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Genetic, ecological and morphological differences among populations of the cactophilic *Drosophila mojavensis* from southwestern USA and northwestern Mexico, with descriptions of two new subspecies

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A variety of molecular markers have consistently shown that little gene flow occurs among the geographically isolated populations of the cactophilic *Drosophila mojavensis* Patterson and Crow of southwestern USA and northwestern Mexico. The molecular studies support previous subspecies designations of *D. mojavensis* (*D. m. mojavensis* from the Mojave Desert and *D. m. baja* from the Baja California peninsula) and, in addition, suggest that two additional subspecies should be recognized (*D. m. sonorensis* from Sonora and Sinaloa, Mexico to southern Arizona and *D. m. wrigleyi* from Santa Catalina Island, California). Here we review evidence from studies on population genetics, ecology and behaviour that supports the subspecies assignments in *D. mojavensis*, and provide descriptions of *D. m. sonorensis* and *D. m. wrigleyi*. We also provide redescriptions of *D. m. mojavensis* and *D. m. baja*. Morphologically, the four subspecies are similar in external appearance, but showed differences in the male genitalia.

Keywords: desert *Drosophila*; geographic isolation; incipient speciation; population differentiation

Introduction

Drosophila mojavensis Patterson and Crow, a member of the *mulleri* complex of the *repleta* species group, inhabits the arid desert regions of southwestern USA and northwestern Mexico (Figure 1), feeding and breeding in necrotic tissue (rots) of a variety of cactus species, although within any particular geographic area a specific local host cactus is generally utilized (Heed 1978; Heed and Mangan 1986; Ruiz and Heed 1988; Ruiz et al. 1990). A great deal has been learned of the ecology, population genetics and reproductive behaviour of the different geographic populations of *D. mojavensis* over the last six decades, and the species has become an important model for understanding the timing of early events involved in the process of speciation, including the contributions of geographic and reproductive isolation, and changes in host plant use, that can ultimately lead to genetic divergence among populations (Mettler 1963; Zouros 1973; Zouros and D'Entremont 1980; Etges and Heed 1987; Ruiz et al. 1990; Markow 1991; Markow and Hocutt 1998; Hocutt 2000; Knowles and Markow 2001; Ross and Markow 2006; Matzkin et al. 2006; Reed et al. 2007). In addition to differences in

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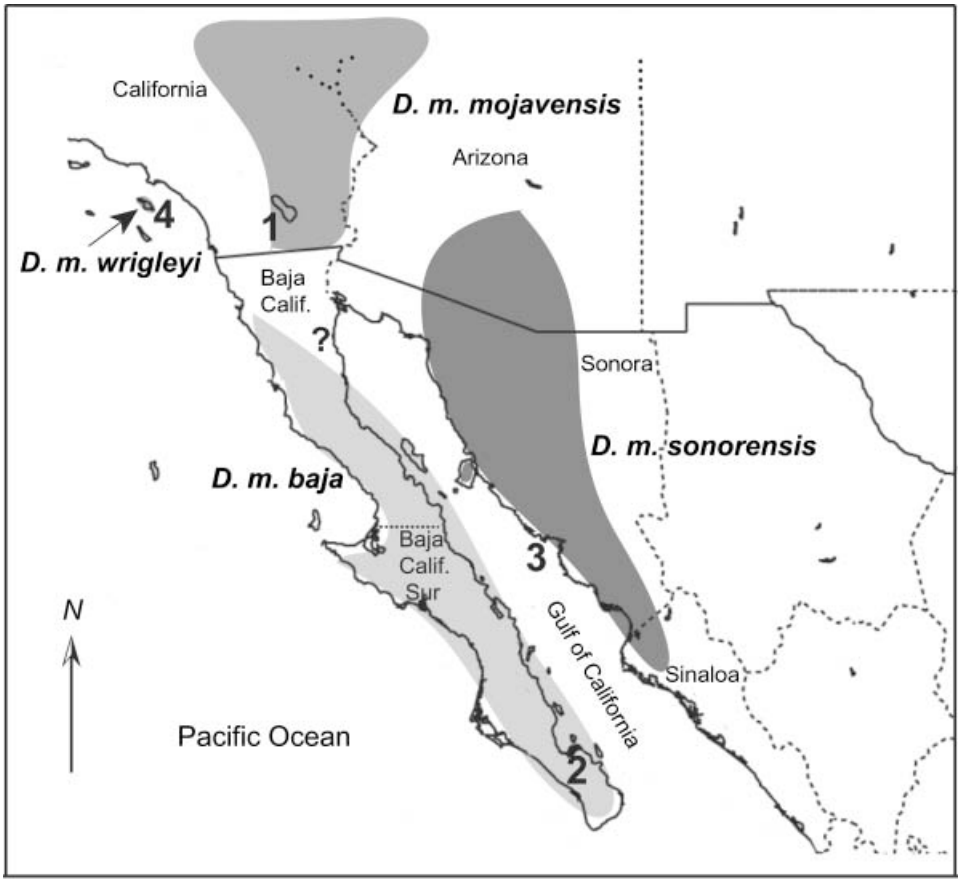

