

It was previously reported that dietary restriction may disturb sex ratio (och Felix Zajitschek, 2012), as well as diet of females (Hu *et al.*, 2012). Deviation from 1:1 sex ratio observed in our experiment was recorded only for flies maintained on carrot substrate after transferring to the new nutritional environments. Such sex ratio distortion may arise at least partially as a consequence of different sex-specific mortality in earlier developmental stages in flies maintained on carrot, *i.e.*, one sex may be more sensitive to different nutritive conditions during development. This assumption should be further tested in the context of sex-specific nutritional requirements during development and adaptations to new nutritive environments.

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**References:** Dyer, K.A., 2012, *Evolution* 66: 973-984; Fakoorziba, M.R., M.H. Eghbal, and F. Eghbal 2012, *Asian J. Anim. Vet. Adv.* 7: 898-903; Filipović, Lj., S. Pavković-Lučić, and T. Savić 2014, V Congress of the Serbian Genetic Society, Kladovo, Serbia, Book of Abstracts pp. 166; Grechanyĭ, G.V., and M.V. Pogodaeva 1996, *Genetika* 32: 1349-1353; Hamilton, W.D., 1967, *Science* 156: 477-488; Hu, H.Y., Z.Z. Chen, B.S. Duan, J.T. Zheng, and T.X. Zhang 2012, *Revista Brasileira de Entomologia* 56: 259-262; Ivanov, Y.N., 2002, *Dros. Inf. Serv.* 85: 96-105; Jaenike, J., 2001, *Annu. Rev. Ecol. Syst.* 32: 25-49; James, A.C., and J. Jaenike 1990, *Genetics* 126: 651-656; Kekić, V., and S. Pavković-Lučić 2003, *Dros. Inf. Serv.* 86: 147; Long, T.A.F., and A. Pischedda 2005, *Proc. Biol. Sci.* 272: 1781-1787; Montchamp-Moreau, C., and D. Joly 1997, *Heredity* 79: 24-30; och Felix Zajitschek, A.M., 2012, Degree project of biology, Master of Science. Uppsala Universitet; Price, T.A.R., Z. Lewis, D.T. Smith, G.D.D. Hurst, and N. Wedell 2009, *Evolution* 64: 1504-1509; Robinson, S.P., L.W. Simmons, and W.J. Kennington 2014, *B.M.C. Evol. Biol.* 14: 51; Santos, M., K. Fowler, and L. Partridge 1994, *Heredity* 72: 515-521; Székely, T., F.J. Weissing, and J. Komdeur 2014, *J. Evol. Biol.* 27: 1500-1512.



### **Reanalysis of polytene chromosomes in *Drosophila mojavensis* populations from Santa Catalina Island, California, USA.**

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One of the four major geographical and host plant associated population groups comprising *Drosophila mojavensis* resides on Santa Catalina Island, California (Heed, 1982; Ruiz *et al.*, 1990; Wasserman, 1992; Etges *et al.*, 1999). Host cacti used include *Opuntia littoralis*, *O. oricola*, and *O. demissa* (*O. oricola* × *O. ficus-indica* hybrids) (Barbour *et al.*, 2007; Beckenbach *et al.*, 2008) as other mainland hosts are absent on Santa Catalina Island. Based on initial observations of polytene chromosomes from larvae of a moderate (n = 30) number of wild-caught females in 1981, these flies were reported to be homokaryotypic for second chromosome 2abcfghqrs (ST) and third chromosome 3abd (ST) similar to mainland California populations in the Mojave Desert (Ruiz *et al.*, 1990).

In recent analyses of chromosomal evolution using the sequenced genome of Santa Catalina Island *D. mojavensis* (*Drosophila* 12 Genomes Consortium, 2007) and the recently sequenced *D. buzzatii* genome (Guillén, 2014; Guillén *et al.*, submitted), inversion breakpoint analyses of the third chromosome suggested that these Santa Catalina Island flies were actually homozygous for an alternate gene arrangement 3f<sup>2</sup> (MU = Mulege). Here we analyzed the karyotypes of the sequenced strain from Santa Catalina Island provided by the UC San Diego *Drosophila* Species Stock Center, stock number 15081-1352.00 and another stock collected from Santa Catalina Island in 2004 by Brian Counterman (SC05) derived from 113 wild-caught adults, including 63 adults reared from *Opuntia* cactus rots. We also made a series of crosses with other populations and conclude that the third chromosome in Santa Catalina Island populations of *D. mojavensis* is uniformly homozygous for gene arrangement 3f<sup>2</sup> (MU).

