also be derived from Baja California (Etges et al. 1999). Thus, the central goal of the present study was to extend study of geographical variation in courtship songs in *D. mojavensis* to a larger number of populations and to assess the genetic architecture of song differences to evaluate the genetic basis of song variation, given its importance to species recognition and potentially to incipient speciation.

MATERIALS AND METHODS

We used 13 geographically diverse populations of *D. mojavensis* from Baja California and mainland Mexico and Arizona (Fig. 1). Almost all populations originated from a sample of more than 30 wild-caught adults (Etges & Ahrens 2001) and flies emerged from cactus rots returned to the laboratory. All flies were maintained as large outbred stocks in 8-dram (25 × 95 mm) glass vials containing banana-yeast-malted barley-agar medium at room temperature. Before song recording, all stocks were mass cultured for one generation in an incubator set to a 14:10 h light: dark cycle at 27 °C during the day and 17 °C at night. All emerged flies were sexed using CO_2 anaesthesia within 3 days of eclosion. Males and females were separated into groups of 10 in vials in the incubator and aged at least 12 days before use to ensure sexual maturity.

Mass crosses were performed with a mainland population from the Santa Rosa Mountains in southern Arizona and a population from Todos Santos, Baja California Sur (Fig. 1). Crosses were started with four to five replicates each of 10 aged virgin males and 10 aged virgin females in fresh food vials. Females were allowed to lay eggs for 3 days and then transferred to fresh food vials. Parentals, F_1 reciprocals and F_2 reciprocals were cultured at one time, but male courtship songs were recorded in several blocks.

Courtship Song and Mating Success

Using a multiple-choice design, we performed within-population courtship trials with wing-clipped male flies to verify that courtship songs influenced mating success in our stocks. Courtship in *D. mojavensis* is fairly stereotypical, with males orienting, licking, and then attempting copulation in response to signs of female acceptance, such as wing spreading and buzzing. Male song production occurs during the orientation and licking phases with the male positioned behind the female (Alonso-Pimentel & Tobin 1992). Aged, virgin adults from Punta Prieta, Baja California Norte and Punta Onah, Sonora (Fig. 1) were used to directly assess the role of courtship song in mating success. We lightly anaesthetized male flies with CO_2, clipped their wings at the base of the marginal vein, and allowed them to recover overnight in vials containing banana food. Courtship trials were performed by placing either 15 winged females and males or 15 winged females and 15 wingless males in a plastic petri dish containing a piece of filter paper moistened with fermented cactus juice and recording the number of copulations for 1 h.

Courtship Song Analysis

Courtship songs were recorded on TDK Pro AM90 audio-cassette tapes using an INSECTAVOX electric microphone (Gorczyca & Hall 1987) connected to a Marantz PMD222 tape recorder. Virgin males were recorded singly in Plexiglas mating chambers (1.5 × 1 × 0.5 cm), with nylon netting on one side, in the presence of two virgin females. We used immature females to reduce courtship latency while ensuring that immediate copulation would not occur. Male courtship songs do not change with age after sexual maturity (Byrne 1999), so we did not control for male age at the time of