Figure 1. Map showing approximate geographic distribution of the four subspecies of *Drosophila mojavensis* in southwestern USA and northwestern Mexico. ?=unconfirmed subspecies at San Felipe, Baja California. Numbers show localities where flies used for laboratory cultures were collected: (1) Mojave; (2) Baja; (3) Sonora; (4) Catalina (see 'Materials and methods' for details).

host plant use, individuals of *D. mojavensis* from different geographic areas vary in several characteristics, including size, colour, chromosome polymorphisms, allele frequencies and DNA sequences (Mettler 1963; Zouros 1973; Johnson 1973, 1980; Etges and Heed 1987; Ruiz et al. 1990; Krebs 1991; Etges 1993; Hocutt 2000; Matzkin and Eanes 2003; Matzkin 2005; Ross and Markow 2006; Machado et al. 2007; Reed et al. 2007; Matzkin 2008). They also exhibit differences in biochemical, physiological and behavioural traits (Starmer et al. 1977; Batterham et al. 1982; Etges and Klassen 1989; Etges and Ahrens 2001; Krebs and Thompson 2005; Pfeiler et al. 2005).

The geographically isolated and genetically differentiated populations of *D. mojavensis* have been given a variety of names, including races, subraces and strains (Zouros 1973; Ruiz et al. 1990; Krebs and Thompson 2005; Pfeiler et al. 2005), with two subspecies, *D. m. mojavensis* from the Mojave Desert and *D. m. baja* from the Sonoran Desert, having been described (Mettler 1963). Population genetic studies

using allozymes and DNA sequence data (reviewed later) have supported the existence of additional subspecies of *D. mojavensis*, one from the Sonoran Desert of mainland Mexico and southern Arizona, USA, and the other from Santa Catalina Island, off the southern California coast (Hocutt 2000; Ross and Markow 2006; Machado et al. 2007; Reed et al. 2007; Matzkin 2008). Subspecies names have been proposed for these two populations in an unpublished doctoral dissertation (Hocutt 2000), and although these names have appeared in the literature (Pfeiler et al. 2005; Ross and Markow 2006), they have never been formally described. There is a need, therefore, to update and stabilize the nomenclature of the genetically differentiated populations of *D. mojavensis*. Here we review genetic, behavioural, ecological and morphological evidence that supports subdividing *D. mojavensis* into separate subspecies, and provide descriptions of the two new subspecies (*D. m. sonorensis* and *D. m. wrigleyi*) and redescriptions of *D. m. mojavensis* and *D. m. baja*.

Materials and methods

We examined 90 adult males and 72 adult females from laboratory cultures of *D. mojavensis* originally collected from the four main geographic regions of its distribution (abbreviated here as Mojave, Baja, Sonora and Catalina): (1) near Borrego Springs, Anza-Borrego Desert State Park, San Diego County, California, USA (Mojave); (2) La Paz, Baja California Sur, Mexico (Baja); (3) Guaymas, Sonora, Mexico (Sonora); and (4) Santa Catalina Island, California, USA (Catalina) (Figure 1). All strains were maintained in mass cultures on standard banana/*Opuntia* medium. External morphological measurements were conducted on both males and females from each of the four populations (the external morphometric and meristic characters examined are given in the redescription of *D. m. mojavensis* under "Subspecies accounts"). Genitalia were also dissected and compared in males from each of the four populations, and in females of the Mojave population.

Calculations of genetic distances (uncorrected *p*-distance) among the four geographic populations of *D. mojavensis* were carried out in MEGA 3.1 (Kumar et al. 2004) using a 658-bp segment of the mitochondrial cytochrome oxidase subunit I (COI) gene (Reed et al. 2007). For this data set, the number of localities sampled from each geographic region (total number of individuals from each region given in parentheses) were two from Mojave ($n=21$), seven from Baja ($n=81$), five from Sonora ($n=64$) and one locality on Santa Catalina Island ($n=9$) (see Reed et al. (2007) for details).

Historical background

Drosophila mojavensis was originally named as a subspecies of *D. mulleri* (Patterson and Crow 1940) based on samples obtained from Mesquite Springs in Death Valley, California, but was elevated to species status by Patterson and Wheeler (1942). A formal description of *D. mojavensis* was later published by Patterson (1943). Mettler (1963) was the first to formally describe subspecies of *D. mojavensis*, designating the populations from the Baja California peninsula and mainland Sonora, Mexico, whose host cacti are pitaya agria (*Stenocereus gummosus*) and organ pipe cactus (pitaya dulce; *Stenocereus thurberi*), respectively, as *D. m. baja*. *Drosophila m. mojavensis* from the Mojave Desert utilizes barrel cactus (*Ferocactus cylindraceus*) as a host (Spencer 1941). The criteria used by Mettler (1963) for designating the two

subspecies included differences in banding patterns on chromosomes 2 and 3, and the observation that flies from Mexico were ''smaller and darker'' than flies from the Mojave Desert.

