The work reported in this paper has been supported in part by a grant from the Ministry of Education and Sciences of Spain to A. M. González.

Literature Cited

Ayala, F. J., and J. R. Powell. 1972. Allozymes as diagnostic characters of sibling species of *Drosophila*. Proc. Nat. Acad. Sci USA 69:1094–1096.

Ayala, F. J., J. R. Powell, M. L. Tracey, C. A. Mourão, and S. Pérez-Salas. 1972. Enzyme variability in the *Drosophila willistoni* group III. Genic variation in natural populations of *Drosophila willistoni*. Genetics 70:113–139.

Barker, J. S. F., and J. C. Mulley. 1976. Isozyme variation in natural populations of *Drosophila buzzatii*. Evolution 30:213–233.

Brewer, G. J. 1970. An Introduction to Isozymes Techniques. Academic Press, N.Y.

Cabrera, V. M., A. M. González, and A. Gullón. 1980. Enzymatic polymorphism in *Drosophila subobscura* populations from the Canary Islands. Evolution 34:875–887.

Cseko, Y. M. T., N. A. Dower, P. Minoo, L. Lowenstein, G. R. Smith, J. Stone, and R. Sederoff. 1979. Evolution of polypyrimidines in *Drosophila*. Genetics 92:459–484.

David, J., F. Lemeunier, L. Tsacas, and C. Bocquet. 1974. Hybridisation d'une nouvelle espèce, *Drosophila mauritiana* avec *D. melanogaster* et *D. simulans*. Ann. Génét. 17:235–241.

Eisses, K. T., H. van Dijk, and W. van Delden 1979. Genetic differentiation within the *melanogaster* species group of the genus *Drosophila* (Sophophora). Evolution 33:1063–1068.

Lemeunier, F., and M. Ashburner. 1976. Relationships within the *melanogaster* species subgroup of the genus *Drosophila* (Sophophora). II Phylogenetic relationships between six species based upon polytene chromosome banding sequences. Proc. Roy. Soc. London (B) 193:275–294.

Loukas, M., S. Tsakas, C. B. Krimbas, E. Zouros, E. Diamantopoulou-Panopoulou, and V. Alevizos. 1974. Appendix to E. Zouros et al. Genetics 78:1223–1244.

Mulley, J. C. 1973. Electrophoretic detection of pyranosidase in *Drosophila buzzatii*. Dros. Inf. Serv. 50:139.

Nei, M. 1971. Interspecific gene differences and evolutionary time estimated from electrophoretic data on protein identity. Amer. Natur. 105:385–398.

———. 1972. Genetic distance between populations. Amer. Natur. 106:283–292.

Shaw, C. R., and R. Prasad. 1970. Starch gel electrophoresis of enzymes. A compilation of recipes. Biochem. Genet. 4:297–320.

Tsacas, L., and J. David. 1974. *Drosophila mauritiana* n. sp. du groupe *melanogaster* de l'Ile Maurice (*Dipt. Drosophilidae*). Bull. Soc. Entomol. France 79:42–46.

Corresponding Editor: G. B. Johnson

GENETIC DISTANCE IN THE SIBLING SPECIES *DROSOPHILA MELANOGASTER, DROSOPHILA SIMULANS* AND *DROSOPHILA MAURITIANA*

A. M. González, V. M. Cabrera, J. M. Larruga, and A. Gullón
Department of Genetics, Faculty of Biology, University of La Laguna, Tenerife, Canary Islands, Spain

Received April 7, 1980. Revised August 10, 1981

Within the *melanogaster* subgroup the species ***Drosophila melanogaster, D. simulans*** and *D. mauritiana* are the most intimately related and laboratory studies have demonstrated that reproductive isolation among them is incomplete (David et al., 1974). This great similarity is also reflected at the cytological and molecular levels. Thus, Lemeunier and Ashburner (1976) in a study of phylogenetic relationships in ihe ***melanogaster*** subgroup, based on sequences of polytene chromosomes, consider ihem to be closely related chromosomally, the *simulans-mauritiana* pair being homosequential. In a study of seven species of the *melanogaster* group, whose members were compared by the differences in the electrophoretic mobility of 18 enzymatic loci, Eisses et al. (1979) found the three possible pairs of these three species to he identical since they do not point out a closer relationship between any two of them.

Likewise, Cseko et al. (1979) in a comparative study of a highly repetitive DNA sequence containing long tracts of polypyrimidine/polypurine DNA, in which 101 species of *Drosophila* were compared, found that within the *melanogaster* subgroup the only species possessing the *melanogaster* sequence are *simulans* and *mauritiana*, although the amount of this sequence varies between them.