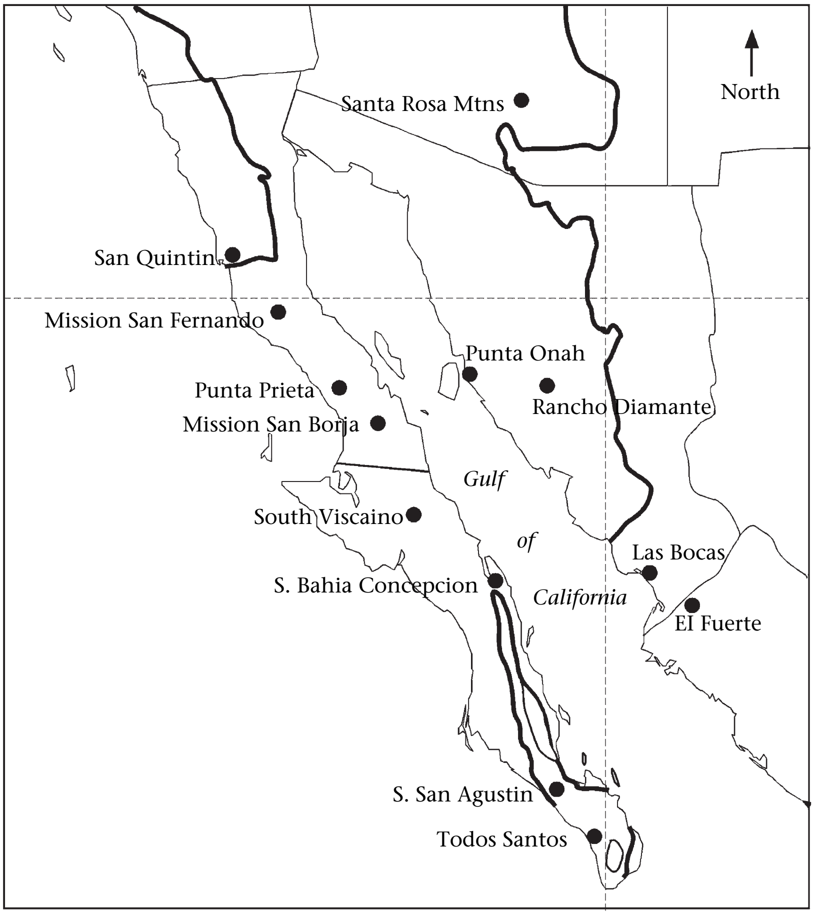

Figure 1. Map of the arid lands of the southwestern United States and northwestern Mexico and the locations of the *D. mojavensis* populations used in this study. The dark line indicates the approximate border of the Sonoran and Mojave Deserts. The populations, their stock numbers, and year of collection are: South Viscaino (A917, 1986), Santa Rosa Mountains, Arizona (A924, 1987), Mission San Borja (MSB94, 1994), Mission San Fernando (MSF94, 1994), Punta Onah (PO94, 1994), South Bahia de Concepcion (A975, 1996), Todos Santos (A977, 1996), Punta Prieta (A979, 1996), South San Agustin (A983, 1996), Las Bocas (A990, 1996), El Fuerte (A991, 1996), Rancho El Diamante, (A993, 1996), and San Quintin (SQ03, 2003).

recording (range 12–32 days). We recorded multiple song bursts from 10 males from each population, and from up to 30 males per generation in the population crosses. For each male, we recorded 25–120 s of song. We monitored ambient temperature inside the INSECTAVOX using a digital thermometer because interpulse interval decreases with increasing temperature (Noor & Aquadro 1998; Byrne 1999).

Recordings were digitized at 11 kHz using Canary sound analysis software (1995, Cornell Laboratory of Ornithology, Ithaca, New York, U.S.A.). All song measurements were made directly from the waveform tracings from Canary. Long interpulse intervals (L-IPI) were calculated as the time in seconds between peaks of two successive pulses (Burnet et al. 1977). Song bursts with short IPIs (<10 ms) noted by Ewing & Miyan (1986) were not common in the populations that we used, so we excluded them from analysis. Mean L-IPI (hereafter IPI) was calculated from at least 30 IPI per male. We also evaluated differences in the coefficient of variation in IPI, mean burst duration (MBD) and the coefficient of variation of burst duration (CVBD) as an index of within-individual variation in burst duration. Preliminary analysis suggested that interburst duration variation was not a significant source of variation and therefore was not included in this study. Time of day of recording was not controlled for.

Statistical Analysis

Differences between populations were assessed for all song variables with populations nested within geographical regions in PROC GLM (SAS Institute 1989). We evaluated the effects of temperature on all song parameters with ANCOVA. Least square means were calculated to estimate the magnitude of song differences between populations.

All generations (parentals, reciprocal F_1s and reciprocal F_2s) were also subject to ANCOVA. Least square means for IPI were potentially biased due to the heterogeneity of slopes from regressions for each generation with temperature, so we evaluated the residuals. IPI and MBD, but not CVBD, data were analysed. All crosses data were

Table 1. Results of courtship trials with winged and wingless males

	Number of copulations	
	Winged females× winged males	Winged females× wingless males
Baja	37	19
Mainland	31	21

Two replicate mating chambers were observed for each of the four combinations of adult *D. mojavensis.*

fitted to a linear genetic model using residual maximum likelihood (REML) to assess the significance of genetic components of the data (Genstat5-Committee 1993). The model contained fixed terms representing the genetic effects distinguished from these crosses and a random effect representing recording block. Genetic terms with three states were fitted in the model as covariates. Genetic terms with only two states remained factors, but did not influence the outcome of the analysis. Other factors were treated as covariates so that heterozygotes were assigned an intermediate value and dominance was included as a separate term. A term for cytoplasmic/maternal effects could not be incorporated into the model because of insufficient degrees of freedom. Wald tests were used to evaluate the statistical significance of each coefficient (b) in the model. Wald tests use a Z statistic, formed as $Z = \widehat{\beta}/\mathrm{SE}$. This Z value is then squared, yielding Wald statistics distributed as a chi-square.