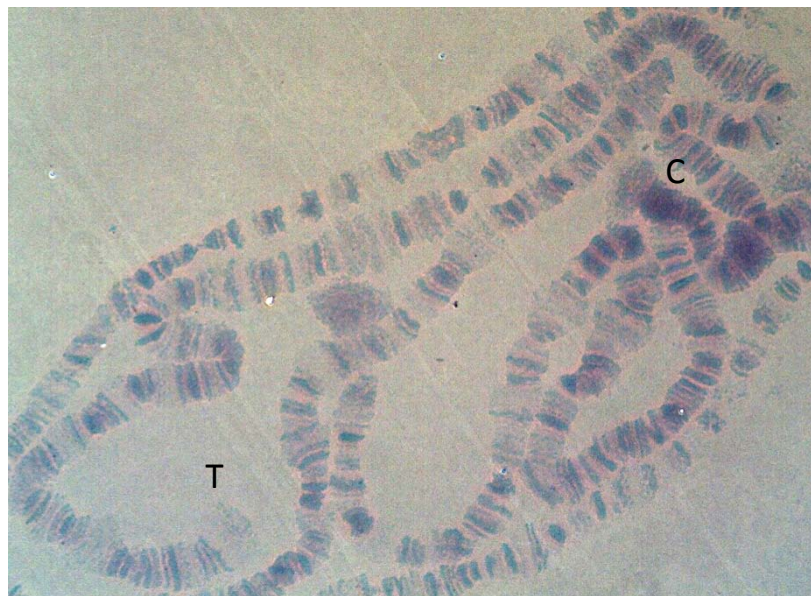


Figure 1. Chromosome 2 ST/ST from Santa Catalina Island. T: Telomere; C: Centromere.

We analyzed 10 larvae from the genome strain. They all had the same karyotype, *i.e.*, homozygous for arrangements 2ST and  $3f^2$  (Figures 1, 2; Table 1). Karyotypes of another 30 larvae were analyzed from the more recently collected SC05 stock and revealed the same chromosome configuration, *i.e.*, all were 2ST/ST  $3f^2/f^2$  homozygotes (Table 1). Confirmation that Santa Catalina Island  $3f^2$  chromosomes were the same

as those observed in other parts of the species range was shown in Santa Catalina Island  $\times$  Punta Prieta reciprocal crosses where third chromosome homozygotes were completely syntenic (Figure 3).

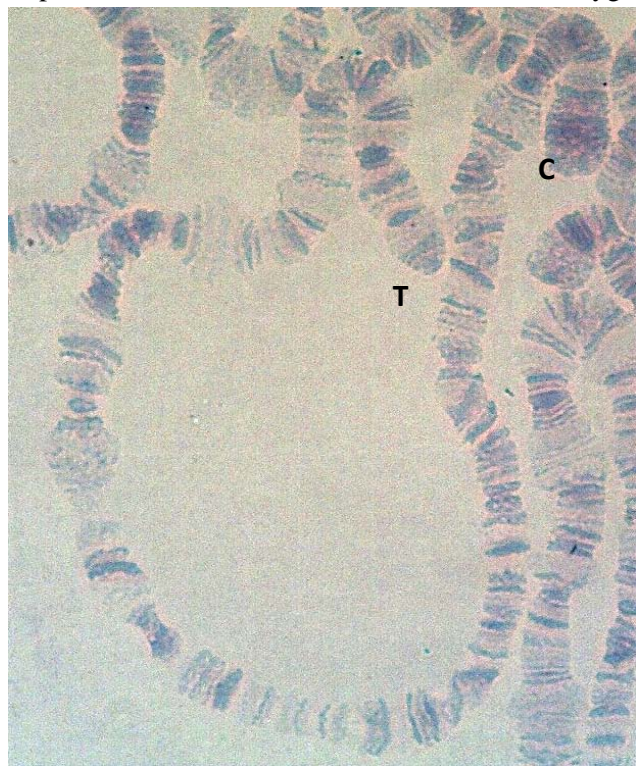


Figure 2. Chromosome  $3f^2/f^2$  from Santa Catalina Island. T: Telomere; C: Centromere.

The identity of  $3f^2$  was also confirmed by comparing the Santa Catalina Island chromosome 3 with a drawing (Figure 4) of a chromosome  $3f^2$  (MU) from stock A564 Santa Rita, Baja California made in 1983 by A. Ruiz.

The other stocks used were: A900 from Santa Rosa Mountains, Arizona; A997 from the Providence Mountains, eastern Mojave Reserve, California; A975 from S. Bahía de Concepción, Baja California; A976 from Santiago BCS, Baja California; PP08 from Punta Prieta, Baja California; LB09 from Las Bocas, Sonora; and PO88 from Punta Onah, Sonora.

