



Figure 1. The frequencies of alleles and genotypes in locus of  $\beta$ -specific esterases in different populations of *Drosophila melanogaster*: A: 1 – the observed frequency of S-allele, 1' – the expected frequency of S-allele, 2 – the observed frequency of F-allele, 2' – the expected frequency of F-allele; B: 1 – the observed frequency of dominant homozygotes, 1' – the expected frequency of dominant homozygotes, 2 – the observed frequency of heterozygotes, 2' – the expected frequency of heterozygotes, 3 – the observed frequency of recessive homozygotes, 3' – the expected frequency of recessive homozygotes. 1 – Odesskaja, 2 – Sadovaya, 3 – Poleskaya, 4 – Priozernaya.

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### Rediscovery of *Drosophila wheeleri* in Sonora, Mexico?

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*Drosophila wheeleri* and *D. aldrichi* are sibling species commonly associated with *Opuntia* cactus in the southwestern United States and Mexico and are members of the large *D. repleta* group. *D. aldrichi* has a much broader distribution than *D. wheeleri*, uses different host cacti, and very likely comprises a complex of cryptic species (Wasserman, 1992; Beckenbach *et al.*, 2008; Oliveira *et al.*, 2008). Samples of *D. aldrichi* have been identified in southern Mexico, as well as El Salvador,

Venezuela, and Brazil (Vilela, 1983), it has been reared from columnar cactus in southern Mexico (Etges, unpubl. data), it has colonized Australia with the introduction of *Opuntia* cactus (Barker, 1982), and it is colonizing Baja California (Castrezana, 2007). The description of *Drosophila wheeleri* (Patterson and Alexander, 1952) includes an interesting account of the first encounter with this species in Sonora, Mexico in 1941. G.B. Mainland and R.P. Wagner collected 55 *D. "aldrichi-like"* adults near cacti around Hermosillo, but then lost the stock in lab culture. The actual description of this species was subsequently based on flies collected in Arcadia, California in 1950 by Warren Spencer. Since then, *D. wheeleri* was largely thought to be restricted to southern California and northern Baja California coincident with its host plants, *Opuntia oricola*, *O. demissa*, and *O. ficus-indica* (Beckenbach *et al.*, 2008). Many years of field collections and rearing thousands of adults from cactus rots suggested that *D. aldrichi* and *D. wheeleri* seemed to be completely allopatric, with the former species distributed from south central Mexico to Texas, a western lineage that extended from southwestern Mexico to as far north as Guaymas, Sonora and Baja California (Jefferson *et al.*, 1974; Beckenbach *et al.*, 2008; Oliveira *et al.*, 2008), and a few isolated locations in Central and South America (see above). How could Patterson and Alexander (1952) have concluded that Mainland and Wagner's 1941 extinct Sonora collection was indeed *D. wheeleri* and why have no other collections ever revealed this species in mainland Mexico?

The purpose of this report is to: 1) describe a possible observation of *D. wheeleri* in northwestern Mexico, identified on morphological criteria, 2) present results of sexual isolation tests with another population of this species, and 3) discuss recently published and unpublished molecular affinities of *D. wheeleri* and *D. aldrichi*. A field collection made in Punta Onah, Sonora in November 2007 unexpectedly revealed 3 adults (1♀ + 2♂) that were first thought to be *D. aldrichi*. Subsequently, a stock derived from these flies was later confirmed to be *D. wheeleri* by S. Castrezana at the Species Stock Center based on the pattern of abdominal banding (Table 1). Although this region of Sonora has been repeatedly collected for over 40 years due to the presence of agria cactus, *Stenocereus gummosus*, a major host of *D. mojaviensis* and *D. arizonae* (Fellows and Heed, 1972;

Heed, 1978; Heed and Mangan, 1986; Etges *et al.*, 1999), no published accounts of *D. wheeleri* in Sonora since 1941 are known. Male genitalia (Vilela, 1983) and polytene chromosomes (Wasserman, 1992) of *D. wheeleri* and *D. aldrichi* are indistinguishable, although a number of other minor characteristics, especially the abdominal banding differences, are reliable indicators for species identification (Patterson and Alexander, 1952). Thus, we may have recovered *D. wheeleri* in Sonora 66 years after its initial collection.

We exploited this finding by determining the degree of sexual isolation between this Sonora population and a population of *D. wheeleri* from Santa Catalina Island near Los Angeles, California. We presumed that levels of sexual isolation with another known population of *D. wheeleri* would be similar to

Table 1. Species collected at baits in Punta Onah, Sonora, November 14 -18, 2007.

Species	Number adults
<i>D. mojaviensis</i>	472 <sup>1</sup>
<i>D. arizonae</i>	825 <sup>1</sup>
<i>D. nigrospiracula/mettleri</i>	937 <sup>2</sup>
<i>D. pseudoobscura</i>	28
<i>D. melanogaster/simulans</i>	33
<i>D. hydei</i>	37
<i>D. packia</i>	15
<i>D. spenceri</i> male (?)	2
<i>D. wheeleri</i>	3
<i>D. "light repleta"</i>	11
<i>D. carbonaria</i> (?)	2
<i>Gitona americana</i>	1
<i>Zaprionus indianus</i>	5

<sup>1</sup> includes many aspirated from agria rots

<sup>2</sup> didn't sort all, probably also includes *D. ritae*.

other geographically isolated populations of the same species rather than to a different species. The latter stock has been in culture since January 2005, founded from 7 baited adults and 8 adults eclosed from *Opuntia* rots collected near Little Harbor, Santa Catalina Island from 12/21/04 - 12/23/04 by B. Counterman. We have yet to analyze postmating isolation with *D. aldrichi*, because it is known that fertile female and sterile male hybrids are obtained in both reciprocal crosses (Patterson and

Alexander, 1952). These crosses need to be repeated. Also, no other data on sexual isolation between these two species are available for comparative purposes.