Subsequent studies in *D. m. baja* revealed the presence of chromosomal inversions in the peninsular populations not present in mainland populations (Sonora and southern Arizona) (Johnson 1973). Additional evidence for a distinction between peninsular and mainland populations was obtained by Zouros (1973) who found large differences in allele frequencies at the alcohol dehydrogenase-2 (*Adh-2*) locus. Zouros (1973), who referred to the two allopatric subspecies as race A (*D. m. mojavensis*) and race B (*D. m. baja*), further subdivided *D. m. baja* into subraces BI (mainland) and BII (Baja California peninsula and several islands in the Gulf of California).

Additional populations of *D. mojavensis* were subsequently discovered on Santa Catalina Island, where they use prickly-pear cactus (*Opuntia* spp.) as a host, and at the Grand Canyon, Arizona where barrel cactus is the host (Heed and Mangan 1986; Ruiz et al. 1990). Ruiz et al. (1990) showed that flies from both of these widely separated geographic regions all shared the standard banding pattern on chromosomes 2 and 3 found in *D. m. mojavensis*, and concluded that they should be assigned to that subspecies. Flies from the Baja California peninsula and mainland Sonora, however, were polymorphic for inversions on chromosomes 2 and 3, supporting the view of Mettler (1963) that they should be recognized as the separate subspecies, *D. m. baja*. Ruiz et al. (1990) also found that, unlike the Baja populations, most mainland Sonora populations were homozygous for the $2q^5$ chromosome inversion and thus were cytologically derived. Although Ruiz et al. (1990) made no additional comment on this difference, their results offered support for the distinction of subraces BI and BII of *D. m. baja* proposed by Zouros (1973).

Genetic differentiation and reproductive isolation

Populations of *D. mojavensis* from throughout the species' range have now been examined using a variety of molecular markers (allozymes, mitochondrial DNA (mtDNA) and nuclear DNA (including microsatellites)) and a great deal has been learned about gene flow, population genetic structure and incipient speciation in this species (Hocutt 2000; Ross and Markow 2006; Machado et al. 2007; Reed et al. 2007; Matzkin 2008).

Based on analyses of allozyme data, and taking into account previously published data on behavioural, ecological, morphological and reproductive differences, Hocutt (2000) first suggested the existence of four rather than two subspecies. In addition to *D. m. mojavensis* and *D. m. baja*, the two additional subspecies proposed by Hocutt (2000) were *D. m. sonora* (here designated *D. m. sonorensis*) for the populations of *D. m. baja* (*sensu* Mettler 1963) from mainland Mexico and southern Arizona (subrace BI of Zouros 1973) and *D. m. wrigleyi* for the population of *D. m. mojavensis* on Santa Catalina Island (Ruiz et al. 1990; also called race C in Pfeiler et al. 2005).

Support for the subspecies assignments proposed by Hocutt (2000) has been provided by several molecular studies that examined variation in mtDNA (COI gene (Reed et al. 2007)) and in nuclear DNA (four microsatellite loci (Ross and Markow 2006), multiple nuclear loci (see Table 2 of Machado et al. 2007), and the glutathione

S-transferase D1 (*GstD1*) gene (Matzkin 2008)) in a number of populations of *D. mojavensis* sampled from each of the four main geographic areas. With minor exceptions, population genetic and phylogenetic analyses in the four studies were in agreement and showed that populations within each main region were panmictic, but strong genetic differentiation was noted between regions, with essentially no gene flow found between the Catalina and Mojave populations. Although analysis of COI sequence data indicated some sharing of haplotypes among Baja and Sonora populations (Reed et al. 2007), the nuclear DNA data set of Machado et al. (2007) provided strong support that populations from each of four geographic areas were reciprocally monophyletic (Machado et al. 2007). Mean pairwise genetic divergences (*p*-distances) calculated using the COI data set ranged from 0.8–1.9% among the four separate populations of *D. mojavensis* (Table 1), supporting their designations as distinct subspecies. Although the nuclear data of Machado et al. (2007) suggest that the Catalina and Mojave populations form a sister lineage, none of the molecular studies conducted to date support the conclusion of Ruiz et al. (1990) that flies from Santa Catalina Island should be assigned to *D. m. mojavensis*. Additional confirmation of significant population structure, and support for the proposed subspecies of *D. mojavensis*, was found between *D. m. baja* and *D. m. sonorensis* using *Adh-2* sequence data (Matzkin 2004), and between *D. m. baja* and *D. m. mojavensis* using male reproductive tract genes (accessory gland protein genes and testis-expressed genes) (Wagstaff and Begun 2005).

Although molecular studies on *D. mojavensis* have focused on adults, support for separate subspecies has also been found in a molecular study on larvae. Using a cDNA microarray, Matzkin et al. (2006) showed that there were significant differences in larval gene expression when *D. m. baja* was reared in the laboratory on organ pipe cactus instead of its natural host, pitaya agria. These results suggest that the different chemical composition of the host cactus breeding site may play a role in genetic diversification in *D. mojavensis*.

