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PROGRESS REPORT

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REQUEST FOR EXTENSION OF

CONTRACT AT(04-3)-34 WITH

THE ATOMIC ENERGY COMMISSION

ENTITLED

"GENETIC STRUCTURE OF NATURAL POPULATIONS"

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We enclose a summary report of our work in progress carried out under contract AT(04-3)-34 during fiscal 1972-1973 (second year of the contract), and include a budget request for extension of the contract for fiscal 1973-1974 (third year).

A. PROGRESS REPORT

I. Genetic Load in Natural Populations.

Experiments have been undertaken to estimate how much of the genetic variability observed in natural populations can be accounted by heterosis, that is, by the higher fitness of heterozygous individuals relative to homozygotes. It is now estimated that 30-60% of all loci are polymorphic, on the average, in natural populations of organisms as different as men, mice, rats, salamanders, fish, horse-shoe crabs, giant clams, and *Drosophila* flies. These estimates of genetic variation are based on the study of genetically controlled biochemical polymorphisms. Some authors have argued that much of this variation must be adaptively neutral since otherwise it would impose an enormous genetic load on the population. The evidence from the study of natural populations seems to indicate, however, that there may be a selective basis for such polymorphisms. One of the mechanisms which might account for the maintenance of such genetic variation by natural selection is heterosis. If there is heterozygous advantage at a large number of loci, then it follows that individuals which are homozygous for a complete chromosome should have considerably lower fitness than normal outbred individuals.

Preliminary experiments carried out in our laboratory with *Drosophila pseudoobscura* and *D. willistoni* have shown that, under population conditions, flies homozygous for randomly sampled (but excluding lethal) chromosomes have fitness which is only about one third, on the average, of the fitness of individuals heterozygous for random combinations of the same chromosomes. In the fall of 1971 we started a large scale experiment with *D. melanogaster* to verify and extend such results. The techniques to establish the appropriate stocks and to maintain the experimental populations, as well as the rationale of the experiment were presented in last year's report. The results during the first six months of the experiment were also reported last year. Here we present a partial summary of our estimates of fitness and of fitness components, together with preliminary speculations concerning the significance of our results.

The experiment is a very large one. A total of 92 experimental populations have been studied in its first phase. Each population was maintained until a stable equilibrium was established between the marker (lethal in homozygous condition) and the wild chromosome, or until the marker chromosome was eliminated. Two kinds of genotypes exist in each experimental population: the homozygous wild flies, +/+, and flies heterozygous for the wild and the balancer chromosome, B/+. The scoring of genotypic frequencies in the experimental populations is made at the adult stage. To estimate the zygotic frequencies, egg samples were taken from each population after an equilibrium had been reached, and these eggs were allowed to develop under near optimal conditions. To correct for any viability differences between the genotypes under near-optimal conditions, crosses of the type B/+ X B/+ were made for each chromosome. If there are no viability differences these crosses should yield B/+ and +/+ flies in the ratio 2:1. Deviations from this expected value permit to estimate viability differences under near optimal conditions. From these latter ratio tests together with the adult

equilibrium frequencies in the experimental populations, and the adult frequencies from the egg samples, we can estimate the fitness, the viability, and the fertility of the +/+ flies relative to the B/+ heterozygotes.

Let the following represent the frequency of B/+ flies:

- r among adults in the ratio test
- h among adults in the egg samples
- h' among zygotes in the egg sample
- H among the adult flies in the cages at equilibrium.

It can be shown that the fitness of +/+ relative to B/+ flies is given by

$$W = \frac{r-h}{r(1-h)} \quad ; \quad (1)$$

their relative viability is given by

$$V = \frac{h'(1-H)}{H(1-h')} \quad ; \quad (2)$$

and their relative fertility is given by

$$F = \frac{H(2-3h')}{2h'(1-H)} \quad (3)$$

We shall report here the results obtained from the study of wild second chromosomes; the study of wild third chromosomes gives results qualitatively identical to those obtained with second chromosomes.

