Biochemical phylogenies of *Drosophila*: protein differences detected by two-dimensional electrophoresis

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Abstract

Approximately 100 abundant proteins were analyzed by two-dimensional electrophoresis (2DE) in eighteen species belonging to three taxonomically different *Drosophila* groups, the *D. melanogaster* species subgroup, the *D. auraria* complex and the *D. virilis* species group. Genetic distances between these species were calculated from a total number of protein differences in the pairs of species. Based on such data of genetic distances a dendrogram was constructed. The genetic distances or phylogeny obtained by 2DE were significantly correlated with those for one-dimensional (conventional) electrophoresis and also with data on mating success between species. It was discussed on the basis of these studies that a technique of 2DE is a useful adjunctive tool in systematics, including the previous reports with different kinds of data.

Introduction

O'Farrell (1975) reported that two-dimensional electrophoresis was a useful tool for detecting polypeptides (proteins) in organisms. This technique, which has been applied for various purposes by many researchers, in particular molecular biologists, involves two independent criteria for the protein separation. In the first dimension proteins are separated according to net charge by isoelectric focusing, and in the second dimension according to molecular weight by sodium-dodecylsulfate (SDS) electrophoresis. In *Drosophila*, the tool has been used mainly for genetic analysis such as genes coding chorion (Spradling *et al.*, 1979) and genetic variation in natural populations (Leigh Brown & Langley, 1979; Ohnishi *et al.*, 1982).

Although their function is mostly unknown, many more proteins can be easily detected by this two-dimensional electrophoresis than by conventional (one-dimensional) electrophoresis. Despite this technical advantage, it has seldom been used to study evolutionary divergence and phylogeny of *Drosophila* (for an exception see Ohnishi *et al.*, 1982). Recently, Imajoh (1981) showed that this technique was fruitful for the study of mosquito speciation, and Aquadro and Avise (1981) also reported that 'it could be a useful adjunctive tool in systematics', using rodent species.

We report here genetic distances measured by two-dimensional electrophoresis among *Drosophila* species classified into three different groups; the *D. melanogaster* species subgroup, the *D. auraria* complex and the *D. virilis* species group. Based on such data, a dendrogram of these *Drosophila* species is constructed and compared with allozyme or protein data detected by one-dimensional electrophoresis and with the data on unsuccessful matings between species. Finally, we discuss how genetic differentiation has occurred in the process of *Drosophila* speciation, referring to the other available information, such as that from inversions and rRNA genes.
Material and methods

This paper deals with six species of the *melanogaster* species subgroup, and four species of the *auraria* complex of the *montium* subgroup (in the *melanogaster* species group of the subgenus *Sophophora*), as well as with eight species of the *virilis* species group of the subgenus *Drosophila*, all of the genus *Drosophila*. Six species of the *D. melanogaster* species subgroup were kindly sent from Bowling Green State University (see Table 1). The *D. auraria* complex includes four species: *D. auraria*, *D. biauraria* and *D. triauraria*, each collected in two different localities of Japan in 1976 and 1978 (see Table 4 for more details), and *D. quadraria* of Texas stock no. 3075.1. The *D. virilis* species group includes eight species with Texas stock nos. as shown in Table 5. The former two groups were taxonomically classified into the *D. melanogaster* species group, being closely related to each other but distantly related to the *D. virilis* species group.

In order to analyze abundant proteins of adult flies by two-dimensional electrophoresis (2DE), six males (more than three days old) were sampled from each species or strain. The two-dimensional electrophoresis procedure is the same as described by Leigh Brown and Langley (1979) or Racine and Langley (1980) following O'Farrell (1975), except for centrifugation (10,000 rpm, 20 min). After the samples were homogenized and centrifuged, 75 microliters of the supernatant were loaded onto an isoelectric focusing gel. The isoelectric focusing (IEF) was carried out in 12 cm long tubes with internal diameter of 2 mm, using the same mixture of pH 3.5–10 and PH 5–8 ampholytes as described by O'Farrell (1975), for a total of 6,000 V-hrs after prerunning for one hour. Sodium-dodecylsulfate (SDS) slab gel electrophoresis in the second dimension was carried out in 10% acrylamide for 4 hours at 200 V. After running, all slab gels were fixed in 45% methanol and 10% acetic acid overnight and stained in the same solution containing 0.02% Coomassie brilliant blue R-250. They were de-stained in a mixture of 25% methanol and 7% acetic acid.

We have detected about 100 abundant proteins in the SDS slab gel. In the comparison between gels, we have detected a charge-change difference of proteins having an identical molecular weight as well as a protein spot which is not found in a given species but in the other species. The total number of such protein differences was counted for each pair of species. When a polymorphic protein locus with two different alleles (twin spots in the gel) within a species was found, the allele frequencies were assumed to be equal at the locus.

Genetic distance was calculated according to Aquadro and Avise (1981)'s equation: \[ D = 1 - F = \frac{(n_x + n_y)}{2n_{xy}}, \] in which \( n_{xy} \) is the number of spots in both species, and \( n_x \) or \( n_y \) is the total number of scored loci (spots) in each species. If either of two alleles at a polymorphic locus in a given species is identical to an allele in different species, the number of shared alleles (\( N_{xy} \)) was counted to be 0.5 at that locus. The relationships between the three groups were determined from comparisons between three representative species, *D. teissieri*, *D. auraria* and *D. virilis*, as seen in Figure 1, and their genetic distances were calculated as for the within-group comparisons. From genetic distance data obtained by two-dimensional electrophoresis, a phylogenetic tree was constructed according to the unweighted pair-group method of clustering by Sokal and Sneath (1963). In brief, the initial requirement is to produce a matrix of genetic distances among all combinations of species or groups, and then the first two species to be clustered are those with the smallest genetic distance. These two species are combined and taken to be a single group, and new estimates of genetic distance between this group and other species or groups are calculated. The same procedure is followed until all species are clustered into a single family.

One-dimensional electrophoresis (SDE) was also carried out, using 10% starch gels to analyze allozyme loci of the *D. melanogaster* species subgroup and the *D. auraria* complex. The electrophoretic procedure is the same as described by Voelker et al. (1980). Four adult flies were used for the analysis of an allozyme pattern in each species or strain. Twenty-six allozyme loci (Acp-1, Adh, Ald, Aldox, Dip-A, B and C, Est-6, Est-C, Fum, Got, G6pdh, aGpdh, Gpt, Hex-A and C, Idh, cMdh, mMdh, Men, Odh, 6Pgdh, Pgi, Pgap, Pgm and Xdh) were surveyed for the former group, and twenty (Acp-1, Adh, Dip-A and B, Est-6, Est-C, Fum, Got, G6pdh, aGpdh, Hex-A and C, cMdh, mMdh, Men, Odh, 6Pgdh, Pgi, Pgap and Xdh) for the latter complex. Data on SDE of the *D. virilis* species group were taken from Nei (1971)'s protein data which are