A METHOD FOR THE DIRECT STUDY OF NATURAL SELECTION

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(With One Text-figure)

I. INTRODUCTION

The evolutionary importance of what we now call genes was recognized by Mendel himself and by the geneticists who rediscovered his principles. In recent years the bearing of genetics on evolutionary problems has been elaborated from two sources. One is the cytological work of Darlington (1936), Dobzhansky (1937), and others. The other is the analytical contributions of Fisher (1931), Haldane (1932), Sewall Wright (1931, 1934), and Tschetverikov (1926), to a reconsideration of the role of natural selection.

More recently, experimental work on the selection of genes has been undertaken. The genetic analysis of free-living populations of *Drosophila* (Tschetverikov, 1927; Dubinin and co-workers, 1934, 1936; Gordon, 1936; Gordon *et al.* unpublished) has provided evidence for the absence of sex-linked, in contradistinction to autosomal, mutant genes which have been shown to be extremely common in the heterozygous condition. Since the main difference is the absence of protection by the dominant wild-type allelomorph, this may be regarded as evidence of more complete elimination of sex-linked mutant genes by natural selection. None the less there is justification for the statement by Robson & Richards (1936) that "the direct evidence for the occurrence of natural selection is very meagre and carries little conviction".

The authors last named accept the criteria advocated by Pearl (1930), who lays down the following:

(*a*) Proof of somatic differences between survivors and eliminated.
(*b*) Proof of genetic differences between survivors and eliminated.
(*c*) Proof of effective time of elimination.
(*d*) Proof of somatic alteration of the race.
(*e*) Proof of the genetic alteration of the race.

The aim and scope of investigations on free-living populations must be distinguished from those of another class of researches which deal with the relative

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viabilities of mutants in *D. melanogaster*. Notable among the latter are the investigations of Gonsalez (1923), and Timofeeff-Ressovsky (1934). Such experiments as these have been carried out under laboratory conditions and furnish no data concerning the behaviour of mutant types in natural populations. In nature, selection does not operate on isolated strains. It is concerned with imperfectly balanced mixed populations.

Laboratory work on spread, elimination or persistence of mutants in competition with wild type has been carried out with somewhat different techniques by Cheruwimov (1932) and by l'Heritier & Teissier (1937). Their experiments lead to seemingly conflicting conclusions. Cheruwimov found that *Bar* maintained itself in a balanced population. L'Heritier & Teissier found that it was rapidly replaced by wild type when small numbers of wild type were introduced into a *Bar* strain.

The experiments to be described in what follows are an attempt to devise methods for obtaining a direct demonstration of natural selection, and for comparing laboratory and field results.

II. MATERIALS AND METHODS

Sturtevant (1921) expressed the view that *Drosophila melanogaster* is not endemic to North America and that it is reintroduced each year from food stores, etc. In the course of sampling at the Field Station of the Department of Entomology, Imperial College of Science at Slough in 1933 and 1934, *D. obscura* and *D. subobscura* appeared before the end of May, while *D. melanogaster* did not appear before the middle of July. The relative incidence of *D. melanogaster* increased

Table I

Date	*D. melanogaster*	*D. obscura* and *D. subobscura*
23 May	0	12
29 May	0	20
10 June	0	45
20 June	0	32
27 June	2	16
3 July	0	6
13 July	1	16
18 July	1	11
25 July	3	54
8 August	3	4
22 August	9	20
31 August	7	86
19 September	7	31
30 September	1	27
7 October	12	36
14 October	11	66
21 October	4	66
28 October	21	54
4 November	1	26
11 November	0	2
19 November	0	1
25 November	0	0

considerably during the summer. Towards the autumn, *D. obscura* and *D. subobscura* again predominated, and later collections yielded *D. obscura* and *D. subobscura* only. Records obtained in 1936 go to confirm the view that *D. melanogaster* appears late and disappears early. In 1936, the peak period for *D. melanogaster* was October, but numbers fell away very rapidly.

Collections made in 1933 at Ross-on-Wye yielded *D. obscura* and *D. subobscura* only. In the summer of 1936 extensive trappings at Studland Bay yielded only one *D. melanogaster* (male) to about 800 *D. obscura* and *D. subobscura*. Collections at a locus in the New Forest yielded no *D. melanogaster* except in traps a few yards from the house, although *D. obscura* and *D. subobscura* were plentiful everywhere. Again in 1938 collections near Aberdeen yielded one *D. melanogaster* (male) to 220 *D. obscura* and *D. subobscura*. Seemingly, therefore, *D. melanogaster* is not endemic to this country and is eliminated each winter, to be repopulated from animals that survive indoors or are brought from abroad with the fruit trade.