We extracted 50 different wild second chromosomes from a freshly collected sample of D. melanogaster flies by standard procedures. Of these, 18 (36%) proved to be lethal or semilethal in homozygous condition. 23 of the other 32 chromosomes were chosen for the experimental populations. The initial frequency of +/B flies was 0.40 in one, and 0.90 in the other population. A stable equilibrium between the balancer and the wild chromosome was reached in every case in a relatively short time (from 6 to 12 weeks). The equilibrium frequencies were, ^{the same} in essentially every case for the two populations started with widely different frequencies. All cages were kept for at least 21 weeks, and in some cases 30 weeks, to verify the stability of the equilibrium frequency. The estimates of the viability, fertility and fitness of the +/+ flies relative to +/B flies are given in columns 2 to 7 in Table 1.

As controls, 12 populations were started with random combinations of the 23 wild chromosomes with each other and with the balancer chromosome. The frequency of B/+ flies was 0.40 in half and 0.90 in the other half of the control populations. In all these control populations, the wild chromosome was eliminated relatively rapidly. By week 23 it had disappeared from virtually all populations. From the rate of elimination of the balancer chromosome, it is possible to estimate the fitness of the wild heterozygous flies relative to the B/+ flies. This fitness was 1.21 ± 0.03 in the populations started with 0.40 frequency of B/+ flies, and 1.31 ± 0.03 in the populations started with 0.90 frequency. Dividing the estimates of fitness given in columns 6 and 7 of Table 1 by the corresponding one of these values, we obtain the fitness of the homozygous +/+ flies relative to wild flies carrying random combinations of wild chromosomes. These estimates of fitness are given in the last two columns of Table 1. Negative values have been changed to zero. The average mean fitness of the wild homozygous

TABLE 1

Fitness and fitness components of 23 wild second chromosomes of Drosophila melanogaster in homozygous condition.

Chromosome	Fitness of +i/+i relative to B/+i						Fitness of +i/+i relative to +i/+j	
	Viability		Fertility		Fitness		to +i/+j	
	0.40	0.90	0.40	0.90	0.40	0.90		
1	1.45	1.23	0.13	0.18	0.19	0.23	0.16	0.18
2	1.14	0.96	0.10	0.32	0.13	0.32	0.11	0.24
3	0.83	0.60	-0.08	0.31	-0.08	0.19	0	0.15
4	1.22	1.00	0.48	0.31	0.38	0.32	0.31	0.24
5	1.25	1.26	-0.09	-0.18	0.11	-0.22	0	0
6	2.11	1.88	0.32	0.30	0.66	0.55	0.55	0.42
7	1.18	1.28	0.21	0.14	0.26	0.19	0.21	0.15
8	1.19	1.53	0.09	0.02	0.11	-0.03	0.09	0
9	0.79	1.04	0.58	0.50	0.60	0.51	0.50	0.39
10	1.44	1.28	0.22	0.14	0.31	0.18	0.26	0.14
11	1.40	1.05	0.08	0.03	0.10	-0.03	0.08	0
12	1.31	0.79	-0.09	0.04	-0.12	-0.03	0	0
13	0.96	0.67	-0.02	-0.09	-0.03	-0.07	0	0
14	1.38	1.27	0.30	0.29	0.40	0.35	0.33	0.27
15	0.64	0.91	-1.78	-1.92	-1.18	-0.53	0	0
16	1.47	1.66	-0.29	-0.21	-0.44	-0.36	0	0
17	4.55	2.91	-0.05	-0.44	-0.22	-1.24	0	0
18	2.08	1.50	0.09	0.31	0.19	0.45	0.16	0.34
19	0.63	0.80	0.62	0.19	0.39	0.14	0.35	0.11
20	0.46	0.26	1.59	2.89	0.73	0.77	0.60	0.59
21	0.92	0.78	0.78	0.89	0.72	0.70	0.60	0.53
22	0.54	2.16	1.02	0.62	0.56	0.18	0.46	0.14
23	1.83	0.92	-0.09	0.24	-0.17	0.20	0	0.15
Mean	1.34	1.21	0.179	0.212	0.156	0.120	0.207	0.178
Standard Error	0.17	0.12	0.126	0.163	0.089	0.090	0.044	0.037

flies relative to random heterozygotes is, then, 0.191 for all 46 experimental populations. If the negative values are included as such in the average, the mean fitness for all wild homozygotes is 0.110.