Reed and Markow (2004) have shown that male sterility produced in hybrid crosses between *D. mojavensis* and its sibling species *D. arizonae* is controlled by genetic factors present at different frequencies in the different populations of *D. mojavensis*, providing further evidence for genetic differentiation among populations. Reed and Markow (2004), however, point out that it is unclear whether these genetic factors play any role in reproductive character isolation within and between the different populations of *D. mojavensis*. For example, significant genetically determined premating behavioural differences have been found between *D. m. sonorensis* and *D. m. mojavensis* (females of *D. m. sonorensis* are much more discriminatory against *D. arizonae* males than are females of *D. m. mojavensis*), and

Table 1. Mean values for uncorrected genetic distances (*p*-distances) among the four geographic populations of *Drosophila mojavensis* based on COI sequences.

	Mojave	Baja	Sonora	Catalina
Mojave	–			
Baja	0.019	–		
Sonora	0.018	0.008	–	
Catalina	0.018	0.012	0.014	–

between *D. m. sonorensis* and *D. m. baja* (Wasserman and Koepfer 1977; Zouros and D'Entremont 1980; Zouros 1981; Markow 1981, 1991; Markow et al. 1983; Krebs and Markow 1989; Krebs 1990; Hocutt 2000). Thus far no detailed studies of postzygotic isolation among *D. mojavensis* subspecies have been performed. Crosses performed as mass matings among various subspecies have been reported to produce fertile offspring (Mettler 1963; Ruiz et al. 1990). It is clear, however, that any existing postzygotic isolation would at this point be incipient and therefore probably undetectable without single pair matings of flies between isofemale lines of each subspecies (Reed and Markow 2004).

Adh-2 polymorphism

As mentioned briefly above, large differences in the frequency of the slow (S) and fast (F) migrating gene products of the *Adh-2* locus led Zouros (1973) to subdivide the Baja and Mexican mainland (Sonora) populations of *D. m. baja* (*sensu* Mettler 1963) into two groups which he termed subraces BI (Sonora) and BII (Baja), here designated as subspecies *D. m. sonorensis* and *D. m. baja*. *Adh-2*^{F} predominated in *D. m. baja*, with frequencies (*f*) ranging from 0.9–1.0, whereas the dominant allele in *D. m. sonorensis* was *Adh-2*^{S} ($f>0.8$). Subsequent allozyme studies (Richardson et al. 1977; Heed 1978; Cleland et al. 1996; Hocutt 2000; Matzkin and Eanes 2003; Matzkin 2004) have confirmed the large differences in *Adh-2* allele frequencies between the two subspecies, with low frequencies of *Adh-2*^{F} (typically 0.0–0.3) consistently found in *D. m. sonorensis*. Because of the likelihood that the *Adh-2* locus is under selection (Matzkin and Eanes 2003), it has been pointed out that evolutionary relationships among the populations based upon allozyme studies may be biased (Ross and Markow 2006; Reed et al. 2007). With *D. mojavensis*, however, several conclusions based on allele frequencies at the *Adh-2* locus agree well with those obtained with the higher resolution mitochondrial and nuclear DNA markers. Specifically, the distinctiveness of *D. m. baja* and *D. m. sonorensis* as originally suggested by Zouros (1973) based on *Adh-2* allele frequencies is supported by the molecular markers. Also, Hocutt (2000) showed that *Adh-2*^{F} was the predominate *Adh-2* allele in *D. m. wrigleyi*, ($f=0.7$; $n=60$), a frequency which is intermediate to that seen in *D. m. baja* ($f\sim0.9$–1.0) and *D. m. mojavensis* from the southern California desert region ($f\sim0.43$–0.55) (Cleland et al. 1996; Hocutt 2000). The distinctiveness of the four subspecies based solely of *Adh-2* allele frequencies is lost only when the population of *D. m. mojavensis* from the Grand Canyon is included. This population shows low *Adh-2*^{F} frequencies ($f\sim0.12$–0.34) which overlap those seen in *D. m. sonorensis* (Cleland et al. 1996; Hocutt 2000).

Morphological differentiation

Few external morphological differences are found between subspecies of *D. mojavensis*. Mettler (1963) stated only that *D. m. baja* (including flies from Baja and mainland Sonora) was "smaller and darker" than *D. m. mojavensis* from the Chocolate Mountains of southeastern California. Subsequent studies of body size (thorax length) have confirmed that males and females from the Baja population are smaller than those from the Mojave and Catalina populations, and, in contrast to Mettler's (1963) observation, are also smaller than flies from the Sonora population (Etges and Heed 1987; Krebs 1991; Etges 1993). Hocutt (2000) also commented that

D. m. mojavensis from the southern California desert tended to be more yellowish than those from Baja (*D. m. baja*) and Sonora (*D. m. sonorensis*), and that flies from southern California and Sonora were larger than those from Baja. Krebs (1991) found that males and females of wild *D. m. wrigleyi* were about 25–30% smaller than field collected flies of *D. m. sonorensis*, but body size of both subspecies was greater in laboratory strains than in wild flies. Overall, these results suggest that body size and colour, although differing among some of the subspecies, are of limited taxonomic value when attempting to differentiate among all four subspecies.