Certain common species of *Drosophila* are easily trapped by preparing a banana agar medium well yeasted, and covering the mouth of the bottle with a wire gauze funnel with a narrow inlet (Fig. 1). If a *D. melanogaster* population of known constitution is released, the population can be sampled at any time. If a gene **A** is dominant to its allelomorph **a**, the possible genotypes with respect to the substitution **A–a** are **AA**, **Aa** and **aa**. The number of flies of genetic constitution **aa** can be ascertained by inspection. Wild type flies are of the constitution **AA** or **Aa**. If these are crossed to flies of constitution **aa**, then there are two possibilities:

(1) $\mathbf{AA} \times \mathbf{aa} = \mathbf{Aa}$ (all wild type).

(2) $\mathbf{Aa} \times \mathbf{aa} = \frac{1}{2}\mathbf{Aa} + \frac{1}{2}\mathbf{aa}$ ($\frac{1}{2}$ wild type and $\frac{1}{2}$ mutant).

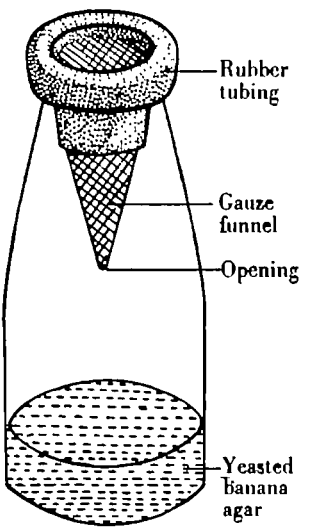

Fig. 1. Baited trap for *Drosophila*.

Matings of single trapped wild-type flies of either sex belonging to class 1 will never yield mutants among the progeny. Crosses between single trapped wild-type males from class 2 and recessives should yield approximately one-half mutant types. Crosses between recessive males and single trapped wild-type females from class 2, which in most cases will have been previously fertilized, will always yield some mutants, because the last fertilization is always effective (Nonidez, 1920; Dubinin, 1928).

III. RESULTS

In 1934 a release experiment was undertaken at Dartington Hall, Totnes. The locus was chosen on account of kind offers of assistance from Mr Pearson, and the encouragement of the managing director, Dr Slater. A population of 36,000 flies was put into large jars. These, together with their eggs, larvae and pupae which had accumulated while the stocks were being built up, were released in an orchard on

the estate on 15 May 1934. The constitution of the population was $\frac{1}{4}$**EE** : $\frac{1}{2}$**Ee** : $\frac{1}{4}$**ee**, where **E** is the wild-type allelomorph of **e**, ebony, a good viable laboratory mutant. Samples were taken from time to time.

Since the experiment was regarded as preliminary, and I was unable to go to Devon frequently, I relied on the kind offices of Mr Pearson, who was unable to spare much time for it. So the data are somewhat meagre. Towards the end of September, 128 days after the release, I made a more extended collection personally. The results of trapping after intervals stated are given in Table II. The only loci which yielded *D. melanogaster* were those in the neighbourhood of the place of release, the laboratory, and the food store. The two latter were about half a mile from the place of release. All the loci, however, yielded *D. obscura* and *D. subobscura*, which, as the Slough records show, are attracted by the same bait as *D. melanogaster*.

Table II

Locus	Place of release			Food store			All other loci		
No. of days after release	Ebony (ee)	Wild type (EE+Ee)	$\frac{Ee}{EE+Ee}$	Ebony (ee)	Wild type (EE+Ee)	$\frac{Ee}{EE+Ee}$	Ebony (ee)	Wild type (EE+Ee)	$\frac{Ee}{EE+Ee}$
10	72	357	12/21	0	0	—	0	0	—
24	0	14	3/4	0	0	—	0	9	1/2
34	1	3	1/2	0	20	7/8	0	2	1/1
41	0	1	1/1	0	0	—	0	0	—
51	0	0	—	1	1	1/1	0	2	2/2
63	0	1	0/1	0	0	—	0	0	—
80	0	1	1/1	0	0	—	1	0	—
93	0	0	—	0	27	6/11	0	21	19/19*
101	0	0	—	0	2	0/1	0	0	—
103	0	2	1/1	0	4	0/2	0	6	0/3
128	0	7	1/7	0	26	4/26	0	24	6/24

* Single trap.

$$\frac{Ee}{EE+Ee} = \frac{\text{Heterozygotes}}{\text{Total tested}}$$

The presence of flies at the food store and the laboratory can be accounted for by the traffic of fruit to the store, and transfer from the store to the neighbouring laboratory, where there are many scents. We see that the last date on which an ebony mutant was trapped was 80 days after release. 93 days after release, a group of wild-type flies from a single trap were all found to be heterozygous for ebony. Of nineteen wild-type flies tested from this trap, all gave ebony when mated to ebony stock. This would best be accounted for if they were sibs of which at least one of the parents was homozygous ebony. This would correspond to a homozygote present about 75 days after release.