It is generally accepted that estimations of genetic differences between populations of the same or different species should be based on the comparison of allelic frequencies nf the largest possible number of loci. The presenl work attempts lo estab-

nogaster subgroup, *D. **melanogaster**, D. simulans* and *D. **mauritiana***, by means of the electrophoretically detected allelic frequencies of 55 loci common to these three species, with the aim of determining the phylogenetic relationship among them.

Material and Methods

Data on *D. **melanogaster*** were obtained from samples of two natural populations, one captured in the locality of Güímar (on the island of Tenerife in the Canarian Archipelago) and the other in the locality of Fernán Nuñct, in the South of the Iberian Peninsula. Those of *D. **simulans*** were obtained from samples of two natural populations, one also collected in Güímar, and the other in Villares, very close to Fernán Nuñez. The males captured were immediately used for electrophoretic analyses. Each female was placed in an individual culturc for five days and was then transferred into fresh vials three consecutive tima in order to obtain abundant offspring. One F_1 larva, pupa or adult from cach was later assayed. For each locus a mínimum of 120 genomes was analyzed. The study of *D. **mauritiana*** was done on individuals of two laboratory strains sent to us in 1978 by Dr. J. McDonald of the Department of Genetics of the University of Melbourne (Australia), and by Dr. L. Tsacas, of the Departmtnt of Genetics of tht C.N.R.S. at Gif-sur-Yvette (France), and kept since then in large population cages. For each locus a minimum of 40 genomes were analyzed.

Preparation of samples, electrophoretic techniques and the preparation of the

the polyacrylamide gels the same trays were used as for starch gels, conveniently sealed so as to facilitate polymerization; the gels were of a continuous grade and in all cases the proportion of bisacrylamide was 5%. The buffers and staining methods were those described in the following references with some minor modifications.

The loci analyzed in starch were Acid phosphatase (*Acph*); Adenylate kinase, two loci (*Ak-2* and *3*); Alcohol dehydrogenase (*Adh*); Aldolase (*Ald*); Aldehyde oxidase (*Ao*); Alkaline phosphatase, three loci (*Aph-3, 4* and *7*); Catalase (*Cat*); Fumarase (*Fum*); Glutamate oxaloacetate transaminase, two loci (*Got-1* and *2*); α-Glycerophosphate dehydrogenase (*α-Gpdh*); Hydroxybutyrate dehydrogenase (*Hbdh*); Hexokinase, three loci (*Hk-1, 3* and *5*); Isocitrate dehydrogenase (*Idh*); Leucine aminopeptidase (*Lap-2*); Malate dehydrogenase (*Mdh*); Malic enzyme (*Me*); Octanol dehydrogenase (*Odh*); 6-Phosphogluconate dehydrogenase (*6-Pgdh*); Phosphoglucomutase (*Pgm*); Phosphohexoseisomerase (*Phi*); Tetrazolium oxidase (*To*); and Xanthine dehydrogenase (*Xdh*). The references for these enzymes are cited in Cabrera et al. (1980). Four diaphorase loci (*Dia-1, 2, 3* and *4*) were run according to Brewer (1970). The tampon system B of Ayala et al. (1972) was used for three peptidase loci (*Pep-1, 2* and *3*), using as dye 3-amine-9-ethylcarbazole (2M) dissolved in N-N-dimethyl formamide. Pyranosidase (*Pyr*) was run according to Mulley (1973).

The loci analyzed in acrylamide were Amylase (*Amy*) according to Barker and Mulley (1976); Glucose-6-phosphate dehydrogenase (*Gόpdh*), as in Cabrera et al. (1980); Tyrosinase (*Tyr*) according to Shaw and Prasad (1970); Larval proteins, nine loci (*Pt-1, 2, 4, 6, 7, 8, 10, 12* and *15*), according to Loukas et al. (1974). Esterase, seven loci (*Est-4, 6, 8, 11, 12, 13*, and *15*): *Est-4* and *6* have been assayed in acrylamide gels at 5%; the remaining esterases have been assayed at 7%. The buffer employed for the esterases has been the continuous Tris-EDTA-borate (Ayala et al., 1972) and the staining method has been as follows: 40 mg betanaphtyl acetate, 50 mg alpha-naphtyl propionate, 20 mg Alphanaphtyl acetate, 100 mg Fast Blue RR salt, 20 mg Fast Black K salt in 150 ml phosphate buffer (0.1 M, pH 6.4).