In the following accounts, comparisons of external morphological characters failed to reveal any characters that could be used confidently to distinguish between the four subspecies of *D. mojavensis* (individual measurements and statistical analyses are available from the second author upon request). Subspecies are distinguished mainly by the shape of the aedeagus (also see Ruiz et al. 1990) and by geographic location (Figure 1), in addition to the molecular differences summarized above.

Subspecies accounts

Drosophila mojavensis mojavensis Patterson and Crow, 1940 (Figures 2 and 3A)

Drosophila mulleri mojavensis Patterson and Crow, 1940:251
Drosophila mojavensis Patterson and Wheeler, 1942:95; Patterson, 1943:158

Diagnosis

Drosophila m. mojavensis can be distinguished from the other subspecies by the shape of the aedeagus (Figure 3A). Internal margin of aedeagus/external margin of aedeagal apodeme index=1.0 (0.99–1.01; n=30); ventral margin of aedeagus/wide aedeagal apodeme index=3.7 (3.65–3.92). Tip of aedeagus with a small pointy protuberance; dorsal and ventral aedeagus margins almost form an isosceles triangle. Aedeagus ventral margin almost straight with a small protuberance in the middle zone.

Redescription

Male. *Head*. Front brownish; frontal length 0.32 (0.30–0.34) mm; frontal index=0.83 (0.76–0.88). Frontal triangle pale brown, more or less distinct. Ocellar triangle slightly prominent and lighter than front, pollinose. Interfrontal setulae in "V" shape. Frontal vittae brownish; orbital plates wider at the or1 level, almost a pointed shape. Orbital setae black, or2 just slightly posterior to or1; or1/or3 ratio=0.90 (0.76–1.07); or2/or1 ratio=0.60 (0.56–0.68); vibrissal index=0.51 (0.41–0.61). Face brownish. Carina broad below and sulcate, central area similar colour as vittae; distal lateral sections light brown. Cheek index ca. 5.70–9.86. Eye red; eye index=1.17 (1.11–1.25). Occiput yellowish, brown above foramen. Antennae tannish brown, pedicel slightly darker, third joint darker; arista with three dorsal, two ventral, and about four small inner branches, plus terminal fork. Proboscis yellowish, clypeus brownish. Palpus pale yellow with about three strong setae along external ventral margin. *Thorax*. Length: 1.01 (0.98–1.04)mm. Mesonotum light

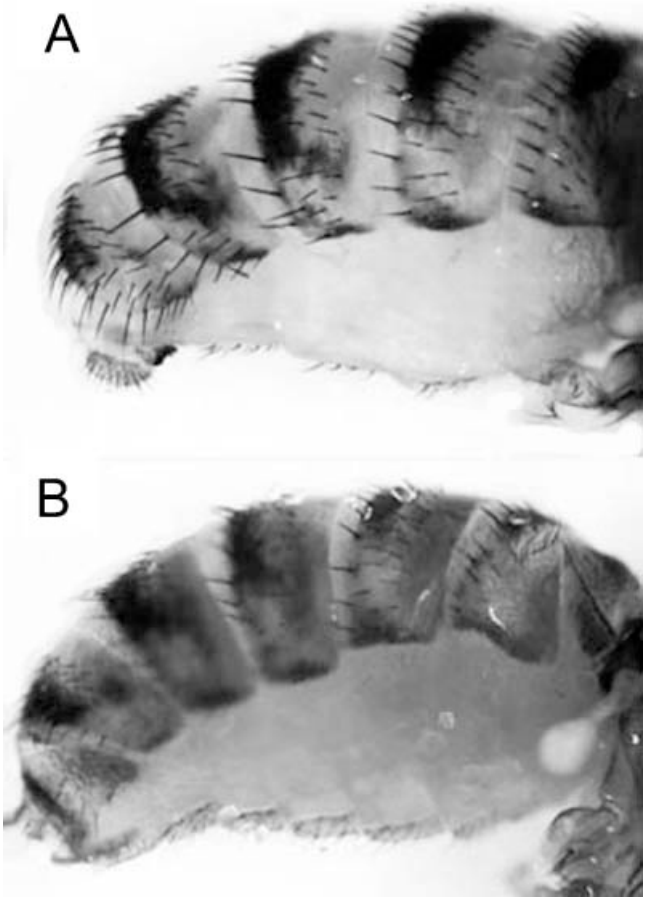

Figure 2. Lateral view of abdominal pattern in *Drosophila m. mojavensis*. (A) Male; (B) female.

