

THE RIBOSOMES OF DROSOPHILA

II. STUDIES ON INTRASPECIFIC VARIATION¹

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ABSTRACT

Electrophoretic comparisons of 40S and 55S ribosomal subunit proteins from 18 strains of *Drosophila melanogaster* revealed the virtual absence of allelic variation. More detailed two-dimensional studies on the large subunit proteins in 6 of the strains demonstrated additional complexity but still no interstrain variation. The significance of these results is discussed with respect to present estimates of genic heterozygosity in natural populations.

THERE has been much interest recently in evaluating the kind and amount of genetic variation within and between species using molecular techniques. Studies employing electrophoretic analysis of soluble proteins (HARRIS 1966; LEWONTIN and HUBBY 1966; O'BRIEN and MACINTYRE 1969; SELANDER, HUNT and YANG 1969; SELANDER *et al.* 1970; BERGER 1971; AYALA *et al.* 1972) comprise the bulk of this work. Each of these studies generally conclude with some statement suggesting that a large proportion, about 40%, of loci within a species is polymorphic, or that significant differences exist between even closely related species in allozyme comparisons (HUBBY and THROCKMORTON 1968; BERGER 1970).

In the present study electrophoretic techniques were utilized to obtain certain information about the kind and amount of intraspecific variation in *Drosophila melanogaster* for those loci responsible for the synthesis of ribosomal proteins. We sought primarily to determine whether present estimates of genic heterozygosity derived from a limited sample of soluble proteins were biased. That is, do the loci which code for the structural proteins of ribosomes show more or less allelic variation than do loci which code for soluble enzymes or hemolymph proteins?

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MATERIALS AND METHODS

Stocks—The *Drosophila* strains used in this study are listed in Table 1 along with information on their origin. *Drosophila* were grown on standard banana-agar medium at 25°. Adults or third instar larvae were collected and stored at -20°.

Ribosomal protein isolation—A complete description of ribosome and subunit protein isolation has been reported elsewhere (BERGER 1972, 1974).

Electrophoresis—Ribosomal proteins were resuspended in 4M deionized urea in preparation for electrophoresis. For one-dimensional electrophoresis in urea-acrylamide disc gels the procedure of LAMBERTSSON, RASMUSON and BLOOM (1970) was used. For two-dimensional studies either of two procedures was employed. In the first method the urea-acrylamide disc gels were polymerized over 15% acrylamide slabs containing .1% sodium dodecylsulfate prepared according to the method of BLATTER *et al.* (1972). The second method exactly followed the procedure of HOWARD and TRAUT (1973), except that electrophoresis in the first dimension (pH 8.2) was extended to 16 hours at 2 ma/gel with constant current. In all cases proteins were visualized by staining with .1% Coomassie Brilliant Blue dissolved in 50% TCA, and destained in methanol, acetic acid, water (5:1:5). Protein concentrations were determined by the method of LOWRY *et al.* (1951) using bovine serum albumin (3x crystallized) as a standard.

RESULTS

Electrophoresis in urea-acrylamide disc gels

Eighteen independently derived strains of *Drosophila melanogaster* were examined electrophoretically in urea-acrylamide disc gels for mobility variation of proteins extracted from either the 40S or 55S ribosomal subunits. Twenty-seven major protein bands of varying staining intensity were observed in the large subunit of each strain (Figure 1). From both side-to-side comparisons and

TABLE 1

Stocks and strains of Drosophila melanogaster examined for ribosomal protein variation

Stock	Source	Code no. or origin
Canton-S	Cal. Inst. Tech.	1
Hikone-A-S	Cal. Inst. Tech.	2
Lausanne-S	Cal. Inst. Tech.	4
Oregon-R-C	Cal. Inst. Tech.	5
Swedish-C	Cal. Inst. Tech.	6
Urbana-S	Cal. Inst. Tech.	7
M(1)n/FM6	Cal. Inst. Tech.	74
M(2)S2 ³ /SM2	Cal. Inst. Tech.	346
M(2)S2 ⁹ /SM5	Cal. Inst. Tech.	347
su(s) ²	Cal. Inst. Tech.	126
su(f)	Cal. Inst. Tech.	145
M(3)40130	Cal. Inst. Tech.	529
su(t)	Cal. Inst. Tech.	609
Hd5	DR. B. CHARLESWORTH	England
Hd14	Univ. of Liverpool	England
Hd16	Univ. of Liverpool	England
Hd20	Univ. of Liverpool	England
A1	Iso-female line	Albany