It must be recalled now that 36% of all wild chromosomes extracted were excluded because they had a zero viability. If these are included in the estimates of average fitness of wild homozygotes, their average fitness becomes 0.122 or 0.070 depending on whether zeros or negative values are used in the cases where the estimated fitnesses are negative.

The decrease in average fitness of flies homozygous for wild chromosomes may be due to a few loci with a relatively large deleterious effect or to many loci each with very small effect. This matter has been discussed by Sved and Ayala (1970, Genetics 66:97-113) and will be discussed at length in the paper we are preparing for publication reporting these results. Here we shall only point out that the fact that all chromosomes tested exhibit very low fitness in homozygous condition suggests that many loci must be involved.

Let us now speculate on the number of polymorphic loci which could be maintained by heterosis in natural populations on the basis of our present results. We shall assume that the fitness of the homozygote relative to the heterozygote at each heterotic locus is 0.99, and that the fitness interaction between loci is multiplicative. Then assuming the mean fitness of the homozygotes to be 0.122, the number of loci at which the wild flies are heterozygous in the third chromosome is 210 ($0.99^{210} \approx 0.122$). If we take the lower estimate of homozygous fitness, the number of loci at which wild flies are heterozygotes is 266 ($0.99^{266} \approx 0.070$). Estimates of the number of polymorphic loci in natural populations of Drosophila range from 30 to 80%; estimates of the proportion of loci at which an individual is heterozygous range from 0.07 to 0.20. If an average wild fly is, according to our estimate, heterozygous in the second chromosome at between 210 and 266 loci, the number of polymorphic loci which could be maintained in the second chromosome by heterosis is between 840 and 1064. The second chromosome of D. melanogaster represents about 40% of the total genome of this organism. About 2100 to 2660 polymorphic loci could, therefore, be maintained in populations of this species by heterosis under the assumptions of our model. It appears, then, that a large fraction of the genetic variation observed in natural populations may be maintained by heterotic natural selection.

Needless to say, our estimates are subject to many potential sources of error. If the difference in fitness between heterozygotes and homozygotes is often greater than 0.01, the number of loci which could be maintained by heterosis would be substantially smaller. On the other hand, differences in fitness much smaller than 0.01 are sufficient to maintain a polymorphism in populations which like D. melanogaster have an effective size probably of many thousands. The possible number of polymorphisms maintained by heterosis becomes, then, many times larger. (With fitness differences between homozygotes and heterozygotes of 0.001, 10 times more polymorphic loci could be maintained; that is, between 21,000 and 26,600 loci.) If there is linkage disequilibrium, heterozygotes at linked loci would tend to be associated. The number of polymorphisms which can, thus, be maintained becomes again considerably increased.

The continuation of this work for the coming fiscal year, includes finishing up the experiments and statistical calculations reported above, and preparing the manuscript for publication. We shall also establish experimental

populations with flies homozygous and heterozygous for wild second and third chromosomes and the appropriate balancers. We know the fitness of flies homozygous for various second or third chromosomes. We want to find out the nature of the fitness interaction between the second and third chromosomes. That is, we want to ascertain how much of the genetic load is due to additive and to epistatic interactions. Our ultimate goal is, of course, to understand the role of natural selection in the maintenance of genetic variation in natural populations. As stated in last year's report, this remains one of the most important, and most actively debated, problems of population and evolutionary genetics.

II. Comparative Studies of Chromosomal and Biochemical Polymorphisms.

Natural populations of Drosophila are more or less strongly polymorphic for allozymes coded by many genes. Many, though not all, species are also polymorphic for gene arrangements in some or in all chromosomes. The behavior of chromosomal and enzyme polymorphisms in natural populations is different in some respects. The reasons for these differences are not well understood, and the present work was undertaken in the hope of throwing some light on the situation.

The incidence of chromosomal polymorphisms is often quantitatively, or even qualitatively, different in geographic populations of a species. We have shown that in D. pseudoobscura chromosomal polymorphisms can be reliably used to diagnose the geographic region of origin of the flies (Powell, Dobzhansky, and Levene, 1973). Species, even morphologically sibling species, can also be diagnosed by their enzymes, as we have shown (Ayala and Powell, 1972). Nevertheless, allozyme frequency differences between geographic populations of a Drosophila species are usually small, though by no means absent.