RESULTS

Wingless males obtained significantly fewer successful matings (50–68%) than did winged males. (Fisher's exact test: $P < 0.01$ in each population; Table 1). Courtship vigour, however, did not differ between winged and wingless males, suggesting that the ability to produce courtship songs is correlated with male mating success in *D. mojavensis*.

ANCOVA results revealed significant geographical variation in courtship song components of the 13 populations, grouped by geographical region, Baja California versus mainland Mexico and Arizona (Table 2). Of all the song parameters analysed, only mean IPI, mean burst duration (MBD) and the coefficient of variation in burst duration (CVBD) showed significant variation among populations. The coefficient of variation in IPI showed little variation, so we did not analyse this further. Ambient recording temperature ($\overline{X} \pm \mathrm{SD} = 22.02 \pm 0.94\,^\circ\mathrm{C}$, $N = 130$, range 17.9–24 °C) had a significant effect on mean IPI ($F_{1,116} = 8.36$, $P < 0.001$) but not on MBD or CVBD. There were significant differences between regions ($F_{1,116} = 200.53$, $P < 0.0001$) and populations nested within regions for mean IPI ($F_{11,116} = 2.80$, $P < 0.01$), with most Baja California populations having significantly longer IPIs than mainland populations (Fig. 2). MBD least square means were significantly larger in mainland populations than in populations from Baja California ($0.307 > 0.239$ s; $F_{1,116} = 47.61$, $P < 0.0001$). Variation in burst duration was significantly greater in populations from Baja California ($F_{1,116} = 12.85$, $P < 0.001$; Table 2, Fig. 3), and was not correlated with the number of bursts recorded per fly ($r = 0.125$, $P = 0.16$), suggesting that the regional differences in CVBD were not an artefact of measuring different numbers of songs per male. Thus, courtship songs of ancestral Baja California populations of *D. mojavensis* were characterized by longer IPIs with shorter, but more variable, burst durations than those of mainland populations.

Inheritance of Courtship Songs

The difference in average IPI ($P < 0.05$; Fig. 4) between males from the Santa Rosa Mountains (least square $\overline{X} \pm \mathrm{SE} = 0.0185 \pm 0.0005$) and those from Todos Santos (0.0216 ± 0.0005) was 0.0031 s. Although MBD did not differ between parental stocks, the reciprocal F_2 generation derived from the progeny of F_1 from the cross of Todos Santos males × Santa Rosa females showed lower

Table 2. Results of ANCOVAs for mean interpulse interval (IPI), mean burst duration (MBD) and the coefficient of variation of burst duration (CVBD)

		IPI		MBD		CVBD	
Source	df	Type III SS	*F*	Type III SS	*F*	Type III SS	*F*
Among populations							
Model	13	0.000327	20.36****	0.302585	7.14****	8775.46	2.62**
Region	1	0.000248	200.53****	0.155096	47.61****	3305.77	12.85***
Pop (region)†	11	0.000038	2.80**	0.149461	4.17****	5407.92	1.91*
Temperature	1	0.000010	8.36**	0.004715	1.45	1.08	0.00
Error	116	0.000143		0.377898		29849.59	
Among parentals, F_1 and F_2 generations							
Model	6	0.00037132	32.77****	0.15003989	6.13****		
Cross	5	0.00006729	7.13****	0.07143939	3.50**		
Temperature	1	0.00018658	98.80****	0.05444236	13.34***		
Error	105	0.00019829		0.42866338			

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$.
†Populations nested within regions.