We analyzed 4 larvae from strain A975. They were all homozygous 2ST/ST and 3ST/ST. Then we made crosses with 10 virgin females from Santa Catalina Island and 10 males from each of the

other stocks of *D. mojavensis*. For the crosses SC05  $\times$  Punta Prieta and SC05  $\times$  Providence Mtns., we made reciprocal crosses with 10-20 adults of each sex. We cytologically analyzed the larvae of the  $F_1$  and the results are shown in Table 1. A  $2q^5$ /ST heterozygote ( $2q^5$  = LP, the La Paz gene arrangement) is shown in Figure 5.  $3f^2$ /ST heterokaryotypes from two different crosses with Santa Catalina Island are shown in Figure 6.

## Discussion

Based on previous cytological evidence (Ruiz *et al.*, 1990), the sequenced genome of Santa Catalina Island *D. mojavensis* was considered to correspond to the ST arrangements on both chromosomes 2 and 3



(Schaeffer *et al.*, 2008). However, our reanalysis of *D. mojavensis* populations on Santa Catalina Island, California, shows that they are homozygous for second chromosome gene arrangement ST and third chromosome gene arrangement  $3f^2$ . Thus, the *D. mojavensis* third chromosome drawing shown in Figure 12 of Schaeffer *et al.* (2008) does not actually correspond to the photomap depicted underneath.



Figure 3. Third chromosome  $3f^2/f^2$  homozygotes from the cross Santa Catalina Island  $\times$  Punta Prieta (PP08), Baja California showing complete synteny. T: Telomere; C: Centromere.

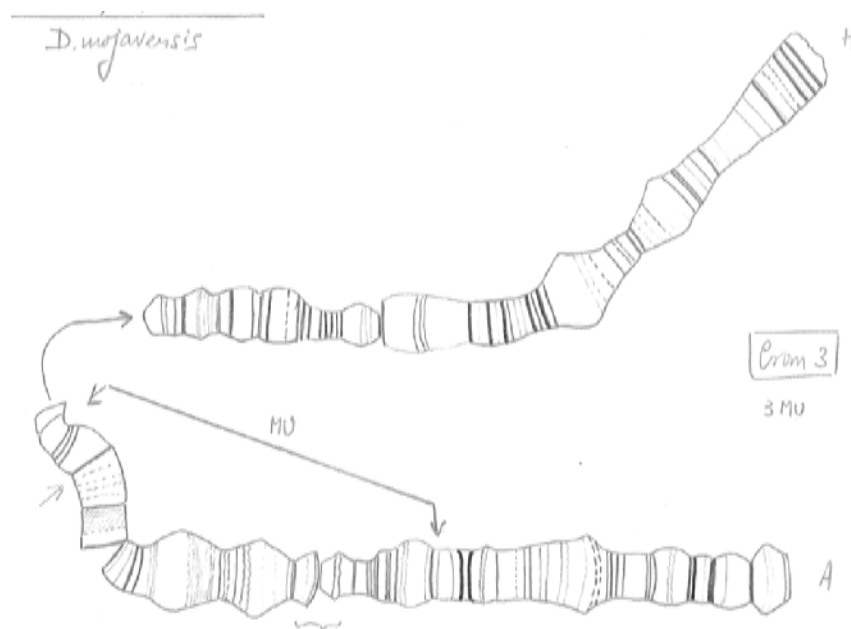


Figure 4. Chromosome  $3f^2$  (MU) from A564 Santa Rita, Baja California.

As previous surveys have suggested that all mainland California, Mojave Desert populations, as well as those in southern Arizona are homozygous 3ST/ST or nearly so (Etges and Heed, 1987; Ruiz *et al.*, 1990; Etges *et al.*, 1999), our

observations suggest a Baja California origin of the Santa Catalina insular populations rather than mainland California. Northern Baja California *D. mojavensis* populations are polymorphic for  $3f^2$  and 3ST, but are currently limited further northwards due to the distribution of its host plant, agria cactus, *Stenocereus gummosus*. No coastal, *Opuntia*-using populations of *D. mojavensis* are currently known in southern California. It is also possible that mainland California and Arizona populations were more polymorphic for the third chromosome in the past and, after colonization of Santa Catalina Island, underwent a reduction in inversion polymorphism with the island founders subsequently going to fixation for  $3f^2$ . Further, statistical parsimony analysis of mtDNA COI sequence variation suggested Baja California as the probable source of Santa Catalina Island *D. mojavensis* populations (Richmond *et al.*, 2013).