Loci *Aph-3*, *Est-8*, *11* and *13* were analyzed in the third larval instar; *Est-12* and *15*, *Got-1* and *Lap-2* in pupa and the rest of the systems in the adult stage.

In order to assure a trustworthy comparison of the electrophoretic migration of the enzymes of the three species studied, homogenized individuals of all of these were put on all of the plates.

When various isoenzymes appear with the same staining on the same plate, the systems of the three species have been considered homologous if they fulfilled the following criteria: 1) The number of the systems from cathode to anode should be the same for the three species. 2) The system should have an optimum activity in the same stage of development for the three species. 3) The system should show equal coloring with the same substrate. 4) The system should have a similar activity during the time of staining (bands could stain sooner or later, and/or to a lesser or greater degree).

Following Eisses et al. (1979), we have denominated the most common enzymatic band of *D. melanogaster* as allele 1.00 in order to determine the differences of electrophoretic migration between allozymes of the three species, and when an allozyme of that same locus in any species migrates more or less than the 1.00 of *D. melanogaster* with respect to the cathode, it is accordingly denominated with a greater or lesser number, respectively.

Results

Table 1 lists the mean values of allelic frequencies and expected heterozygosities for each locus in the three species, as well as the percentage of polymorphic loci at the 99% level (if the frequency of the most common allele is equal to or lower than 0.99) and the mean heterozygosities (*H*).

Table 2 shows for each pair of species the

Table 1. *Allelic frequencies and expected heterozygotily (H) at 55 loci in* D. melanogaster, D. simulans *and* D. mauritiana

Gene	Allele	D. melanogaster	D. simulans	D. mauritiana
Acph	.91	–	0.223	0.025
	.95	–	0.736	0.975
	1.00	1.000	0.041	–
	H	0.000	0.405	0.049
Adh	.79	–	0.004	–
	.87	–	0.996	–
	.95	0.093	–	1.000
	1.00	0.907	–	–
	H	0.168	0.008	0.000
Ak-3	.97	–	0.019	–
	.98	–	0.981	1.000
	1.00	1.000	–	–
	H	0.000	0.037	0.000
Ak-4	.86	0.004	–	–
	1.00	0.996	1.000	1.000
	H	0.008	0.000	0.000
Ald	.96	–	0.032	–
	1.00	1.000	0.968	1.000
	H	0.000	0.062	0.000
Amy	.92	–	0.145	–
	.94	–	0.813	1.000
	.96	–	0.042	–
	.98	0.010	–	–
	1.00	0.990	–	–
	H	0.020	0.316	0.000
Ao	1.00	0.994	–	–
	1.03	0.006	0.767	1.000
	1.05	–	0.233	–
	H	0.008	0.357	0.000
Aph-3	.96	0.174	0.994	1.000
	1.00	0.826	0.006	–
	H	0.287	0.012	0.000
Aph-4	.90	0.021	0.071	0.125
	.95	0.005	–	–
	1.00	0.881	0.929	0.875
	1.04	0.093	–	–
	H	0.214	0.132	0.219
Aph-7	.98	–	0.083	–
	1.00	0.626	0.894	0.875
	1.02	0.368	0.023	0.125
	1.04	0.006	–	–
	H	0.472	0.193	0.219
Cat	1.00	1.000	1.000	1.000
	H	0.000	0.000	0.000
Dia-1	1.00	1.000	1.000	1.000
	H	0.000	0.000	0.000
Dia-2	1.00	1.000	1.000	1.000
	H	0.000	0.000	0.000
Dia-3	.91	0.083	–	–
	1.00	0.917	1.000	1.000
	H	0.152	0.000	0.000
Dia-4	.91	0.015	–	–
	1.00	0.966	0.996	1.000