The results of the final collection are summarized in Table III, with calculations of the frequency of the ebony gene q.

If p is the frequency of the gene **E**, q is the frequency of **e**, and $p+q=1$, the constitution of such a population mating at random may be written p^2**EE** + $2pq$**Ee** + q^2**ee**. This is $(1-q)^2$**EE** + $2q(1-q)$**Ee** + q^2**ee**. This relation describes the frequency of the

Table III. *Frequence of gene* **e** *at final collection* (q)

Locus	**EE** $=a$	**Ee** $=b$	q
Place of release	6	1	0·08 ± 0·07
Food store	22	4	0·08 ± 0·04
All other loci	18	6	0·14 ± 0·05
All loci	46	11	0·11 ± 0·03

zygotes at fertilization, and does not apply to the surviving adults of a population in which there is intensive selection. Most individuals who carry the mutant gene belong to the class **Ee** rather than to **ee**. If **ee** is much less viable—and this seems to be so—the estimate of q based on **ee** will diverge greatly from the true value of q based on **Ee**. If the total number of homozygous wild type in the sample is a, and if the total number of heterozygotes is b, then $\frac{(1-q)^2}{2q(1-q)}=\frac{a}{b}$, from which $q=\frac{b}{2a+b}$. The standard error of q can be obtained by the Method of Maximum Likelihood.

The likelihood equation is

$$L=a\log\frac{1-q}{1+q}+b\log\frac{2q}{1+2},$$

and since

$$\sigma_q^{-2}=\frac{d^2L}{dq^2},$$

σ_q^{-2} is found to be

$$\frac{q(1+q)^2(1-q)}{2n(1+q-q^2)},$$

where $n=a+b$, the total number of flies analysed. The value of q is given in Table III. For all loci, $q=0{\cdot}11\pm0{\cdot}03$.

This value of q is the gene frequency of ebony after several generations of selection in natural conditions. The corresponding value (q_0) in the initial generation exposed to selection was $\frac{1}{2}$. According to a well-known theorem of selection, the values of $q_1, q_2, q_3, \ldots, q_n$, the gene frequencies in successive discrete generations of complete selection (i.e. selection which eliminates *all* recessive parents), would be $1/3$, $1/4, 1/5, \ldots, 1/2+n$ after n generations. The experiment occupied sufficient time for the emergence of about six successive generations. Under conditions of complete selection without overlapping generations, we should anticipate that the final value of q should be about $1/8$. This value is close to that estimated from the observations recorded. Hence it is necessary to ask how overlapping of generations would increase or decrease the expectation deduced for high values of k, where k is the proportion of recessives eliminated before reaching maturity. Haldane (1927) has worked out the effect of overlapping of generations for slow selection, and has shown it to be similar to where generations do not overlap. Table IV has been calculated for fast selection ($k=1$, $\frac{3}{4}$ and $\frac{1}{2}$; 0, 10, 50 % overlapping). An arithmetic example for $k=\frac{1}{2}$, and 10 % overlap is given below Table IV. The values are the proportion q of the recessive gene in the parents of the $n+1$th generation.

Table IV

k	% overlap	Value of q						
		$n=0$	1	2	3	4	5	6
1	0	0·5	0·333	0·250	0·200	0·167	0·143	0·125
	10	0·5	0·333	0·257	0·209	0·176	0·152	0·134
	50	0·5	0·333	0·294	0·239	0·211	0·184	0·165
$\frac{3}{4}$	0	0·5	0·385	0·308	0·255	0·217	0·188	0·162
	10	0·5	0·385	0·311	0·259	0·221	0·193	0·171
	50	0·5	0·385	0·321	0·271	0·236	0·208	0·181
$\frac{1}{2}$	0	0·5	0·429	0·371	0·324	0·287	0·256	0·231
	10	0·5	0·429	0·367	0·319	0·281	0·254	0·228
	50	0·5	0·429	0·351	0·304	0·266	0·236	0·212

