Table 1. Number of substitutions per site according to the Kimura's two-parameters model.

		T4		T8	
		D. mel Toonda	*D. sim Leticia*	*D. mel Canton S*	*D. sim CA-1*
T8	*D. mel Toonda*	0.0000	0.0408	0.6163	0.6566
	D. sim Leticia		0.0000	0.6064	0.6363
T4	*D. mel Canton S*			0.0000	0.0312
	D. sim CA-1				0.0000

The estimate of the duplication time using Li and Graur's method was 50 MYA (Figure 1). Since most of the estimates consider that *Drosophila* subgenus diverged from the *Sophophora* subgenus 40 MYA, the duplication of the ancestral gene happened likely before the splitting.

To carry out studies about the role of these genes in other species of *Drosophila*, this result should be taken into account.

References: Cariou, M.L., 1987, Genet. Res. 50: 181-185; Hilton, H., R.M. Kliman and J. Hey 1994, Evolution 48: 1900-1913; Li, W-H., and D. Graur 1991, *Fundamentals of Molecular Evolution*, Sinauer Associates, Inc.; Villares, R., and C.V. Cabrera 1987, Cell 50: 415-424.

Noor, Mohamed A. F. Section of Genetics and Development, Cornell University, Ithaca, NY 14853. Courtship songs: a noninvasive method of identifying North American *obscura*-subgroup *Drosophila* males in field collections.

After collecting *obscura*-subgroup *Drosophila* flies along the west coast of North America, one is faced with a challenge when identifying the species. Three of the native North American species are morphologically identical: *Drosophila pseudoobscura*, *D. persimilis*, and *D. miranda*. Generally, females are reared in the laboratory, and their offspring are identified using chromosome squashes or allozymes (Anderson *et al.*, 1977). However, wild-caught males are often not identified and are listed as "*pseudoobscura/ persimilis/ miranda*" in most publications. I report here that male courtship songs can be used to unambiguously identify the species of captured males. Previous studies have noted differences between *D. pseudoobscura* and *D. persimilis* in courtship song elements (Waldron, 1964; Ewing, 1969; Noor and Aquadro, in press), and here I show both that the song of *D. miranda* differs from that of the other two species and that songs can be used to reliably determine the species of wild-caught *obscura*-subgroup *Drosophila* males.

I reared laboratory lines of *D. pseudoobscura* (Flagstaff) and *D. persimilis* (Mount St. Helena) on cornmeal/yeast/agar medium at 21°C. These stocks have been used extensively in laboratory behavioral investigations (e.g., Noor, 1996). Two isofemale lines of *D. miranda* (Mather and Mount St. Helena) were also cultured, both only 2 generations removed from the wild. Individual males from these lines were then paired with conspecific females and recorded in an Insectavox (Gorczyca and Hall, 1987). Courtship songs were analyzed using CANARY (Cornell University Laboratory of Ornithology) software. Interpulse interval (IPI) was defined as the length of time from the beginning of one sound pulse to the beginning of the next in milliseconds. Intrapulse frequency is the frequency of sound within each song pulse in cycles per second. Table 1 presents the results with their standard errors. *D. miranda* strains have a lower intrapulse frequency and a longer interpulse interval than either *D. pseudoobscura* or *D. persimilis* (see Figure 1). The IPI and frequency observed in the *D. pseudoobscura* and *D. persimilis* song are perfectly consistent with those observed in previous studies (Waldron, 1964; Ewing, 1969; Noor and Aquadro, in press). These two song characters can be used together to unambiguously assign wild-caught males to species, but it is important to note the strong temperature-dependence of these phenotypes (particularly IPI- see Noor and Aquadro, in press). Correspondingly, if one is using an Insectavox, one should switch the inside light off, as this light can heat the box substantially, sometimes causing an overlap in song elements between individuals of different species recorded at different times (slight song overlap observed by Noor and Aquadro (in press) in *D. pseudoobscura* and *D. persimilis* resulted from this heating). An individual of known species should be recorded under the same conditions and at the same time to correct for environmental effects.

I used this technique to identify 19 *obscura-subgroup Drosophila* males captured at Mather, California, in June, 1997. These males were brought to the laboratory, isolated for 1 day

Table 1. Courtship song parameters

Recording temp.	Species	IPI (ms)	Frequency (cy/sec)
14°C	*D. pseudoobscura*	57.9 ± 0.7	191 ± 5
14°C	*D. persimilis*	70.0 ± 0.7	300 ± 9
14°C	*D. miranda* (Mather)	116.9 ± 7.4	136 ± 4
19°C	*D. pseudoobscura*	37.7 ± 0.2	213 ± 2
19°C	*D. persimilis*	57.6 ± 1.2	403 ± 12
19°C	*D. miranda* (MSH)	67.7 ± 2.0	155 ± 2