Our remaining observations about karyotypic diversity among populations of *D. mojavensis* are largely in agreement with past surveys. The following observations are tentative, however, due to moderate sample sizes.  $F_1$  larvae from crosses involving mainland Sonora populations, *i.e.*, SCI  $\times$  LB09 and SCI  $\times$  PO88, were all heterozygous for chromosome 2,  $2q^5/ST$  and 3 chromosome 3,  $3f^2/ST$ , indicating that these stocks are likely homozygous for  $2q^5$  (LP) and 3ST. The cross involving an Arizona population SCI  $\times$  A900

produced F<sub>1</sub> larvae with both 2q<sup>5</sup>/ST and 2ST/ST, but all were 3f<sup>2</sup>/ST suggesting this stock is fixed for 3ST but still segregating for second chromosome arrangements 2ST and 2q<sup>5</sup>. This stock was collected and karyotyped in 1975 and 1985 and was fixed for 2ST in both collections (Etges and Heed, 1987) suggesting possible laboratory contamination.

Table 1. Chromosomal constitution of *Drosophila mojavensis* stocks and the progeny in crosses in this study.

| Populations  | Chr 2               | Chr 3                           | n <sup>1</sup> |
|--|---------------------|---------------------------------|----------------|
| Santa Catalina Island - 15081-1352.00                        | 2ST/ST              | 3f <sup>2</sup> /f <sup>2</sup> | 10             |
| Santa Catalina Island - SC05                                 | 2ST/ST              | 3f <sup>2</sup> /f <sup>2</sup> | 30             |
| South of Bahía de Concepción, Baja California - A975         | 2ST/ST              | 3ST/ST                          | 4              |
| Providence Mountains, CA - A997                              | 2ST/ST              | 3ST/ST                          | 28             |
| Crosses  |                     |                                 |                |
| Santa Catalina Island × Las Bocas, Sonora - LB09             | 2q <sup>5</sup> /ST | 3f <sup>2</sup> /ST             | 19             |
| Santa Catalina Island × Santa Rosa Mountains, AZ - A900      | 2q <sup>5</sup> /ST | 3f <sup>2</sup> /ST             | 7              |
|  | 2ST/ST              | 3f <sup>2</sup> /ST             | 3              |
| Santa Catalina Island × Punta Onah, Sonora - PO88            | 2q <sup>5</sup> /ST | 3f <sup>2</sup> /ST             | 10             |
| Santa Catalina Island × S. Bahía de Concepción, BC - A975    | 2ST/ST              | 3f <sup>2</sup> /ST             | 5              |
| Santa Catalina Island × Santiago, Baja California Sur - A976 | 2q <sup>5</sup> /ST | 3f <sup>2</sup> /ST             | 8              |
| Santa Catalina Island × Punta Prieta, Baja California - PP08 | ??                  | 3f <sup>2</sup> /ST             | 4              |
|  |                     | 3f <sup>2</sup> /f <sup>2</sup> | 10             |
| Santa Catalina Island × Providence Mountains, CA - A997      | 2ST/ST              | 3f <sup>2</sup> /ST             | 15             |

<sup>1</sup> number of larvae karyotyped.



Figure 5. An F<sub>1</sub> heterozygous 2q<sup>5</sup>/ST karyotype from the cross SCI × PO88.

In Baja California stocks, all four larvae from South of Bahía de Concepción, Baja California (A975) were homozygous for 2ST and 3ST, and all SCI × A975 F<sub>1</sub>s were homozygous for 2ST and heterozygous for (3f<sup>2</sup>/ST) suggesting this stock is homozygous for 2ST and 3ST. Offspring of wild-caught females from this region in Baja California collected in 1971 and 1974 (Johnson, 1980) were polymorphic for 2ST, 2q<sup>5</sup> (LP), and SL (San Lucas gene arrangement) suggesting the A975 stock has lost polymorphism in laboratory culture. Similarly, F<sub>1</sub> larvae from crossing SCI to the stock derived from Santiago, Baja California Sur (A976) were all chromosome 2 heterozygotes, 2q<sup>5</sup>/ST, and chromosome 3 heterozygotes, 3f<sup>2</sup>/ST, suggesting this stock is fixed for 2q<sup>5</sup> and 3ST. However, a collection of wild flies in 1982 revealed that the

frequency of  $2q^5 = 0.98$  and  $3f^2 = 0.94$ ,  $n = 82$  (Etges *et al.*, 1999) suggesting that our sample sizes were low, and inversion frequencies have changed in culture over many years.



Figure 6.  $F_1$  heterozygous  $3f^2/ST$  karyotypes from A. SCI  $\times$  LB09, and B. SCI  $\times$  Providence Mtns. crosses. T: Telomere; C: Centromere.

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