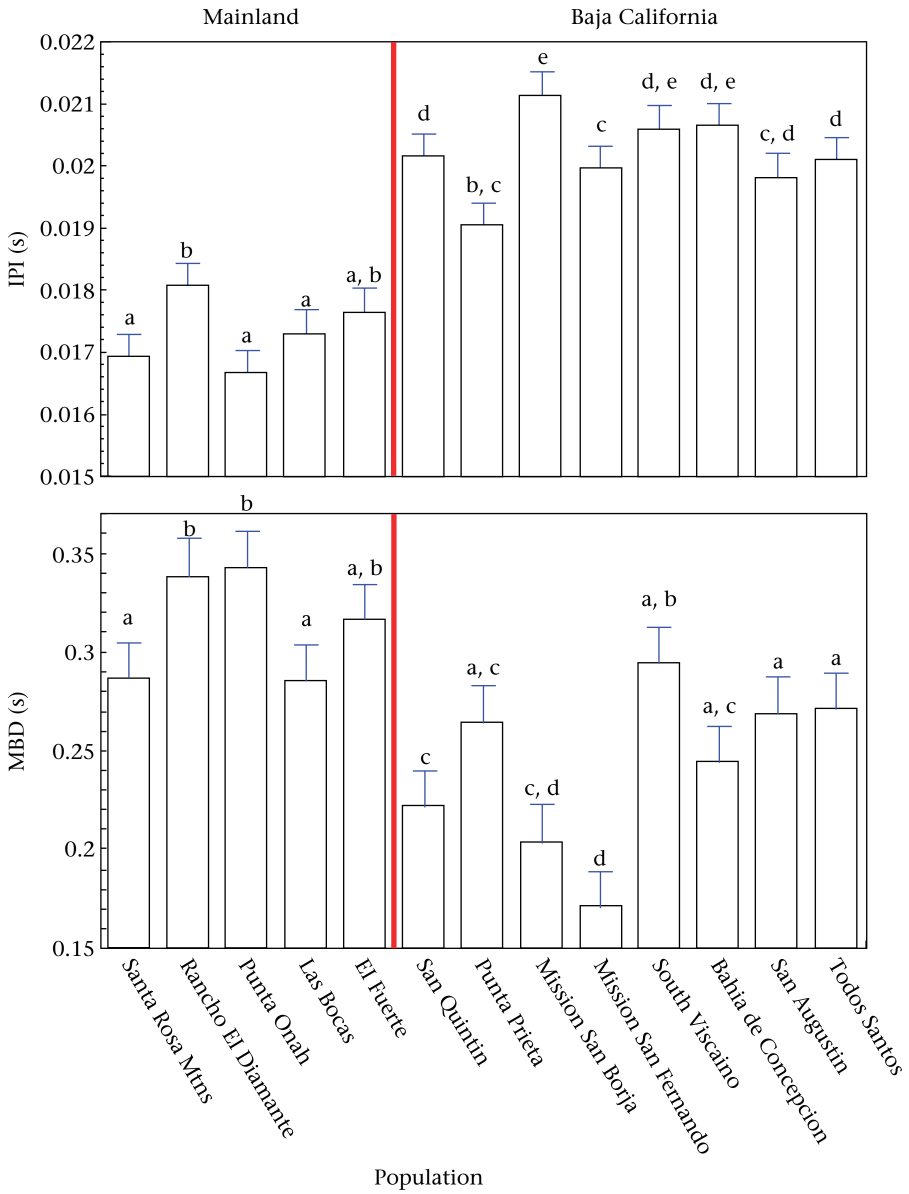

Figure 2. Least square means + SD of interpulse interval (IPI) and mean burst duration among the 13 populations of *D. mojavensis* in this study. $N = 10$ males for each population. Nonsignificant means share the same letter. Populations are grouped into geographical regions: mainland or Baja California.

MBD than did the other crosses (Fig. 4). Temperature significantly affected IPI ($F_{1,105} = 98.80$, $P < 0.0001$) and MBD ($F_{1,105} = 13.34$, $P < 0.001$) in parental stocks and crosses. Log transformation of temperature did not remove the heterogeneity of slopes among groups (results not shown), so all results are based on analysis of residuals (Fig. 5). Inspection of the residuals indicated that they were normally distributed for both IPI and MBD, so these data were not log transformed for the REML analysis. The recording 'block' effect was not statistically significant. Fitting the fixed effects model (Table 3) to these data by sequentially adding and removing the genetic terms resulted in significant additive, autosomal and dominance effects on IPI variation in these populations (Table 3). The difference in IPI ± SE residuals between males from Baja California (1.334 ± 0.160) and those from Arizona (−0.630 ± 0.233) was −1.96, equivalent to the autosomal effect from the REML model (−2.013 ± 0.776; Wald statistic = 24.73, $P < 0.001$), but this was modified by a dominance effect of 0.57 ± 0.218 (Wald statistic = 6.77, $P = 0.009$) or about 30% of the difference between parentals. Thus, genes influencing the difference in IPI between males from the Santa Rosa Mountains, Arizona and Todos Santos, Baja California populations of *D. mojavensis* were largely additive, with significant dominance towards shorter, mainland IPIs.