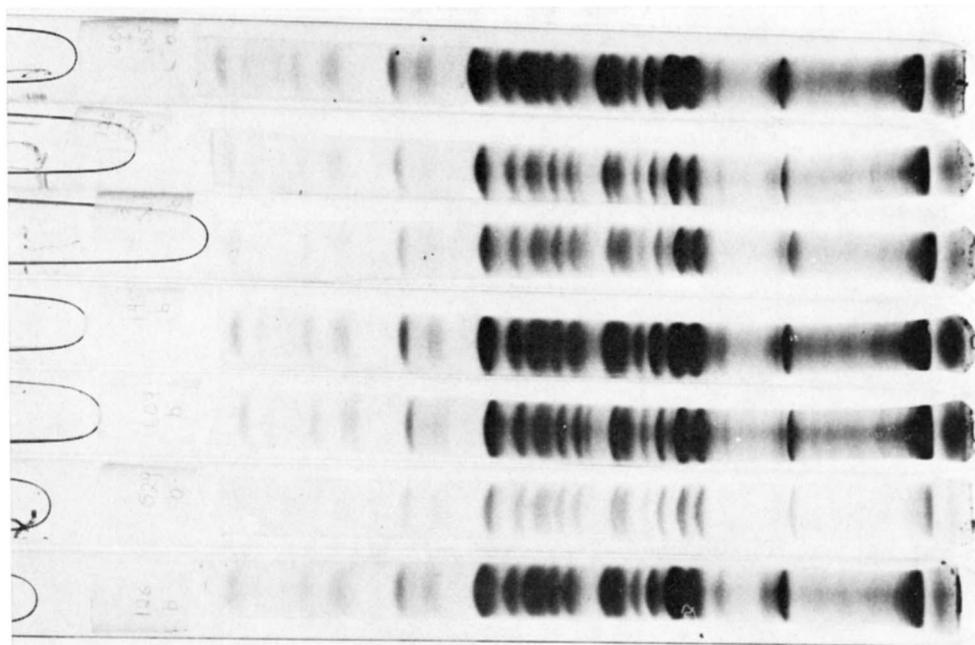


FIGURE 1.—Urea-acrylamide disc gels of 55S subunit proteins. Approximately 150 μ g of large subunit protein were layered onto each tube (.5 cm \times 15 cm) and electrophoresis applied for 6 hours at 2 ma per tube, or until the pyronin yellow marker dye had reached the gel's bottom. From top to bottom the gels contain strains: 126; 529; 609; 145; Swed. B; 126 + 529; 609 + 145 protein. Migration is cathodal toward the left.

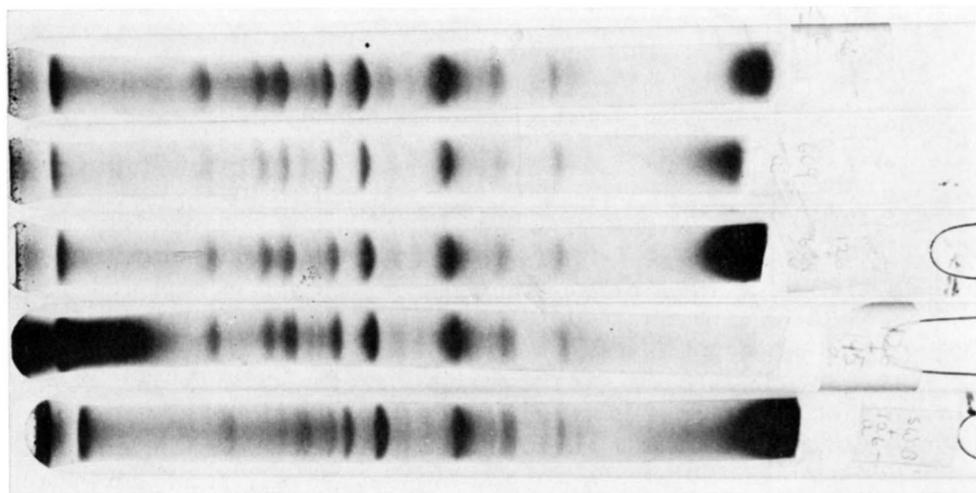


FIGURE 2.—Urea-acrylamide disc gels of 40S subunit proteins. Approximately 100 μ g of small subunit protein were layered onto each tube. The samples from top to bottom include strains: 126; 609; 126 + 609; 126 + 145; 126 + Swed. B. Migration is cathodal, toward the right.