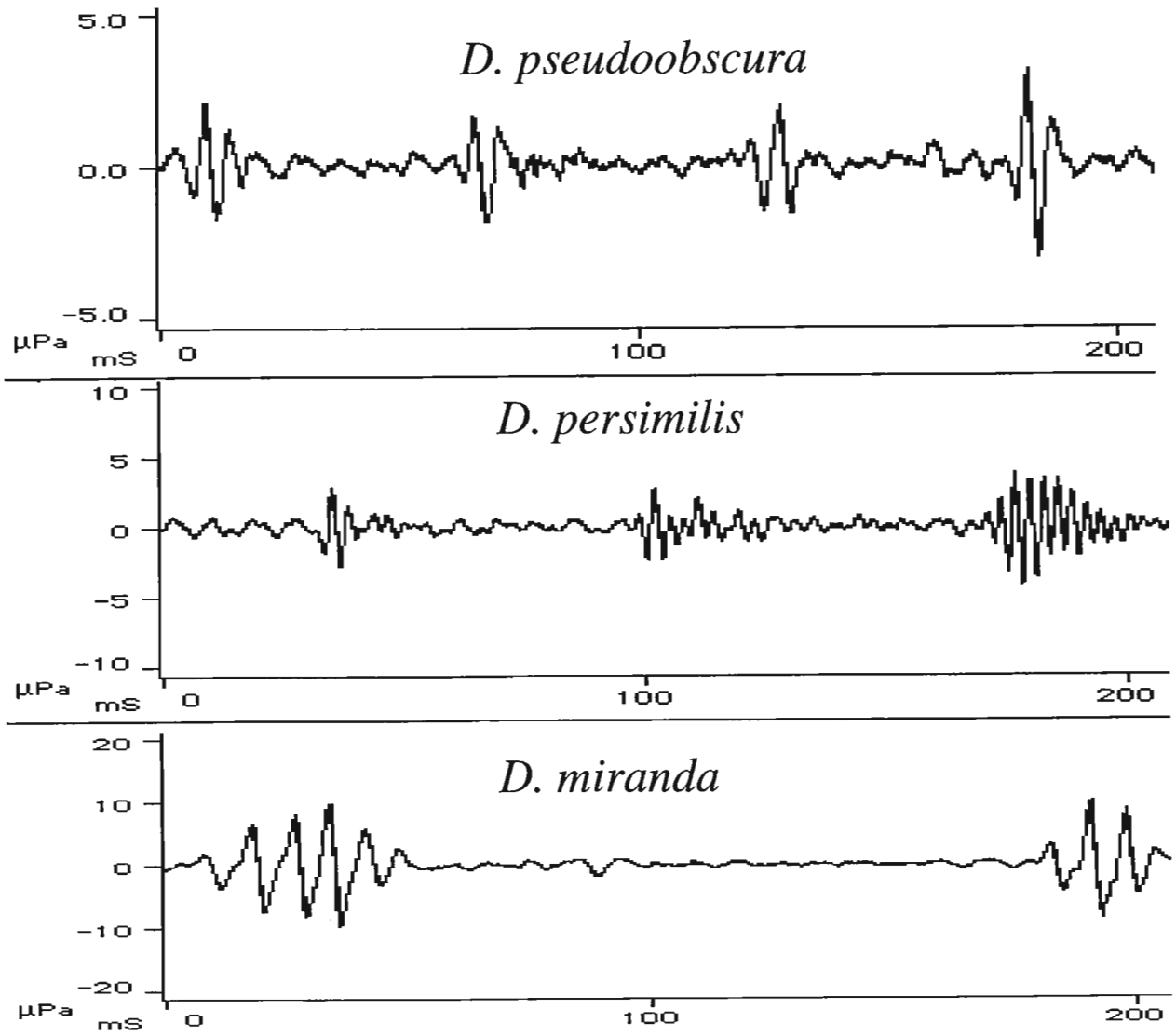

Figure 1.

to prevent crowding-induced courtship inhibition (Noor, 1997), and paired with females in an Insectavox. Figure 2 shows the plot of their mean courtship song IPI and frequency. It is clear that one individual has a lower IPI and frequency than the others. Hence, this individual is likely a *D. pseudoobscura* male. No individuals have very long IPI's but short frequencies, suggesting that there were no *D. miranda* males captured at this time. These data accord with the expected species proportions from previous collections at Mather (Noor, 1995), suggesting the validity of this technique of species identification. I further tested this technique on male offspring from 24 wild-caught females from Mather and Mount St. Helena, California. This technique accurately identified all the males as *D. pseudoobscura* or *D. persimilis*, as shown by subsequent crosses.

Courtship songs can thus be used to determine the identity of wild-caught North American *obscura*-subgroup *Drosophila* males, and may also be used for identifying females if their male offspring are cultured. This method is superior to allozymes and chromosome squashes in that the fly in question does not need to be injured to determine the species identity, and the techniques are both simple and inexpensive once an Insectavox is obtained.

