

an unfavourable environment, producing smaller adults because the feeding time of the larvae was curtailed. In these situations, conclusions about the interaction must be subjective. A reduction in body size may lower the reproductive output of the female but, when considering animals whose natural habitat is ephemeral and unpredictable, a reduction in development time may represent an important facilitative effect.

The interaction which occurs between two species can be very specific to a given set of conditions (Thompson, 1988; Dunson and Travis, 1991), and it appears that describing the interaction between these two *Drosophila* species in a single manner is almost meaningless (see Arthur, 1986; Hodge, 1995). Compared to variability in nature, only a narrow band of different environments have been used here and these produced four of the six theoretical outcomes between a pair of interacting species. Experiments such as this one produce useful information on the possible range of interactions that can occur between two species and may aid in clarifying the mechanisms via which the interspecific effects are produced (see Tilman, 1987). It is then desirable to put the results into a more realistic context and determine which scenarios are most likely to occur under natural conditions.

References: Arthur, W., 1980, *Biological Journal of the Linnean Society* 13:109-118; Arthur, W., 1986, *Philosophical Transactions of the Royal Society, Series B.* 313:471-508; Arthur, W., and S. Cassey 1992, *Ecological Entomology* 17:354-358; Ayala, F.J., 1966, *American Naturalist* 100:81-83; Dunson, W.A., and J. Travis 1991, *American Naturalist* 138:1067-91; Hodge, S., 1995, *Interspecific facilitation in Drosophila systems*. Unpublished PhD thesis, University of Sunderland, UK; Hodge, S., and N. Wilson 1997, *The Entomologist* 116:93-103; Merrel, D.J., 1951, *American Naturalist* 85:159-169; Miller, R.H., 1954, *Dros. Inf. Serv.* 28: 137; Mitchell, P., and W. Arthur 1990, *Journal of Animal Ecology* 59:121-133; Moore, J.A., 1952, *Evolution* 6:407-420; Moth, J.J., and J.S.F. Barker 1976, *Oecologia* 23:151-164; Park, T., 1954, *Physiological Zoology* 27:177-238; Thompson, J.N., 1988, *Annual Review of Ecology and Systematics* 19:65-87; Tilman, D., 1987, *American Naturalist* 129:769-774.

Gandarela, Manuel R., and Emilio Valadé. Dpto. de Biología Fundamental, Facultad de Biología, Universidad de Santiago de Compostela, Spain. Estimation of duplication time between genes *scute* and *asense*.

characteristic of a family of transcriptional regulators. Their products confer on cells the capacity to become neural precursors. Besides its neurogenic function, *sc* is also involved in the establishment of the X:A ratio.

It is possible to estimate the date of the duplication event which gave rise to these two members of the gene

The *achaete-scute* gene complex (AS-C) is involved in the development of sensory organs and the central nervous system of *Drosophila*. The AS-C is a gene family containing four genes with neurogenic functions: *achaete* (*ac*), *scute* (*sc*), *lethal of scute* (*l'sc*) and *asense* (*ase*). AS-C genes encode related proteins containing the basic-helix-loop-helix (bHLH) domain

family. Li and Graur (1991) describe a method to estimate the duplication time of two paralogous genes from the sequences of these two genes from two species when the divergence time between these species is known. In this work we give an estimation of the duplication time between *sc* and *ase*.

We amplified by PCR and sequenced a conserved region of *sc* gene from one strain of *D. melanogaster* (Toonda, Australia) and another one of *D. simulans* (Leticia, Colombia). To estimate the duplication time we included two sequences of *ase* obtained from literature: *D. melanogaster* Canton S (Villares and Cabrera, 1988) and *D. simulans* CA-1 (Hilton *et al.*, 1994). We used 3 million years ago (MYA) as the time of divergence between *D. melanogaster* and *D. simulans*. This value is an average of several estimates based on paleobiogeographic, allozymic, immunological and nucleotide data (Cariou, 1987).

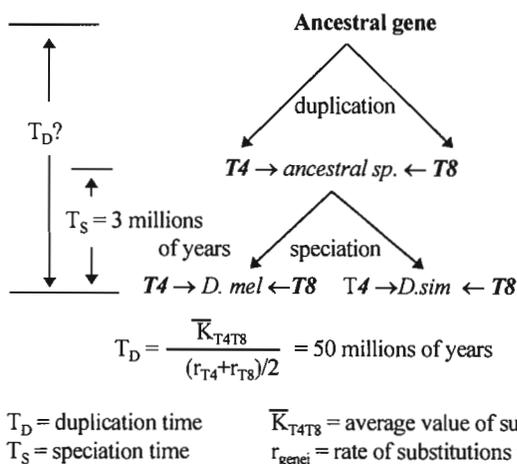


Figure 1. Model for estimating the time of the gene duplication event (Li and Graur, 1991). The matrix of Kimura's two-parameters distances (Table 1) were used to estimate T_D . We consider 3 MYA as the time of speciation between *D. melanogaster* and *D. simulans* (Cariou, 1987).

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

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

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.

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

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.

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

Table 1. Courtship song parameters

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