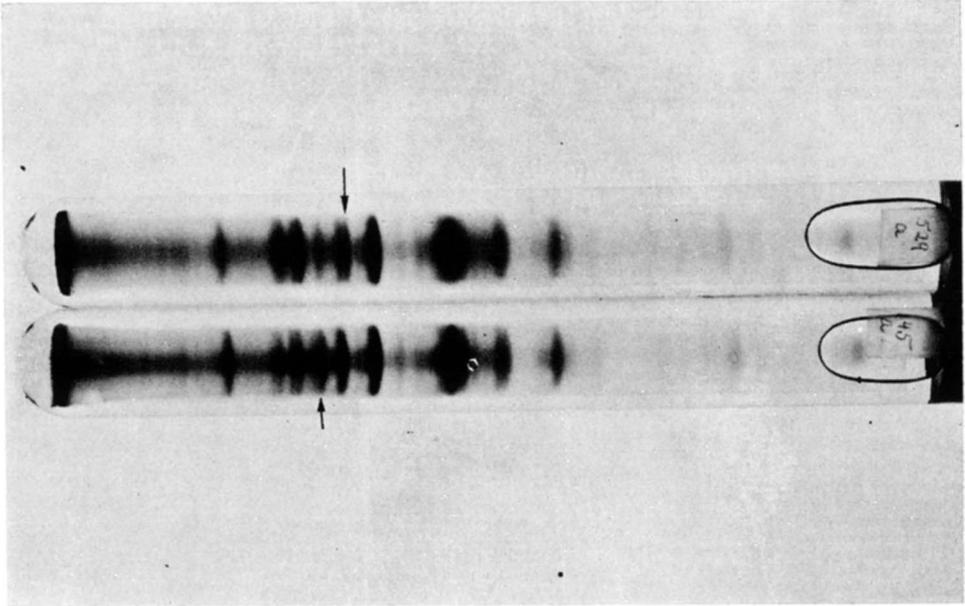


FIGURE 3.—Urea-acrylamide disc gel of strain 529 (top) and 145 (bottom) small subunit proteins showing variable position of SP-6. The 145 pattern typifies the pattern of the other 16 strains. Migration is toward the right.

studies involving the coelectrophoresis of pairs of strains, we judged the patterns in all the strains to be identical. Twenty-seven major protein bands of varying intensity were seen in the small subunit preparations of each strain (Figure 2). In all but one case the banding patterns were identical. In one strain (Figure 3) a variant of small subunit protein 6 (SP-6) was found to migrate in a position between SP-7 and SP-8. This variation is now being scrutinized by genetic analysis.

Two-dimensional electrophoresis (Method 1)

The relatively small number of protein bands resolved by one-dimensional electrophoresis indicated that several of the bands must be heterogeneous. This was especially suspected in the large subunit patterns. In order to further resolve the complexity of our subunit proteins we utilized a second electrophoretic procedure. Using a 15% acrylamide-0.1% sodium dodecylsulfate (SDS) gel system we were able to separate subunit proteins solely by molecular weight. As seen in Figure 4, the majority of ribosomal proteins have molecular weights greater than cytochrome-c (12,500 daltons) but lower than ovalbumin (43,000 daltons). By first separating proteins in urea disc gels and then polymerizing the unstained gels to 15% acrylamide-0.1% SDS slabs for two-dimensional separation we were able to distinguish 37 protein spots from large subunit protein bands 1-25 (Figure 5). Despite this increased complexity the patterns in the 6 strains tested remained identical. Several minor spots were observed in some but not all prepar-