More than three decades of observations on chromosomal polymorphisms in D. pseudoobscura disclosed that changes in the frequencies of some polymorphisms occur with time in some populations. (For a review, see Dobzhansky, 1971, in "Ecological Genetics and Evolution", pp. 109-133). Two kinds of changes have been observed - cyclid ones correlated with the succession of seasons, and more long-term ones lasting for several years or decades. It has been demonstrated that these changes are due to natural selection, although the mode of operation of this selection is still insufficiently known. Starting in April 1971, we have undertaken a comparative study of chromosomal and enzyme polymorphisms, designed to find out whether temporal changes occur in both. With respect to the enzyme polymorphisms this study bears on the claim made by many authors that the allozyme variation is neutral with respect to adaptation, and that therefore allozyme frequencies are not governed by natural selection.

Samples of two sibling species, D. pseudoobscura and D. persimilis are taken at approximately monthly intervals during the breeding seasons of the flies in two Californian localities: McDonald Ranch, Napa County, elevation about 750 feet, and Mather, Tuolumne County, elevation about 4800 feet.

The chromosomal polymorphisms in D. pseudoobscura and D. persimilis in the Mather locality have been recorded at intervals since 1945. Our results since the spring of 1971 confirm the patterns previously observed.

In the McDonald population, two chromosomal arrangements, KL and MD, are most common in D. persimilis. Observations during our second year of sampling confirm the preliminary results given in last year's report. Seasonal cyclic oscillations are apparent. MD chromosomes are significantly more frequent during spring and KL chromosomes are more frequent during summer and early fall months than are their alternatives, as shown in Fig. 1.

There are as many as eight gene arrangements in the third chromosomes of D. pseudoobscura in the McDonald population. The present indications (see Table 2) are that ST chromosomes are most frequent early in the season, and least frequent in summer and in autumn. TL, and perhaps CH, chromosomes show frequency changes that appear to be the reverse of those in ST. Changes in AR chromosomes seem to be irregular. More observations are needed to show whether or not the frequency changes are seasonally cyclic.

Our study of allelic frequencies at loci coding for enzymes in the above populations started with a survey of more than 30 loci. Recently we have reduced our study to the eight most favorable loci. We report here our preliminary results for two loci, Pgm-1 (phosphoglucumutase-1) and Me-2 (malic enzyme-2). The former is sex-linked, the latter is autosomal.

Tables 3 and 4 report the observed frequencies of the two commonest alleles of the Me-2 and Pgm-1 genes at McDonald. The columns headed "s.d." give the binomial standard deviations for the most common alleles (the s.d.'s for the less common alleles, especially the rare alleles not included in the Tables, are smaller). The allelic frequencies may be different in samples taken in the same locality at different times, though in no case do they seem to be as variable as the frequencies of the chromosomal inversions considered above. Moreover, some of the changes may be seasonal and cyclic. Thus, in D. pseudoobscura, Pgm-1¹⁰⁰ increases in frequency throughout the spring in the McDonald locality, reaching a maximum around June-July; then it gradually decreases reaching its minimum yearly frequency in the last autumn sample; Pgm-1¹⁰⁴ follows a reverse trend (Table 3). These trends of change in the allelic frequencies at Pgm-1 may be cyclic since similar patterns were observed in 1971 and in 1972. Seasonal changes in the frequency of alleles at the Pgm locus seem to occur also in D. persimilis in the McDonald locality. Pgm-1¹⁰⁴, which is the most common allele in this species, reaches its highest frequencies in June-July, and the lowest in October-November (Table 3).

Frequency changes which might also be seasonal occur at the Me locus in the McDonald locality (Table 4). In D. pseudoobscura, Me-2¹⁰⁰ has its lowest frequencies in mid-summer and increases in frequency towards the fall. The reverse change occurs in Me-2¹⁰⁴. These trends were observed in both years 1971 and 1972. Trends in allelic frequencies, if they exist, are less clear-cut in D. persimilis. We shall continue this study for at least two more years. The statistical analysis of the other loci surveyed is, at present, in progress.