brownish-yellow; setae arising from brown spots; eight rows of acrostichal setulae. Upper/lower postpronotal setae (h index)=1.20 (1.19–1.20); anterior/posterior dorsocentral setae (dc index)=0.63 (0.60–0.65). Scutellum dark brownish with margin slightly clear. Basal setae slightly convergent, apical scutellar convergent; basal/apical scutellar setae (scut index)=0.82 (0.73–0.90). Pleura brownish, shining, with a narrow, dark brown stripe from upper margin of katepisternum to below procoxa. Faint darker brown stripes, one visible along upper margin, and a second stripe on the middle part of anepisternum; this strip prolonged faintly to the anepimeron. Anterior/posterior katepisternal setae (sterno index)=0.72 (0.64–0.80), median katepisternal seta about 52–61% of the anterior one. Haltere yellow with slightly brownish colouration on anterior side of knob. Legs yellow, with a faint brownish postbasal band on tibia which it is darker on hind leg. Procoxa and all fifth tarsal segments slightly darker than the rest of the legs; preapical setae on all tibiae, apical seta on mesotibia. *Abdomen* (Figure 2A). Pale yellow; apical band on tergites well defined in dorsal area with interruption between sides. On the upper margin of the lateral area, apical band curves slightly and expands as a diffuse extension to

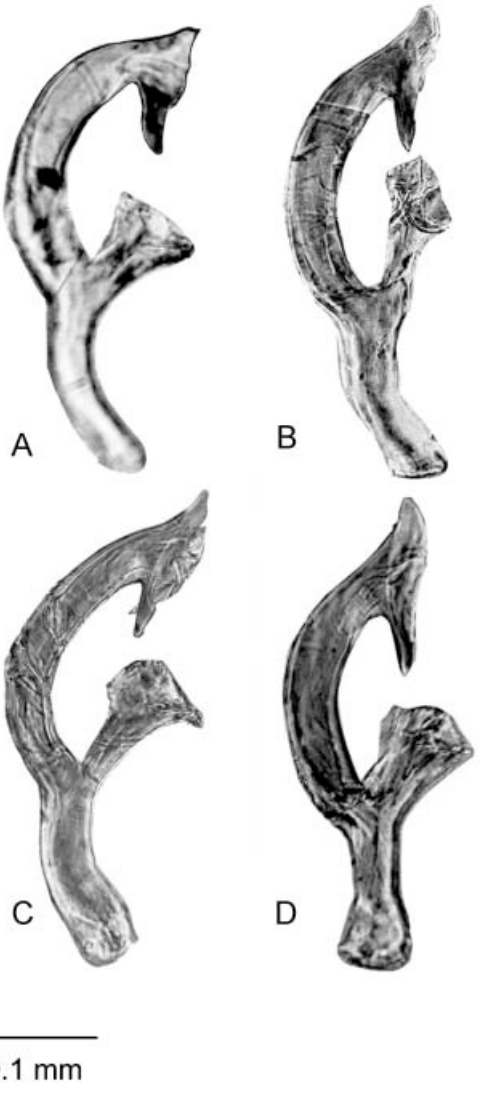

Figure 3. Photographs showing lateral views of the aedeagus and aedeagal apodeme in the four subspecies of *Drosophila mojavensis*. (A) *D. m. mojavensis*; (B) *D. m. baja*; (C) *D. m. sonorensis*; (D) *D. m. wrigleyi*.

almost touch the margin of the anterior tergite. Background colour in lateral area brownish-yellow, diffuse; most of the margin in lateral areas from tergites 1–4 covered by a dark irregular spot; colour of lateral area spots lighter than apical band; colour gradually disappears from ventral to dorsal area; spot almost completely disappears at posterior margin of tergite 5. Testes yellow with 2.5 inner and 3 outer coils. *Wings*. Hyaline, veins brown. Apex of subcostal break slightly black with two well-developed setae. Third costal section with heavy bristles on its basal third. Crossveins clear. Wing length 1.70 (1.60–1.80)mm, length to width ratio=1.82

(1.73–1.88). Indices: Costa (C index)=2.91 (2.69–3.06), C-III/C-IV (ac index)=2.27 (2.00–2.57), C-III/M-III (4C index)=0.92 (0.80–1.05), M-IV/M-III (4v index)=1.81 (1.67–1.91), CuA (apical section)/dM-Cu (5x index)=1.26 (1.00–1.44), CuA (apical section)/M-III (M index)=0.48 (0.43–0.53), Basal R_{4+5}/M-III (prox. X index)=0.67 (0.64–0.71). *Genitalia*. Cerci with few microtrichose, many long setae, and fused anteriorly to epandrium. Epandrium barely microtrichose on dorsal area; three to five setae on the apical margin in the medial section; ventral lobe enlarged and bare, with filament-like setae, forming a rounded toe that partially overlaps surstylus laterally. Surstylus not microtrichose; 9–11 peg-like prensisetae almost rounded on tip, with the two anterior setae slightly larger and pointed; three to four inner setae larger than prensisetae; seven to eight outer setae. Cerci barely microtrichose; hypandrium slightly longer than epandrium. Aedeagus fused to aedeagal apodeme (Figure 3A). Aedeagal apodeme upper half smaller than aedeagus. Ventral rod almost twice as long as width of aedeagal apodeme.