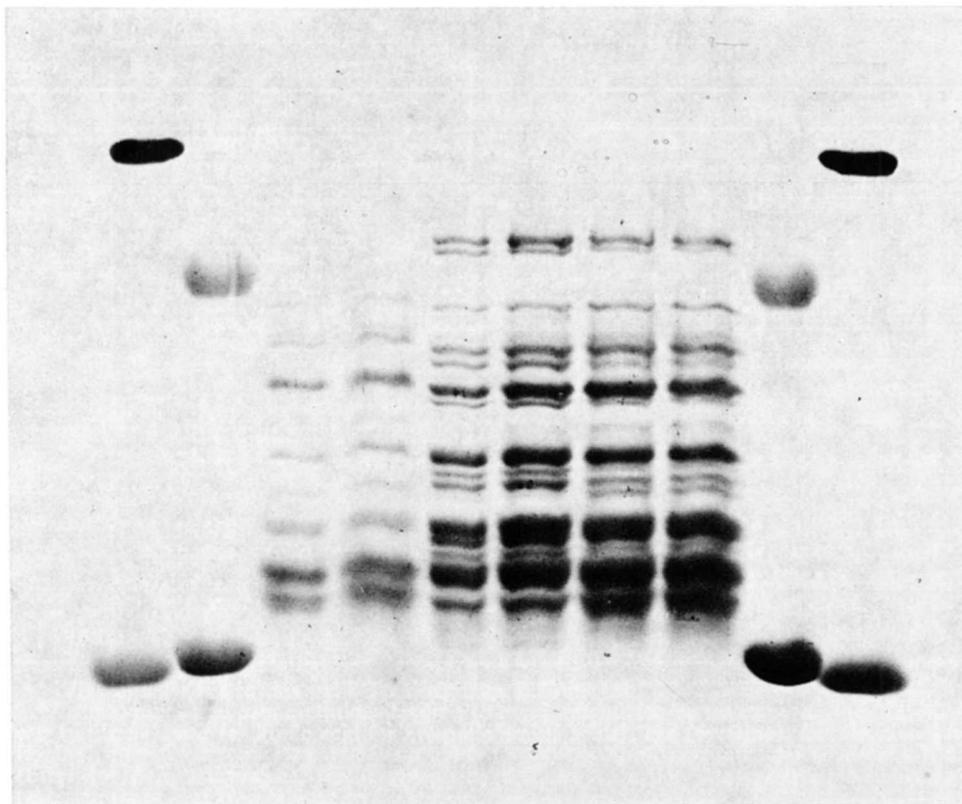


FIGURE 4.—SDS-acrylamide gel of *D. melanogaster* subunit proteins. The outermost pockets contain bovine serum albumin and cytochrome C. The next pair contains ovalbumin and myoglobin. From left to right, the remaining 6 pockets contain 40S, 40S, 55S, 55S, 80S and 80S protein. Approximately 20 μ g of 40S protein, 40 μ g of 55S protein, and 60 μ g of 80S protein were applied. Migration is anodal, toward the bottom.

ations and were not considered further. A diagrammatic summary is seen in Figure 6.

Analyses of proteins SP-1 to SP-19 by a second dimension revealed only a single protein complexity. SP-8 now appeared as two spots of approximate molecular weights 23,000 and 25,000. A diagrammatic representation is seen in Figure 7. Again no additional allozyme variation was detected by this procedure in the six strains tested.

Using similar procedures MARTINI and GOULD (1971) have resolved 36 major spots in the large subunit, and 26 major spots in the small subunit of rabbit reticulocyte ribosomes.