The difference in MBD ± SE residuals between males from the Santa Rosa Mountains (0.380 ± 0.234) and those from Todos Santos (0.118 ± 0.195) was not as large (0.262), with, intriguingly, a significant Y chromosome

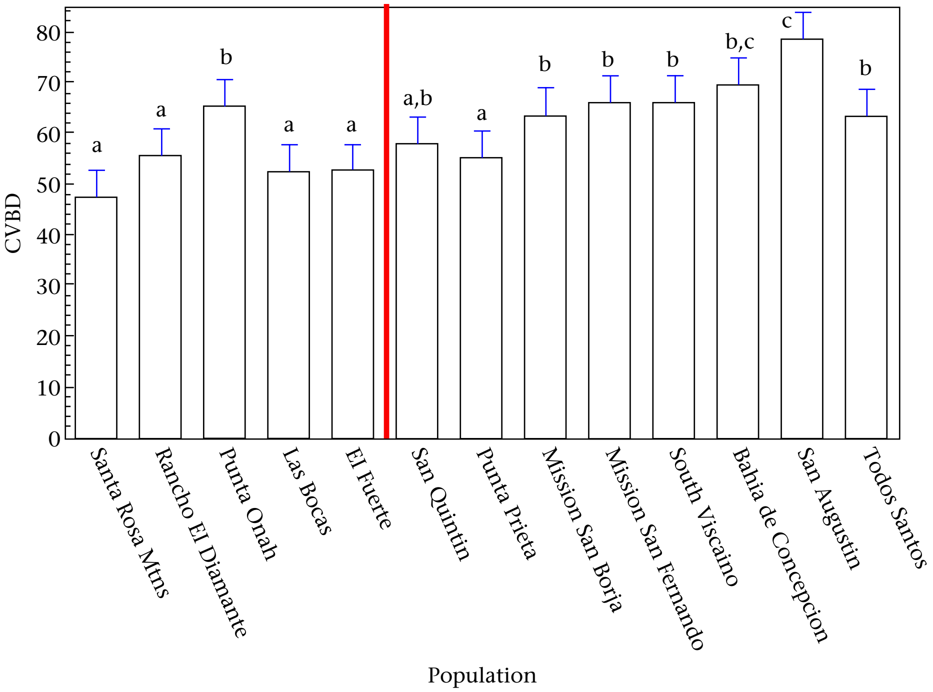

Figure 3. Least square means + SD of the coefficient of variation of burst duration (CVBD) among the 13 populations of *D. mojavensis*. Figure annotations are the same as in Fig. 2.

effect (Wald statistic = 4.01, $P = 0.045$), and to a lesser degree, some influence of the X chromosome (Wald statistic = 3.12, $P = 0.077$). These effects may be partially correlated (Table 3). The overall Y effect on MBD differences was 0.685 ± 0.342, similar in magnitude but opposite in direction to the additive effect.

DISCUSSION

Differences in courtship songs between males from Baja California and mainland populations of *D. mojavensis* were consistent with a suite of other genetic, behavioural, physiological and ecological differences that characterize this polymorphic species (Heed 1978, 1982; Etges et al. 1999; Filchak et al. 2005). Regional differences in mean IPI were more consistent than those for burst duration or variation in burst duration (Table 2, Figs 2, 3). Populations from southern Arizona to northern Sinaloa, Mexico showed little variation in IPI with almost no overlap with Baja California populations. There was also no relationship between latitude and IPI (linear regression: slope = −0.0002, $F_{1,12} = 1.032$, $P = 0.331$) or MBD (slope = 0.0000003, $F_{1,12} = 0.182$, $P = 0.678$) although CVBD was marginally significant (slope = −1.863, $F_{1,12} = 4.01$, $P = 0.070$). Thus, factors associated with latitudinal climatic variation do not seem to explain the observed variation in these courtship song parameters, unlike other traits including egg-to-adult development time and adult thorax size (Etges 1990). Mean values for IPI and MBD are consistent with Byrne's (1999) findings, although she included only two populations from Baja California, three populations from mainland Mexico and Arizona, and two from southern California. These California populations originated from Santa Catalina Island (near Los Angeles, California) and Vallecito (Mojave Desert, California) and were characterized by longer IPIs, similar to those of Baja California populations and those of *D. arizonae*. Furthermore, MBD of the southern California populations tended to be shorter than those of mainland populations and those of *D. arizonae* (Byrne 1999), suggesting that courtship songs of these populations are more similar to those of Baja California populations than they are to those of mainland Mexico and southern Arizona.