Female. Identical to male, except as follows. Abdomen similar to male except lateral light area on tergite 5 diffuse, darker than male (Figure 2B). *Wings*. Wing length 1.78 (1.62–1.87)mm, length to width ratio=2.01(1.93–2.05). Indices: Costa=3.20 (2.45–3.43), C-III/C-IV=2.34 (2.13–2.57), C-III/M-III=0.86 (0.77–1.03), M-IV/M-III=1.64 (1.51–1.74), CuA (apical section)/dM-Cu=1.19 (1.00–1.38), CuA (apical section)/M-III=0.52 (0.46–0.58), Basal R_{4+5}/M-III=0.80 (0.70–1.00). *Genitalia*. Valve of oviscapt distally rounded, ventrally almost straight, with ca. 6 distal and 12–13 marginal, peg-like, mostly roundish-tipped, outer ovisensi1la; inner ovisensi1la: four thin, trichoid-like, distally positioned, and one long, straight, subterminal.

Distribution and host cactus

Mojave Desert (southern California to northwestern Arizona; Figure 1). *Host cactus*: barrel cactus (*Ferocactus cylindraceus*).

Remarks

Referring to many newly described species of *Drosophila*, including *D. m. mojavensis*, Patterson (1943) commented that type specimens are widely scattered in private collections and museums. Whether a holotype was designated is not stated in either Patterson and Crow (1940) or Patterson (1943), but a ''cotype'' (either a syntype or paratype) of *D. m. mojavensis* from Mesquite Springs, Death Valley, California is deposited in the American Museum of Natural History, New York. We did not examine the ''cotype''.

Material examined

External and internal (genitalia) measurements were conducted on 30 males and 12 females from laboratory culture ANZA406 started with flies from California (USA), near Borrego Springs, Anza-Borrego Desert State Park, San Diego County, April 2006, collected by L.K. Reed and T.A. Markow. Voucher specimens (all from isofemale line culture ANZA406-4): 10 males and 12 females deposited at the San

Diego *Drosophila* Stock Center collection at the University of California, San Diego, La Jolla, California (acquisition nos. 461–470 (males) and 471–482 (females)); 10 males and 10 females deposited at the Smithsonian Diptera Collection, United States National Museum of Natural History (USNM), Washington, DC.

Drosophila mojavensis baja Mettler, 1963
(Figure 3B)

Drosophila mojavensis Patterson, 1943:158

Diagnosis

Drosophila m. baja can be distinguished from the other subspecies by the shape of the aedeagus (Figure 3B). Internal margin of aedeagus/external margin of aedeagal apodeme index=2.04 (1.36–2.30; n=20); ventral margin of aedeagus/wide aedeagal apodeme index=5.30 (4.08–5.89). Dorsal margin of aedeagus in the anterior part has a depressed curve that is less pronounced than in *D. m. sonorensis*; aedeagus tip is pointed and looks like a spine (some individuals appear to have two small spines).

Distribution and host cactus

Baja California peninsula and the islands of the western Gulf of California (Figure 1, but see remarks below). *Host cactus*: pitaya agria (*Stenocereus gummosus*).

Remarks

In Mettler's (1963) brief description, no mention is made of type specimens or a type locality. Flies assigned to *D. m. baja* by Mettler (1963) were collected over a wide geographic area of northwestern Mexico, including the states of Baja California Sur (La Paz and Mulegé), Baja California (near Cabo San Miguel on the Gulf of California) and mainland Sonora (Sonoita and Magdalena). Here we are restricting the definition of *D. m. baja* to include only the peninsular populations (with the possible exception of flies from the San Felipe region in northeastern Baja California) and those from islands in the western gulf; the Sonoran populations are here assigned to *D. m. sonorensis* (see following account). In the absence of evidence that types were ever designated, and to avoid confusion in future taxonomic studies of the *D. mojavensis* subspecies group, we have designated a *neotype* of *Drosophila mojavensis baja* from La Paz, Baja California Sur (see Material examined) in accordance with Article 75 of the Code of the International Commission of Zoological Nomenclature (ICZN). This designation is necessary to clarify the taxonomic status of *D. m. baja* and to fix a type locality.

The subspecies assignment of flies from the San Felipe region in northeastern Baja California needs to be confirmed with molecular markers and examination of genitalia, but some evidence suggests that *D. m. sonorensis*, or possibly *D. m. mojavensis*, might be found there. Richardson et al. (1977) reported that the *Adh-2*^{S} allele was fixed in flies from San Felipe, suggesting that they belonged to *D. m. sonorensis*, although number of individuals analyzed was not given (San Felipe was not sampled in the *Adh* study of Zouros (1973)).

Material examined

Neotype. Male: Baja California Sur (Mexico), La Paz, February 2001, L. Matzkin, deposited at the San Diego *Drosophila* Stock Center collection at the University of California, San Diego, La Jolla, California (acquisition no. 527 from laboratory culture MJBC 113 started from flies collected on *Stenocereus gummosus*). *Neoparatypes* (same collection data as neotype): 14 males and 15 females deposited at the San Diego *Drosophila* Stock Center collection (acquisition nos. 528–541 (males) and 542–556 (females)); 10 males and 10 females deposited at the Smithsonian Diptera Collection, United States National Museum of Natural History (USNM), Washington, DC. External measurements were conducted on 20 males and 20 females from laboratory culture MJBC 113; internal (genitalia) measurements were conducted on 20 males.