Two-dimensional electrophoresis (Method 2)

A second two-dimensional electrophoresis system was employed in which proteins are first separated in 4% acrylamide disc gels at pH 8.2, and then in a

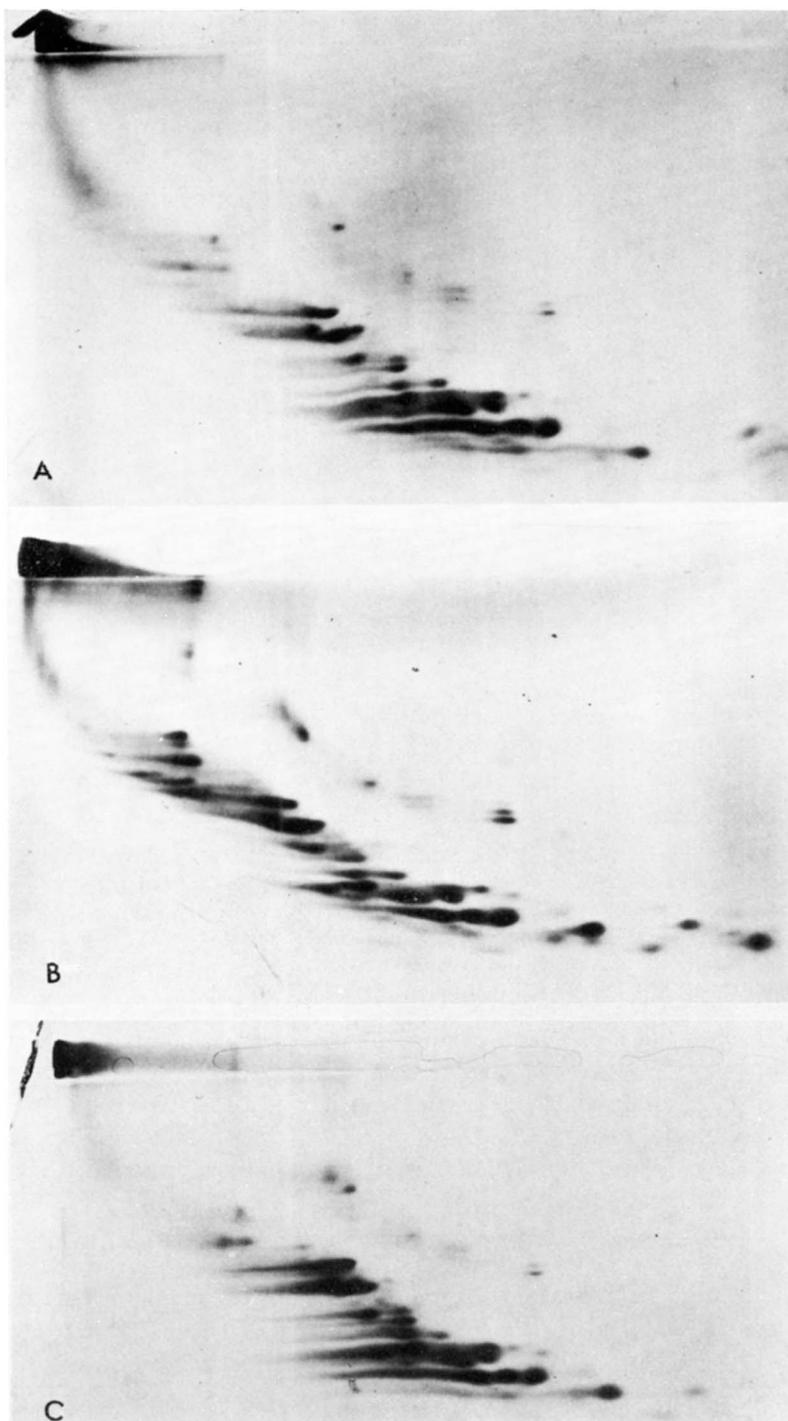


FIGURE 5.—Two-dimensional electropherograms (Method 1) of 55S subunit proteins. Approximately 300 μg of protein were layered in the first dimension. Strains shown are (A) Swedish B; (B) 145; (C) 529. Polarity is shown in Figure 6.