Both populations were mass reared on banana food in 8 dr shell vials in an incubator programmed on a 14:10 LD cycle and 27°C during the day and 17°C at night. All eclosing adults were sexed under CO<sub>2</sub> anesthesia and stored in same sex groups for at least 10-12 days in the same incubator. The day before the mating trials began, these virgins were transferred to food vials containing food coloring so that unmated flies and copulating pairs could be identified later. Food color was alternated between trials. The following day, 10 males and females of each population were introduced into a 50 ml Erlenmeyer flask turned on its side and plugged with cotton. Courtship was observed for 30 min or until 10 copulating pairs were aspirated out of the observation chamber into individual vials in order to identify males and females of each population. A total of 1300 adults were observed in 34 courtship trials performed from June to August 2008 (a few preliminary trials were started with fewer adults). Estimates of sexual isolation and sexual selection were obtained with JMATING (Carvajal-Rodríguez and Rolán-Alvarez, 2006) and associated mating statistic probabilities were generated by 10,000 bootstrap iterations.

Table 2. Numbers of copulations in 34 multiple mating trials with *D. wheeleri* from Punta Onah, Sonora (PO) and Santa Catalina Island, California (SC).

Mating combination				Total
PO♀ X PO♂	PO♀ X SC♂	SC♀ X PO♂	SC♀ X SC♂	
76	51	64	99	290

A total of 290 copulations were observed (Table 2) that yielded an estimate of sexual isolation,  $I_{psi} \pm SD$ , of  $0.206 \pm 0.058$ ,  $P = 0.0002$ . Here, random mating yields a sexual isolation coefficient of zero, while complete assortative mating would be one, so these populations are characterized by weak, yet significant premating isolation. Estimates of sexual selection based on numbers of mated vs. unmated males and females revealed significantly greater mating success of Punta Onah males than Santa Catalina males ( $P = 0.021$ ). Thus sexual isolation of these allopatric populations of *D. wheeleri* is similar to that of inter-population sexual isolation between another Sonoran Desert species, *D. mojavenensis*, from Baja California and mainland Mexico (see Etges, 1992; Etges and Ahrens, 2001).

Thus, morphological similarity and low sexual isolation with California *D. wheeleri* suggest that the rediscovered Sonora *D. wheeleri* is conspecific with it. However, further data are needed on the genetic continuities/discontinuities with *D. aldrichi* populations to the south, just under 200 km from Punta Onah (Jefferson *et al.*, 1974). Recent phylogenetic analysis of *D. wheeleri* and *D. aldrichi* populations based on nuclear and mtDNA gene regions has revealed that California *D. wheeleri* populations are monophyletic, but *D. aldrichi* is a paraphyletic assemblage of at least two lineages in Mexico (Beckenbach *et al.*, 2008; Oliveira *et al.*, 2008). The more basal lineage extends from Oaxaca and Puebla in southern Mexico northeastwards into Texas. This clade also contains the lineage introduced into Australia. The “western” lineage of *D. aldrichi* is a sister group to *D. wheeleri*, and extends northwestwards from Oaxaca and Chiapas into Sonora and southern Baja California making the phylogenetic position of Sonoran *D. wheeleri* of great interest. Paradoxically, the sequence of mtDNA *cox1* of *D. wheeleri* from Sonora has been found to be identical to that of *D. aldrichi* from Las Bocas in southern Sonora (D. Oliveira, unpubl. data). Either this Sonoran *D. wheeleri* is actually *D. aldrichi*, or there may be some introgression with Sonoran *D. aldrichi*. The latter possibility suggests that these two forms are still exchanging genes and would be an ideal system for studying speciation. Also, the line of *D. aldrichi* from Venezuela (see above) is also

included in this poorly resolved “western” clade of *D. aldrichi* based on *cox1* data (D. Oliveira, unpubl. data). Therefore, the identity of our recently discovered “*D. wheeleri*” from Sonora is still tentative, and resolution will require further collections and additional sequence data to resolve the phylogenetic relationships within this species cluster.

Cultures of these species are available from the author, and Sonoran “*D. wheeleri*” has been deposited at the *Drosophila* species stock center.

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### **Isoenzymatic analysis of South American species of the *Drosophila tripunctata* group (Diptera, Drosophilidae).**

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### **Abstract**

The *Drosophila tripunctata* species group is endemic of the Neotropical region, is the second largest Drosophilidae group in this region, and the largest forest group of the genus. The South-Center region of the Parana State, Brazil, is characterized by a wet, cool season, and represents the most preserved redoubt of Araucaria rain forest remnants. In this work we tested four allozyme