III. Genetic Modification of the Reaction of Drosophila to Light and to Gravity.

Drosophila pseudoobscura is, on the average, photoneutral and geoneutral when tested in Hirsh-Hadler mazes. As reported previously, by artificial selection it is possible to select populations for definitely positive as well as for negative responses. The selected populations are, however, not stable.

Figure 1. Percentage frequencies of MD (triangles) and KL (circles) gene arrangements in the third chromosomes of *Drosophila persimilis* in samples taken in the McDonald

TABLE 2

Percentage frequencies of the four commonest gene arrangements in the third chromosomes of Drosophila psuedoobscura in the McDonald locality. N = chromosomes examined; * ostensible minimum, and ** ostensible maximum annual frequencies..

Month	ST	TL	AR	CH	N
Apr. 1971	**57.4	*12.2	11.5	12.8	144
May "	47.9	22.3	17.0	7.3	242
June "	40.6	24.4	15.3	**14.9	242
July "	*37.9	23.6	23.0	10.0	330
Sept. "	45.8	**34.7	8.3	6.9	72
Oct. "	43.1	22.4	13.8	6.9	58
Nov. "	43.7	23.4	25.0	*4.7	64
Feb. 1972	**62.0	17.5	10.5	*3.5	200
Mar. "	52.7	*15.2	20.4	5.9	270
Apr. "	42.7	21.0	19.7	9.0	300
May "	46.4	18.8	18.8	9.9	282
June "	42.4	17.2	26.0	9.2	250
July "	43.2	24.7	14.2	**11.5	296
Aug. "	49.1	20.3	16.8	7.5	346
Sept. "	32.1	32.5	17.9	7.7	246
Oct. "	*28.5	**33.0	23.5	8.0	200

TABLE 3

McDonald locality. Percentage frequencies of the two commonest alleles at the sex-linked Pgm-1 locus in Drosophila pseudoobscura and Drosophila persimilis. N = number of genomes examined; s.d. = standard deviation of the allelic frequencies; * ostensible minimum, and ** ostensible maximum annual frequencies.

Month	<u>Drosophila pseudoobscura</u>				<u>Drosophila persimilis</u>			
	100	104	s.d.	N	100	104	s.d.	N
Apr. 1971	61.5	37.7	3.1	252	28.3	70.3	3.9	138
May "	67.5	31.6	2.5	348	28.3	70.8	4.4	106
June "	70.5**	29.0	3.4	176	25.0*	75.0**	7.2	36
July "	69.2	28.9*	2.6	308				
Sept. "	64.1	35.9	6.0	64	31.2	68.7	8.2	32
Oct. "	63.3	36.7	6.2	60				
Nov. "	55.0*	45.0**	6.4	60	32.8**	65.6*	3.4	192
Feb. 1972	68.3	31.7	4.2	120				
Mar. "	63.6	34.7	3.1	242	25.3	72.7	3.6	150
Apr. "	66.4	33.2	2.7	304	23.8	75.6	3.4	160
May "	63.5	35.8	3.0	260	26.3	73.7	5.1	76
June "	71.0***	28.8*	2.3	396	20.0*	78.8*	4.6	80
July "	69.2	30.3	2.3	390	22.1	77.9	5.0	68
Aug. "	64.2	35.6	2.3	436	29.6	70.4	4.6	98
Sept. "	65.0	34.7	2.6	326	27.5	71.6	2.5	324
Oct. "	62.3*	37.4**	2.8	302	33.3**	66.1*	2.5	366

TABLE 4

McDonald facility. Percentage frequencies of the two commonest alleles at the autosomal Me-2 locus in Drosophila pseudoobscura and Drosophila persimilis. N = number of genomes examined; s.d. = standard deviation of the allelic frequencies; * ostensible minimum, and ** ostensible maximum annual frequencies.