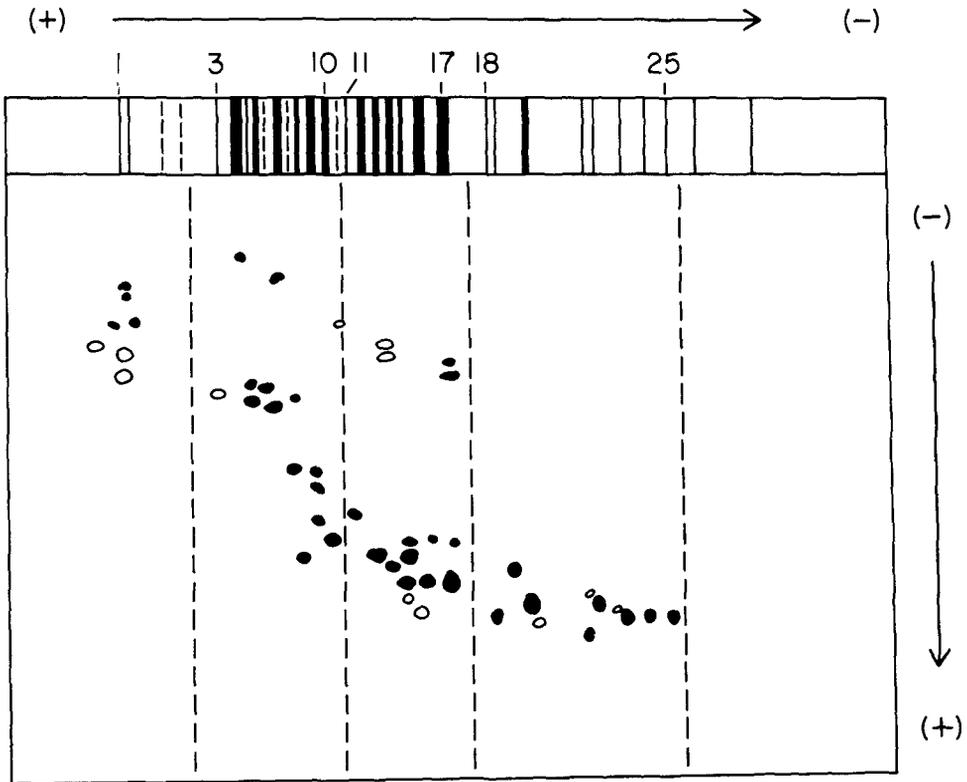


FIGURE 6.—Diagrammatic summary of 55S subunit proteins from Figure 5.

slab in the second dimension at pH 4.5. Thus in both dimensions separation is primarily by net charge. Under these conditions all but one large subunit protein migrates cathodally in the first dimension, and all are cationic in the second dimension (Figure 8). A total of 38 protein spots were seen in the 55S proteins for all 6 strains tested. Again, no interstrain variations were observed. Analysis of small subunit preparations using this procedure revealed no mobility variation in the six strains tested.

DISCUSSION

Several independent estimates of genetic variation have been reported for *D. melanogaster* utilizing gel electrophoresis of soluble enzymes (BERGER 1970; O'BRIEN and MACINTYRE 1969). On the average polymorphism can be detected at about 40–50% of the loci studied, a value comparable to that found in many other national species (LEWONTIN and HUBBY 1966; SELANDER, HUNT and YANG 1969; SELANDER *et al.* 1970). Clearly, then, the striking monomorphism observed for ribosomal proteins in this study stands in contrast and requires some interpretation. Three come to mind.

First, the sample size in the study was small; only 18 strains were surveyed. Although this bias will underestimate variation for rare alleles it is probably not sufficient to explain the identities observed, since studies made on equivalent