Song Differentiation and Divergence Among Populations

Geographical differences in male courtship songs, particularly IPI and MBD, are consistent with the pattern of sexual isolation between Baja California and mainland populations. The degree of premating isolation between ancestral Baja California populations of *D. mojavensis* isolated by the Gulf of California from mainland Mexico and Arizona populations has long been characterized as weak, but usually significant (Zouros & d'Entremont 1974; Markow 1991), depending on preadult rearing environments (e.g. cactus type versus laboratory food; Brazner 1983; Etges 1992). Our results suggest that courtship songs may be involved in mate recognition, along with epicuticular hydrocarbons (Stennett & Etges 1997; Etges & Ahrens 2001), and may influence patterns of assortative mating among populations of *D. mojavensis*. Epicuticular hydrocarbon profiles are region and sex specific, and serve as contact pheromones during courtship (Etges & Ahrens 2001). Parallel differentiation in courtship

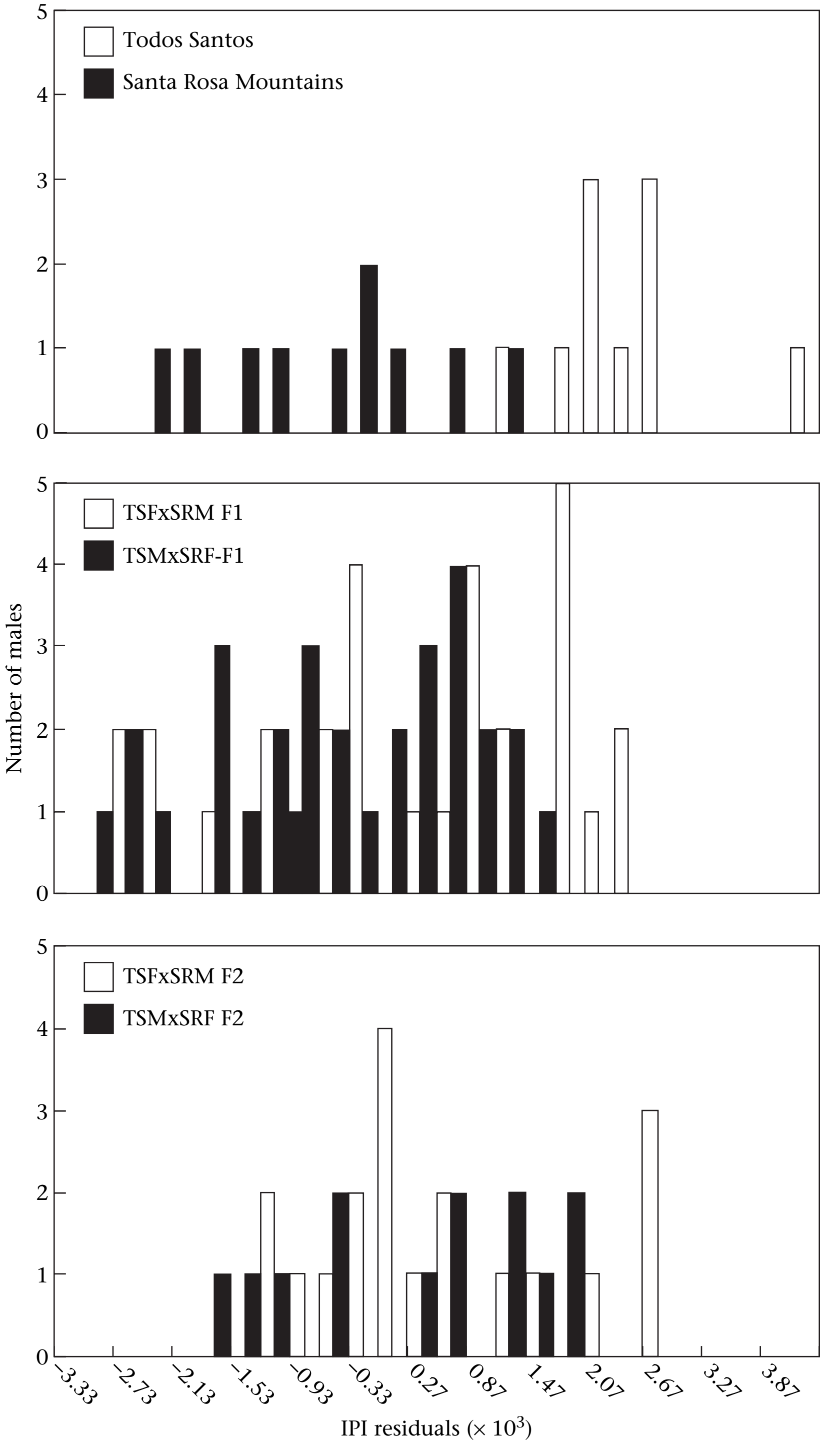

Figure 4. Residuals of log (interpulse interval, IPI) for males from the parental populations and the reciprocal F_1 and F_2 crosses.