Month	<u>Drosophila pseudoobscura</u>				<u>Drosophila persimilis</u>			
	100	102	s.d.	N	102	104	s.d.	N
Apr. 1971	82.2	10.9	2.4	258	6.0	94.0	2.0	134
May "	82.2	11.4	2.4	264	7.8	91.1	3.0	90
June "	84.1	10.1	3.1	138	10.7**	89.3*	5.8	28
July "	80.2*	13.5	2.1	348	4.0	96.0	2.0	100
Sept. "	81.8	13.6**	5.8	44	2.6*	97.4**	2.6	38
Oct. "	83.3	10.0	6.8	30				
Nov. "	85.9**	7.7*	3.9	78	4.1	94.1	1.6	220
Feb. 1972	88.2**	9.1	3.1	110	4.5	93.2	3.8	44
Mar. "	76.3	13.1	2.8	236	5.1	89.7	2.6	136
Apr. "	78.8	9.8	2.3	306	3.2*	90.4	2.4	156
May "	79.9	9.8	2.4	274	3.9	96.1**	2.2	76
June "	83.3	9.5	1.9	390	9.2**	85.5*	4.0	76
July "	78.8	12.2	2.1	392	5.9	91.2	3.4	68
Aug. "	78.7*	12.6**	2.0	436	8.5	88.3	3.3	94
Sept. "	80.6	12.0	2.2	324	4.6	94.8	1.2	326
Oct. "	84.0	7.8*	2.1	306	8.0	90.7	1.5	364

Relaxation of the selection leads to a convergence of formerly very different populations towards approximate neutrality. Mathematical analysis of the data by Professor Howard Levene led to estimates of the "homeostatic drive", which is really the natural selection tendency to restore the status quo ante. It seemed possible to find such an intensity of artificial selection which will just balance the homeostatic drive, and thus will produce no change in the population's behavior. An experiment of this kind has actually been done, and the expectation has been verified. The data are being analyzed mathematically by Professor Levene.

The geo- and photopositive and negative populations do not differ in appearance from each other or from unselected control. An attempt is being made to test their reactions to various environmental influences. This work is being done in cooperation with Professor Charles Judson, of the University of California, Davis. The responses to light and to gravity of the selected populations were tested at 15°, 20°, 25°, and 27°C (the selection was carried out at 25°). Temperature variations do not change the behavior of the flies with respect to light and to gravity. Experiments on the phototactic behavior of the selected populations at different light intensities are in progress. Intensities of 800, 300, 150, 25, 7, 0.2 foot candles, as well as absence of light, are being tried. As expected, in the absence of light the selected photopositive and photonegative populations do not differ in behavior. In the range 7-300 foot candles the differences are about the same, while at 0.2 foot candles the difference is reduced but is still distinctly present. The results at 800 foot candles are unclear and the experiments are being repeated. Experiments on the influence of light of different wave lengths are in preparation.

IV. Genetics of a Geographically Isolated Population of *Drosophila pseudoobscura*.

Prof. Alice Hunter, formerly of the Universidad de los Andes, in Bogota, Colombia, has discovered in 1960-1962 a colony of *D. pseudoobscura* in the Andes near Bogota. This colony is isolated from the main body of the species (British Columbia to Guatemala) by a gap of some 1500 miles. Dobzhansky, Hunter, Pavlovsky and Wallace showed in 1963 that the Bogota population resembles in chromosomal composition that of Guatemala, and differs sharply from the populations living farther North. Prakash in 1972 found that the crosses of Bogota ♀ X United States ♂ give sterile male F₁ hybrids. He conjectured that the Bogota colony came from human introduction around 1960, and that this colony has become an incipient new species. We have begun to analyze the genetic nature of the male hybrid sterility in this case. In the first place, it has been found that Bogota males have a Y-chromosome clearly different from that in most U.S.A. males. Crosses have then been made in which the chromosomes of the U.S.A. males have been marked by one or more mutant marker genes. This work is now in midcourse; what can be stated for certain is that the sterility is an outcome of interaction of several genes in different chromosomes.

B. PUBLICATIONS UNDER THIS CONTRACT NOT INCLUDED
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C. PROPOSED BUDGET FOR THIRD YEAR PROJECT, October 1973 - September 1974.