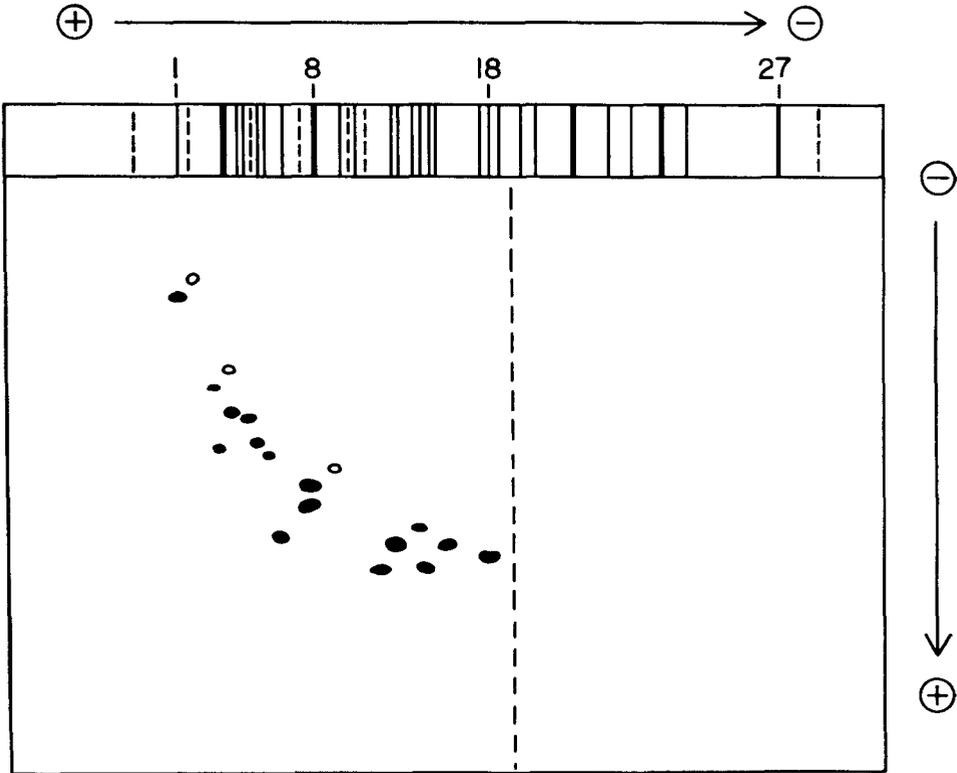


FIGURE 7.—Diagrammatic summary of 40S subunit proteins resolved by two-dimensional electrophoresis (Method 1).

samples of *Drosophila* for soluble enzymes (BERGER 1971) have revealed extensive polymorphism. Moreover, in the present study a large number of genetic systems were examined simultaneously and the virtual absence of variation is significant. The SP-6 variant seen in one strain has not yet been confirmed by genetic analysis but may represent an interesting exception. We have no idea whether each protein resolved represents the product of an independent structural locus or whether a single ribosomal protein may be modified to produce several isozymes. Thus our first approximation of 67 loci may be an overestimate.

A second interpretation of these results is that the techniques employed fail to detect existing variation. The biases here are threefold: firstly, small mobility differences may not be resolved; secondly, if net charge is an important phenotype of these proteins certain amino acid substitutions may occur but not be detected; and thirdly, the complexity of the protein patterns may be too great for the resolution of variations. We cannot argue against the first two biases except to point out that essentially the same techniques we employ were used by several others (LEBOY, COX and FLAKS 1964; KALTSCHMIDT *et al.* 1970; BOLLEN *et al.* 1973) to detect intraspecific ribosomal protein variation in prokaryotic systems. As far as the problem of complexity is concerned, we have utilized several different electrophoretic systems to screen for polymorphism and have