songs between Baja California and mainland populations and the kinds of genetic differences responsible for them strongly implies that sexual isolation among these geographically isolated populations involves multiple cues. Playback experiments examining song differences between Baja California and mainland Mexico populations need to be performed to verify whether song variation is a causal component of premating isolation among populations.

Such geographical variation in courtship songs is consistent with the evolutionary history of *D. mojavensis* in the Sonoran Desert and ecological patterns of host plant use. *Drosophila mojavensis* is restricted to the Sonoran and Mojave Deserts and adjacent regions, and is sympatric with *D. arizonae* in a relatively small region of Sonora, Sinaloa and Arizona, where they occasionally share host plants (Wasserman & Koepfer 1977; Ruiz et al. 1990; Etges & Jackson 2001). Baja California populations of

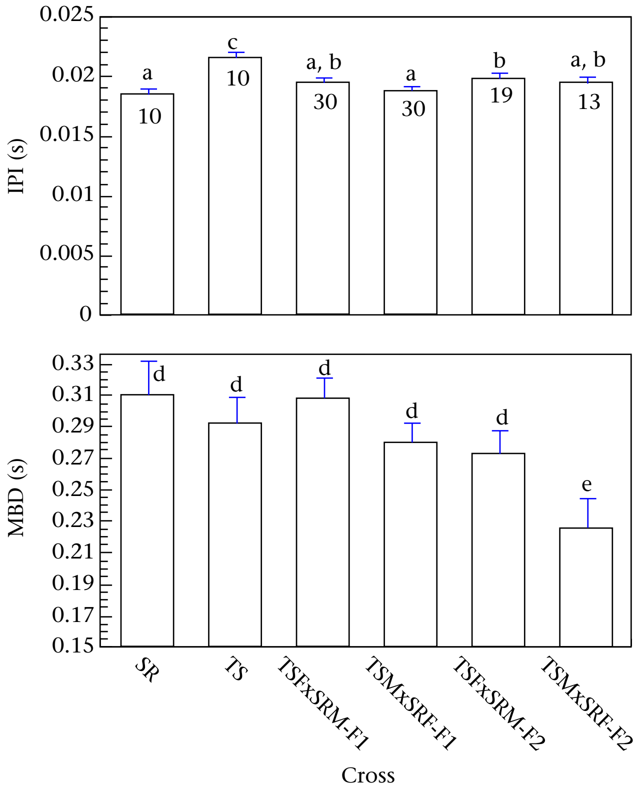

Figure 5. Least square means + SD of interpulse interval (IPI) and mean burst duration (MBD) among the cross generations of *D. mojavensis*. Nonsignificant means share the same letter. SR: Santa Rosa Mountains; TS: Todos Santos; F: females; M: males. Numbers in the bars are the sample sizes for both IPI and MBD.

D. mojavensis, which are considered ancestral, colonized mainland Mexico and Arizona by switching from agria, *Stenocereus gummosus*, to organ pipe cactus, *S. thurberi* (Heed 1978; Etges et al. 1999). Cina cactus, *S. alamosensis*, is sometimes shared with *D. arizonae* in Sonora and Sinaloa. Thus, the significantly shorter IPIs of mainland Mexico and Arizona populations of *D. mojavensis* (Fig. 2) are consistent with the character displacement model: IPIs of *D. arizonae* are much longer and sometimes fall within the range of Baja California *D. mojavensis* (Byrne 1999).

Table 3. Fixed genetic model used in the REML analyses to assess the types of effects influencing genetic differences in two parameters of *D. mojavensis* male courtship song, interpulse interval (IPI) and mean burst duration (MBD)

Cross*	Autosomes	X	Y	Dominance
M×M	1	1	1	0
B×B	0	0	0	0
M×B	0.5	1	0	1
B×M	0.5	0	1	1
MB×MB	0.5	0.5	0	0.5
BM×BM	0.5	0.5	1	0.5
IPI fitted effects	−2.013	−0.198	0.247	−0.568
SE	0.776	0.388	0.314	0.218
MBD fitted effects	−0.674	0.251	0.685	−0.074
SE	0.842	0.421	0.342	0.821

*Mainland (M) and Baja California (B) populations of *D. mojavensis*. Female genotypes are listed first in each cross.