Table 1. *Continued*

Gene	Allele	D. melanogaster	D. simulans	D. mauritiana
	1.01	0.019	0.004	–
	H	0.066	0.008	0.000
Est-4	1.00	1.000	1.000	1.000
	H	0.000	0.000	0.000
Est-6	1.00	0.773	0.190	–
	1.02	0.003	0.005	–
	1.05	0.210	0.800	0.529
	1.07	0.014	0.005	–
	1.11	–	–	0.471
	H	0.358	0.323	0.498
Est-8	.99	0.010	–	–
	1.00	0.979	–	–
	1.01	0.011	–	–
	1.04	–	1.000	–
	1.06	–	–	1.000
	H	0.041	0.000	0.000
Est-11	1.00	0.927	0.979	1.000
	1.03	0.073	0.021	–
	H	0.135	0.041	0.000
Est-12	.98	0.040	–	–
	1.00	0.960	0.979	–
	1.01	–	0.021	–
	1.03	–	–	1.000
	H	0.038	0.041	0.000
Est-13	1.00	1.000	1.000	1.000
	H	0.000	0.000	0.000
Est-15	1.00	1.000	1.000	1.000
	H	0.000	0.000	0.000
Fum	.99	0.010	–	–
	1.00	0.990	1.000	1.000
	H	0.020	0.000	0.000
Got-1	.95	–	–	0.306
	1.00	1.000	–	–
	1.06	–	1.000	0.694
	H	0.000	0.000	0.425
Got-2	.91	0.005	1.000	1.000
	1.00	0.995	–	–
	H	0.010	0.000	0.000
α-Gpdh	1.00	0.582	–	–
	1.02	0.418	1.000	1.000
	H	0.487	0.000	0.000
Gόpdh	.96	0.025	1.000	1.000
	1.00	0.967	–	–
	1.02	0.006	–	–
	1.04	0.002	–	–
	H	0.064	0.000	0.000
Hbdh	.94	–	1.000	1.000
	1.00	1.000	–	–
	H	0.000	0.000	0.000
Hk-1	1.00	0.685	0.996	1.000
	1.06	–	0.004	–
	1.10	0.315	–	–
	H	0.431	0.008	0.000
Hk-3	1.00	1.000	1.000	1.000
	H	0.000	0.000	0.000

TABLE 1. *Continued.*

Gene	Allele	*D. melanogaster*	*D. simulans*	*D. mauritiana*
Hk-5	*1.00*	1.000	1.000	1.000
	R	0.000	0.000	0.000
Idh	*95*	0.004	—	0.944
	100	0.996	—	—
	1 02	—	1.000	0.028
	1 0V	—	—	0.028
	H	0.008	0.000	0.107
Lap-2	*.96*	—	0.072	—
	Va	0.087	0.914	—
	99	0.085	—	1.000
	100	0.822	0.014	—
	1.04	0.006	—	—
	H	0.309	0.159	0.000
Mdh	*VJ*	—	0.004	—
	100	0.993	0.996	1.000
	1 09	0.007	—	—
	HL	0 014	0.008	0.000
Me	*1.00*	1 000	1.000	1.000
	H	0.000	0.000	0.000
Odh	M	*0 011*	—	—
	1 00	*0 978*	1.000	1.000
	H	0 043	0.000	0.000
Pep-1	*99*	—	1.000	1 000
	1 00	1.000	—	—
	H	0.000	0.000	0.000
Pep-2	*96*	—	1.000	1.000
	1 00	1 000	—	—
	H	0.000	0 000	0.000
Pep-3	*97*	0.101	—	—
	1 00	0.983	0.180	—
	1 03	0.014	0.820	1.000
	H	0.033	0.295	0.000
6Pgdh	*96*	0.006	0.988	1.000
	VI	0.006	—	—
	1.00	0.963	0.012	—
	1 02	0.019	—	—
	1.04	0.006	—	—
	H	*0.012*	0.024	0.000
Pgm	*90*	0.028	—	—
	94	0.085	0.115	—
	96	—	0.049	—
	1 00	0.884	0.836	0.919
	1 05	0.003	—	0.081
	H	0.210	0.285	0.149
Pbf	*94*	0.004	—	—
	1.00	0.996	1.000	—
	1 01	—	—	1.000
	a	0.008	0.000	0.000
Pur.	*1.00*	1.000	1.000	1.000
	R	0.000	0.000	0.000
To	*85*	0.011	—	—
	94	*0.007*	—	—
	1 W	*0 902*	1.000	1.000
	H	0 179	0 000	0.000