References: Anderson, W.W., F.J. Ayala, and R.E. Michod 1977, J. Hered. 68:71-74; Ewing, A.W., 1969, Anim. Behav. 17:555-560; Gorczyca, M., and J.C. Hall 1987, Dros. Inf. Serv. 66: 157-160; Noor, M.A.F., 1995, Pan-Pacif. Entomol. 71:71-74; Noor, M.A.F., 1996, Anim. Behav. 52:1205-1210; Noor, M.A.F., 1997, J. Insect Behav. 10:305-312; Noor, M.A.F., and C.F. Aquadro, *in press*, Anim. Behav.; Waldron, I., 1964, Science 144:191-193.

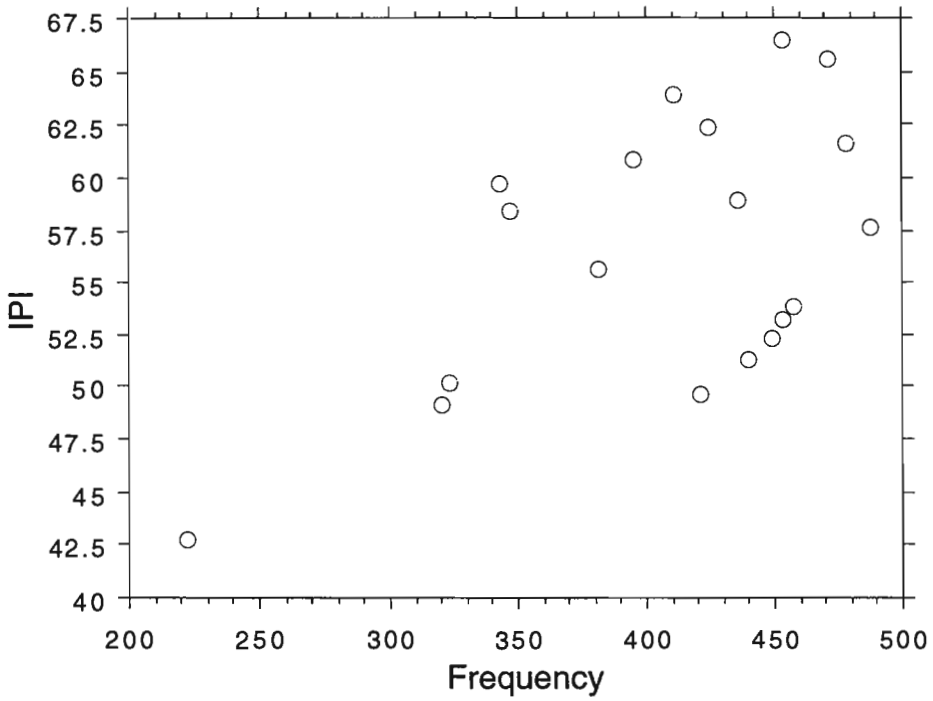

Figure 2. Plot of courtship song elements of wild-caught flies from Mather, California.

Noor, Mohamed A.F.[1], **John R. Wheatley**[2], **Kris A. Wetterstrand**[1], **and Hiroshi Akashi**[3]. [1]Section of Genetics and Development, Cornell University, Ithaca, NY 14853. [2]Department of Psychology, Indiana University, Bloomington, IN 47405. [3]Section of Evolution and Ecology, University of California, Davis, CA 95616. Western North America *obscura*-group *Drosophila* collection data, summer 1997.

We report here our collection data for *obscura*-group *Drosophila* species in Utah, Arizona, and California. Most notable is the rapid introduction and/or rise in frequency of *D. subobscura* in central Utah. Our collection in 1993 at this site in Utah yielded only *D. pseudoobscura*, suggesting this introduction is very recent. Also noteworthy is the drop in the relative abundance of *D. azteca* after a steady increase over several years (Noor, 1995). Finally, four of the ten *D. persimilis* females captured at Mount St. Helena appear to have been inseminated by males possessing the sex-ratio gene arrangement, hence producing all female offspring. In contrast, only 1 of the 30 *D. persimilis* females captured at Mather had been inseminated by an apparently sex-ratio male.

Obscura-group *Drosophila* were collected from five sites in the western United States in June/July, 1997:

American Fork, Utah- 40°26.71'N, 111°42.74'W- July 9-10, flies were collected from the Uinta National Forest in American Fork Canyon. (AFC)

Flagstaff, Arizona- 34°56.58'N, 111°29.53'W- June 20-22, flies were collected from the immediate vicinity of Mormon Lake. (FLAG)

Mather, California- 37°53.12'N, 119°50.78'W- June 26-29, flies were collected immediately outside the cabin maintained by the Carnegie Institute of Washington. (MATHER)

Mount St. Helena, California- 38°39.18'N, 122°35.96'W- July 1-3, flies were collected at Robert Louis Stevenson state park north of Calistoga. (MSH)

Paradise, California- 39°46.20'N, 121°37.58'W- July 6-7, flies were collected from Bille Park. (PARA)

We used buckets of fermenting bananas to attract flies for capture. These buckets were left out of doors overnight, and fresh bananas were added to the fermenting bananas daily. Males were identified to species using morphological criteria (Beckenbach and Prevosti, 1986), and females were identified using chromosome squashes of