Genetics of Song Differences

Our genetic analysis suggests that average IPI is influenced largely by autosomal genes that act additively, not unlike other *Drosophila* species (Pugh & Ritchie 1996; Ritchie & Kyriacou 1996; Aspi 2000), with dominance towards shorter mainland IPIs. The architecture of IPI differences in *D. mojavensis* probably depends on the choice of populations used in the crosses. The genetic basis of IPI differences between Baja California and Arizona (mainland) populations is also consistent with genetic analyses of courtship success. Krebs (1990) conducted courtship trials with F_1 males from mainland (Santa Rosa Mountains and Sonora, Mexico) and Baja California (Punta Prieta and San Lucas, just north of Bahia de Concepcion; see Fig. 1) population crosses, and found that mainland females mated with reciprocal F_1 and mainland males with equal frequencies. Krebs concluded that mating success in *D. mojavensis* is controlled by autosomal loci that are influenced by dominance towards mainland male courtship proclivity. In a related study, Sonora–Baja F_1s also behaved like Sonora males in courtship trials with *D. arizonae* (Markow 1981). The striking consistencies in these studies with our REML analysis of IPI variation suggest that courtship success in trials involving mainland and Baja California populations may be determined by male courtship songs: the genetic basis of IPI variation and courtship success is largely the same. Unfortunately, genetic analyses of epicuticular hydrocarbon differences are not yet available.

In other species with homogametic XX females and heterogametic XY males, Y-linkage effects are rare but not unknown, such as Y chromosome effects on mating success in *D. melanogaster* (Chippindale & Rice, 2001) and pulse train length in *D. virilis* (Huttunen & Aspi 2003). Y-linkage effects are sometimes seen for a range of traits, including male secondary sexual morphology polymorphisms in some species of fish (reviewed in Lindholm & Breden 2002), but the reasons for this are not clear (they have a large nondegenerate Y). Theory implies that linkage might arise due to sexual conflict, or that linkage of female preference genes can facilitate some forms of sexual selection, especially in female heterogametic systems (Hastings 1994; Reeve & Pfennig 2003; Kirkpatrick & Hall 2004; Lindholm et al. 2004), although Z or X linkage seems more powerful than Y or W. Analysis of assortative mating between *D. mojavensis* and *D. arizonae* implied that male behaviours rather than female preferences showed evidence of Y linkage and interaction with the fourth chromosome (Zouros 1981). In courtship trials, hybrid males carrying the *D. arizonae* Y chromosome copulated with *D. arizonae* females at rates similar to those of *D. arizonae* males, but hybrid males carrying a *D. mojavensis* Y chromosome did not. The proportion of the *D. mojavensis* Y chromosome that is functional is unknown, but clearly worthy of further study. The magnitude of the Y-linked effect that we detected is similar to that of the autosomal effect, but in the opposite direction, suggesting that an epistatic interaction effect between Y and its genetic background is the most important factor in these crosses. Further genetic analysis is needed, ideally with introgressed chromosomes (Pantazidis et al. 1993). Studies of male fertility in *D. mojavensis* hybrids, and between different strains, would help to futher resolve the genetic basis of courtship song differences revealed here. In playback experiments (Byrne 1999), burst duration influenced mating success of wingless *D. arizonae* males with *D. arizonae* females, suggesting that the effect of the Y chromosome on burst duration may be common to both *D. mojavensis* and *D. arizonae*.

Genetic analysis using simple crosses has provided insight into the kinds of genetic factors influencing courtship songs in *Drosophila* and other insects (Ritchie & Kyriacou 1994; Saldamando et al. 2005; reviewed in Gleason 2005). Our results suggest that conclusions about estimates of genetic effects depend on the parental populations used in the crosses, particularly in a species like *D. mojavensis* that is genetically and geographically differentiated across its range (Etges et al. 1999). This unsurprising result will require a measured interpretation about the generality of results from investigations into the genetic basis of differences between recently diverged populations showing low levels of reproductive isolation, but this level of analysis is a prerequisite to understanding divergence in elements of mate recognition systems before speciation is complete (Colegrave et al. 2000). Future quantitative trait locus (QTL) analyses of courtship song and epicuticular hydrocarbon variation among these populations of *D. mojavensis* should resolve and complement our results, but will also depend on the populations used for analysis.

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