Drosophila mojavensis sonorensis Castrezana, **new subspecies**
(Figure 3C)

Drosophila mojavensis baja Mettler, 1963 (in part)
Drosophila mojavensis sonora Hocutt, 2000

Type material

Holotype. Male: Sonora (MEXICO), Guaymas, June 1999, L. Matzkin, deposited at the San Diego *Drosophila* Stock Center collection at the University of California, San Diego, La Jolla, California (acquisition no. 557 from laboratory culture MJ 122). *Paratypes* (same collection data as holotype): 15 males and 10 females deposited at the San Diego *Drosophila* Stock Center collection (acquisition nos. 558–572 (males) and 573–582 (females)); 10 males and 10 females deposited at the Smithsonian Diptera Collection, United States National Museum of Natural History (NMNH), Washington, DC.

Diagnosis

Drosophila m. sonorensis can be distinguished from the other subspecies by the shape of the aedeagus (Figure 3C). Internal margin of aedeagus/external margin of aedeagal apodeme index=1.21 (1.19–1.27; n=15); ventral margin of aedeagus/wide aedeagal apodeme index=4.33 (4.26–5.16). Compared to *D. m. mojavensis*, ventral margin of aedeagus in *D. m. sonorensis* is larger with stronger protuberances; dorsal margin of aedeagus has a depressive curve resulting in a narrow aedeagal tip.

Distribution and host cactus

Sonora and Sinaloa, Mexico to southern Arizona, USA (Figure 1). *Host cactus*: organ pipe (*Stenocereus thurberi*), except in the region of El Desemboque, Sonora where pitaya agria (*Stenocereus gummosus*) is also utilized.

Remarks

High frequencies of the *Adh-2*^{S} allele in flies from islands adjacent to Sonora in the eastern Gulf of California, including Tiburón Island (Richardson et al. 1977; Heed

1978), suggest that they belong to *D. m. sonorensis*. Also, Etges (1993) found *D. mojavensis* on pitaya agria in the El Desemboque region of mainland Sonora, about 5km from Tiburón Island, which from their size and behaviour were typical of the mainland population, *D. m. sonorensis*.

Material examined

External measurements were conducted on 20 males and 20 females from laboratory culture MJ 122; internal (genitalia) measurements were conducted on 15 males.

Etymology

Subspecies name suggested by Hocutt (2000) was *sonora* for the state of Sonora, Mexico, the geographic centre of its distribution, here changed to *sonorensis* to follow ICZN recommendations.

Drosophila mojavensis wrigleyi Castrezana, **new subspecies**
(Figure 3D)
Drosophila mojavensis mojavensis Ruiz, Heed and Wasserman, 1990

Type material

Holotype. Male: California (USA), Santa Catalina Island, 20 October 2007, V. Carlin-Harris, deposited at the San Diego *Drosophila* Stock Center collection at the University of California, San Diego, La Jolla, California (acquisition no. 483 from collection CI 1007). *Paratypes* (same collection data as holotype): 15 males and 15 females deposited at the San Diego *Drosophila* Stock Center collection (acquisition nos. 484–498 (males) and 499–513 (females)); 10 males and 10 females deposited at the Smithsonian Diptera Collection, United States National Museum of Natural History (NMNH), Washington, DC.

Diagnosis

Drosophila m. wrigleyi can be distinguished from the other subspecies by the shape of the aedeagus (Figure 3D). Internal margin of aedeagus/external margin of aedeagal apodeme index=1.37 (1.35–1.49; n=15); ventral margin of aedeagus/wide aedeagal apodeme index=3.97 (3.47–4.27). Ventral margin of aedeagus is almost straight; anterior dorsal margin has a slight depressive curve with small protuberances appearing like a saw with two teeth.

Distribution and host cactus

Currently known only from Santa Catalina Island off the coast of southern California, USA (Figure 1). *Host cactus*: prickly-pear (*Opuntia* spp., including *O. littoralis*).

Remarks

Fasolo and Krebs (2004) found that *D. m. wrigleyi* showed significantly greater thermal tolerance than both *D. m. sonorensis* from southern Arizona (Santa Rosa

Mountains) and San Carlos, Sonora, and *D. m. baja* from Baja California Sur (Ensenada de los Muertos). Fasolo and Krebs (2004) also noted that preliminary mtDNA studies using 16S rRNA showed *D. m. wrigleyi* possessed three apparently unique base substitutions compared to flies here assigned to *D. m. baja* and *D. m. sonorensis*.

Material examined

External measurements were conducted on 20 males and 20 females from collection CI 1007; internal (genitalia) measurements were conducted on 15 males.

Etymology

Subspecies name suggested by Hocutt (2000) in honor of the Wrigley family, and especially William Wrigley, Jr., for their efforts in protecting Santa Catalina Island.

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