<u>Salaries</u>	<u>Pct. FTE</u>	<u>Amount</u>
Post-graduate Research Geneticist (Dr. M.L.Tracey)	1.00	11,268
Laboratory Assistant (Mrs. Mary Dunham)	0.50	<u>3,534</u>
		14,802
<u>Employee Benefits</u> (13% of salaries)		<u>1,924</u>
(A) TOTAL PAYROLL		<u>16,726</u>
<u>Supplies and Materials</u>		
Drosophila culture supplies	2,000	
Chemicals (mostly for enzyme assays)	4,500	
Glassware, population cages, computer time, etc.	<u>1,000</u>	
		7,500
<u>Equipment</u>		
Maintenance and replacement of existing equipment		1,000
<u>Publication Costs</u>		2,000
<u>Travel - Domestic</u>		2,000
(B) TOTAL SUPPLIES, EQUIPMENT, ETC.		<u>12,500</u>
TOTAL DIRECT COSTS (A+B)		29,226
University Indirect Costs (51.5% salaries, exclusive of employee benefits)		7,623
TOTAL FOR PROJECT		\$ 36,849

D. BUDGET JUSTIFICATION

Salaries. The University of California will provide the salaries of the coprincipal investigators (Th. Dobzhansky, 25% of full time; F. J. Ayala, 20% of full time), of Mrs. Olga Pavlovsky, Staff Research Associate, Step 5 (50% of full time for this project), and of a typist (25% of full time).

The personnel in this project for whom support is requested include a postdoctoral trainee (Dr. M. L. Tracey, who has been carrying out most of the experimental research on the "Genetic Load" program), and of a Laboratory Assistant (50% of full time) in charge of making the Drosophila medium, washing glassware, etc. In view of our failure to obtain support for a full time technician in previous years, we are not including such request now.

Supplies, equipment and publication costs. The quantities requested are essentially the same as awarded for last year. They represent our needs, based on past experience, for Drosophila medium, starch, chemicals for electrophoresis, glassware for electrophoresis and for Drosophila cultures, replacement as needed and maintenance of equipment, page charges, etc.

Travel. Our investigation of temporal and spatial changes in the chromosomal and allozyme frequencies of natural populations of Drosophila requires samples at approximately monthly intervals in at least two localities. We anticipate an average of three collecting days for two persons for each of twelve months (72 man collecting days at \$20 each, \$1440), plus car rental and other expenses (36 collecting days at \$15 per day, \$540).

Note that the small increase in the level of support requested over last year's award is to account for cost-of-living salary increases, and for travel. Due to the budget restrictions, the principal investigators have personally contributed a substantial part of the expenses for Drosophila collecting. We hope that this situation can be corrected for next year.

UCD-34P-200X1

COLLEGE OF AGRICULTURAL AND
ENVIRONMENTAL SCIENCES
AGRICULTURAL EXPERIMENT STATION
DEPARTMENT OF GENETICS

DAVIS, CALIFORNIA 95616

July 30, 1973

U.S. Atomic Energy Commission
San Francisco Operations Office
2111 Bancroft Way
Berkeley, California 94704

Gentlemen:

Enclosed are six copies of the Progress Report and Request
for Extension of Contract AT(04-3)-34, entitled "Genetic Structure
of Natural Populations".

Sincerely yours,

Theodosius Dobzhansky
Co-Principal Investigator

Francisco J. Ayala
Co-Principal Investigator

R. W. Allard, Chairman
Genetics Department

A. G. Marr, Dean
Research Development

MASTER

DISTRIBUTION OF THIS DOCUMENT IS UNLIMITED

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UNIVERSITY-~~TYPE~~ CONTRACTOR'S RECOMMENDATION FOR
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UCD-34P200 x1

2. TITLE

"Genetic Structure of Natural Populations"

3. TYPE OF DOCUMENT (Check one):

- ☒ a. Scientific and technical report
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Title of conference _____

Date of conference _____

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6. SUBMITTED BY: NAME AND POSITION (Please print or type)

Dale J. Cook, Director, Office of Information Services

Organization

U.S. Atomic Energy Commission

Signature

Date

Aug. 16, 1973

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8. PATENT CLEARANCE:

- ☒ a. AEC patent clearance has been granted by responsible AEC patent group.
☐ b. Report has been sent to responsible AEC patent group for clearance.
☐ c. Patent clearance not required.

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