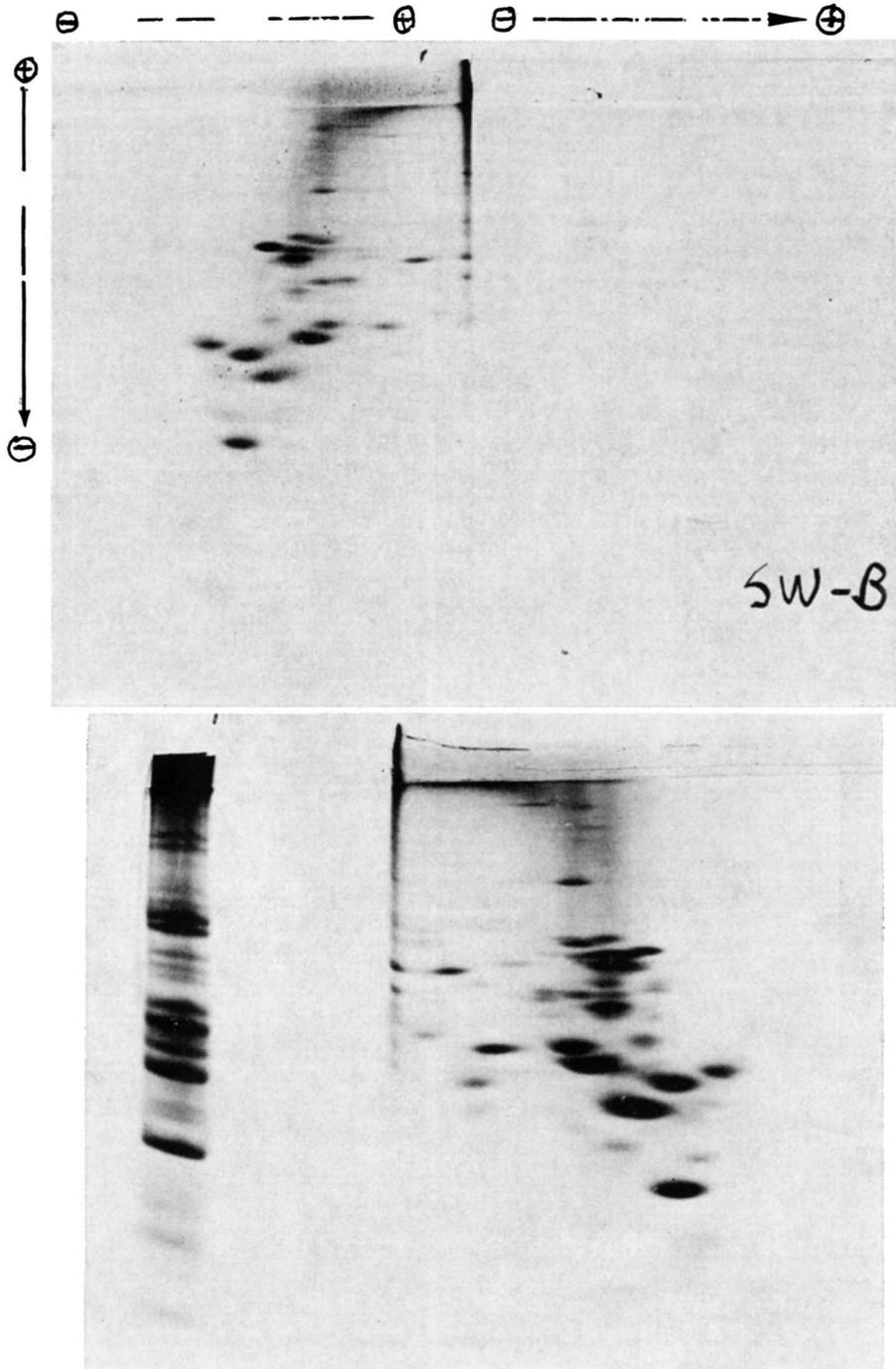


FIGURE 8.—Two-dimensional electrophoresis of 55S ribosomal proteins (Method 2). In the top panel polarities of migration in the pair of disc gels are shown. Only 1 spot appears in the anodally migrating gel. In the lower panel are 55S proteins of the strain 145. Included here is a pocket to the left which designates the banding patterns in the second dimension gel alone.

uncovered only a single case. It is perhaps encouraging to note that the total number of protein spots we see in our two-dimensional gels of *Drosophila melanogaster* corresponds closely with the careful measurement of MARTINI and GOULD (1971) for rabbit reticulocyte subunits. As these authors point out, this total can account for most of the proteins predicted on the basis of subunit mass and percent protein composition. We have made a detailed study of *Drosophila virilis* ribosomes (BERGER 1974) and have drawn similar conclusions.

A third and perhaps more appealing interpretation of the data is that ribosomal protein genes as a group are exceptionally conservative during evolution. The steric restrictions imposed by membership in a three-dimensional organellar array, and the functional restrictions associated with the role of ribosomes in translating a universal triplet code, offer two possible hindrances against extensive amino acid substitution. Although one cannot distinguish between these alternative arguments, it becomes important, in a sense, to do so. If steric restrictions alone promote evolutionary conservatism, it then becomes necessary to re-evaluate the many standing estimates of heterozygosity. Structural protein (ribosomes, mitochondria, membranes, chromosomal) loci constitute some unknown but undoubtedly significant proportion of the total genome, and if they are as a class invariant, then estimates of species variability would be far lower than we now suspect. If the conservatism we see is restricted to components of the translational machinery, then the significance of our findings to the general question of variation is questionable. Continued analysis of other structural proteins is clearly required.

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