TABLE 1 *Continued*

Gene	Allele	*D. melanogaster*	*D. simulans*	*D. mauritiana*
	1.03	0 073	0 123	0 150
	H	0 135	0.216	0 255
Xdh	*.94*	—	0.003	1 000
	1 00	1.000	0.997	—
	H	0 000	0.006	0.000
Pt-1	*1 00*	1 000	1 000	1 000
	H	0 000	0 000	0 000
Pt-2	*1.00*	0 979	0 928	1 000
	1 02	0 021	0 072	—
	H	0 041	0 134	0 000
Pt-4	*1 00*	1 000	1 000	1 000
	H	0 000	0 000	0 000
Pt-6	*1.00*	1 000	1 000	1 000
	H	0 000	0 000	0 000
Pt-7	*1 00*	0 990	0 986	0.882
	1.01	0 010	0.014	0 118
	H	0 020	0 028	0 208
Pt-8	*1.00*	0.610	0 024	0.125
	1 01	—	0.845	0.625
	1 03	0 378	—	—
	1 04	0 012	0 131	0 250
	H	0 485	0 268	0 531
Pt 10	*.99*	0 023	—	—
	1 00	0 977	0 981	—
	1.02	—	0 019	1 000
	H	0 045	0 037	0 000
Pt-12	*1 00*	1 000	1 000	—
	1 02	—	—	1 000
	H	0 000	0 000	0 000
Pt-15	*1.00*	0 990	1 000	1 000
	1.04	0.010	—	—
	H	0.020	0 000	0 000
Total loci		55	55	55
Mean heterozygosity		0 084	0 062	0 048
Standard error		0.018	0 015	0 016
Percentage of polymorphic loci		49 09	34.55	18 18

values between them, calculated according to Nei (1971) and in parentheses under the preceding values those calculated according to Nei (1972).

DISCUSSION

When the heterozygosities and the proportion of polymorphic loci in the three species are compared, the low polymorphism of *D. mauritiana* stands out. It is not possible to know, however, if this is

TABLE 2. *Estimates of* I *(below the diagonal) and* D *with the standard error of* D *(above the diagonal) in three sibling species of the* Drosophila melanogaster *group according to Nei (1971) and in parentheses according to Nei (1972)*

	melanogaster	*simulans*	*mauritiana*
melanogaster	—	0.452 ± 0.102 (0.395)	0.606 ± 0.123 (0.564)
simulans	0.636 (0.673)	—	0.179 ± 0.060 (0.197)
mauritiana	0.545 (0.563)	0.836 (0.821)	—

of St. Mauritius (Tsacas ontl David, 1974) and therefore has a population size presumably smaller than that of thr cosmopolitan *D. melanogaster* and *D. simulans*, or to the fact that our data for *mauritiana* have been obtained from the study oí only two laboratory strains which may have lost part of thcir natural polymorphism.

Due to tlie morphological similarity of the three species we have thought it interesting to determine the diagnostic loci according to the criteria of Ayala and Powell (1972). *Est-8* antl *Idh* seem to be of particular interest since they permit the assignation oí one individual from any of the three species with error less than .001.

The statistics of Nei (1972) should always be preferred to calculate genetic distances, but we have also used calculations based on ihe most common allozymes (Nei, 1971), because our data for *D. mauritiana* arise from laboratory strains and thcreforc it could be questioned if its polymorphism is representative. In the study of Eisses el al. (1979) the distances according to Nci (1971) between pairs of the three species *D. melanogaster, U. mauritiana* and *D. simulans* were all the same (0.325), while wc find that those between *melanogaster* and *simulans* (0.452) and between *melanogaster* and *mauritiana* (0.606) are noticeably greater, antl the onc belwecn *simulans* and *mauritiana* is lower (0.179). This means that, if we admit constant evolution rates, the last pair of species are more closely related to each other lhan to *melanogaster*. In addition, the value found for the latter two species is one of the lowest found between siblings. Using thr be seen (Table 2) that the relationships among the species do not change.

Thc greater proximity found by us between *D. mauritiana* and *D. simulans* agrees with the report by David et al. (1974), that the viability and fertility of hybrids among these three species was greatest between *simulans* and *mauritiana*. Lemeunier and Ashburner (1976), reporting on the sequences of band of polytene chromosomes, found that *D. simulans* and *D. mauritiana* are homosequential and differ from *melanogaster* by a great paracentric inversion on the right arm of chromosome 3 and by some small inversions on chromosome X and on the right arm of chromosome 2.

Finally il seems interesting to point out that if we use the data from Eisses et al. (1979) to construct a dendrogram according to Farris (1972), the relationships between the above mentioned species are the same as we have found.

SUMMARY

The genetic distance (Nei, 1971, 1972) among three sibling species of the *melanogaster* subgroup, *D. melanogaster, D. simulans* aná *D. mauritiana*, has been obtained by means of the electrophoretic analysis of allozymes and larval proteins in 55 loci common to these three species. *Drosophila simulans* and *D. mauritiana* are more similar to each other than to *D. melanogaster*.

ACKNOWLEDGMENTS

The authors are indebted to Di. J. McDonald and Dr. L. Tsacas for supply-