We start with

$$\tfrac{1}{4}\mathbf{EE}+\tfrac{1}{2}\mathbf{Ee}+\tfrac{1}{4}\mathbf{ee}.$$

In the parents of this generation, $p_0=0{\cdot}5$, $q_0=0{\cdot}5$. Now, if $\frac{1}{2}$ of the recessives are eliminated, the breeding population is

$$\frac{\tfrac{1}{4}\mathbf{EE}+\tfrac{1}{2}\mathbf{ee}+\tfrac{1}{8}\mathbf{ee}}{\tfrac{8}{7}}, \text{ i.e. } \tfrac{2}{7}\mathbf{EE}+\tfrac{4}{7}\mathbf{Ee}+\tfrac{1}{7}\mathbf{ee},$$

whence $$p_1=(\tfrac{2}{7}+\tfrac{2}{7}), \quad q_1=(\tfrac{2}{7}+\tfrac{1}{7}).$$

Offspring of this is

$$(0{\cdot}571^2\mathbf{EE}+2\times 0{\cdot}571\times 0{\cdot}429\mathbf{Ee}+0{\cdot}429^2\mathbf{ee}),$$

i.e.

$$0{\cdot}3265\mathbf{EE}+0{\cdot}4898\mathbf{Ee}+0{\cdot}1837\mathbf{ee}.$$

Now $\frac{1}{2}$ of the recessives are eliminated, and 10 % of phenotypically wild type of previous generation survive; therefore effective breeding population is

$$0{\cdot}9(0{\cdot}3265\mathbf{EE}+0{\cdot}4898\mathbf{Ee}+\tfrac{1}{2}0{\cdot}1837)+0{\cdot}1(0{\cdot}3333\mathbf{EE}+0{\cdot}6667\mathbf{Ee}),$$

i.e.

$$0{\cdot}32718\mathbf{EE}+0{\cdot}50749\mathbf{Ee}+0{\cdot}08267\mathbf{ee},$$

where

$$p=\frac{32718+25375}{32718+50749+8267}, \quad q=\frac{25375+8267}{32718+50749+8267},$$

$$p_2=\frac{58093}{91735}=0{\cdot}633, \quad q_2=\frac{33642}{91735}=0{\cdot}367.$$

etc., etc.

The following assumptions have been made:

(1) *Overlapping is confined to the phenotypically wild type of both genetic kinds.*

This will be true for very high values of k, for if the recessive is mainly eliminated before reaching maturity, then it is unlikely that any will survive to overlap with the succeeding generation. For low values of k, this would mean increasing the value of k over the whole life cycle, and the assumption is clearly not justifiable.

(2) *Fertility is independent of age.*

It will be seen that for $k=1$, and $k=\frac{3}{4}$, the effect of overlapping is to reduce the rate of elimination of **e**, but for $k=\frac{1}{2}$ it increases the rate of elimination. In the latter instance the assumption of no overlapping of the recessives is not justifiable.

The conclusion which emerges from this is that the final q estimated from the observed frequencies of the genotypes is very close to q estimated from the initial value of q in the population with complete selection of the recessive. In short, the simplest conclusion to be drawn is that, under the natural conditions studied, selection for the recessive type was complete. It must however be borne in mind that there is indirect evidence for the mating of a homozygous ebony individual. If such occurrences are found to be frequent, then the question of selection of the heterozygote and of assortative mating is definitely raised. The possibility of dilution from other sources has not been definitely excluded, since the trappings were not very extensive, but the absence of *D. melanogaster* at loci distant from the place of release would tend to exclude this.

IV. DISCUSSION

The experiment performed in 1934 was mainly intended to be exploratory. Other populations were released in 1935 but, owing to a prolonged drought in the spring and early summer immediately after release, these were unsuccessful because all the *D. melanogaster* were wiped out. I subsequently found that mixed deciduous woods with thick undergrowth and in damp situations were the most suitable *Drosophila* areas. In 1936, the woods at Studland Bay were investigated and found to be suitable, but, owing to my removal to Aberdeen in the summer of 1937, I was unable to continue the work.

If the locus of the experiment has been thoroughly tested previously to release, the question of dilution can be largely eliminated. In addition, methods of internal control can be developed. Dilution can be excluded if the population is released at a central point in the area and *D. melanogaster* is found in collections (*a*) ***initially***, at the place of release and there alone: (*b*) ***subsequently***, in positions radiating therefrom.

The experiment described is not on a sufficiently large scale to warrant any conclusions of a detailed nature concerning the viability of the gene *ebony*, nor to throw any light on the fitness of the heterozygote. The latter question is also raised by the anomalous results on *ebony* by l'Heritier and Teissier (1937) who found that eventually ebony remained at a constant proportion in the population. If no dilution took place, the experiment described conforms to Pearl's requirements, stated earlier.

V. SUMMARY

1. Decisive experiments on selection of mutants of *Drosophila melanogaster* can be carried out under natural conditions in Britain where this species is not indigenous.

2. This communication records an experiment in which a balanced population containing 25 % ebony mutants was released in South Devon.

3. The frequency of the genotype among the descendants was estimated after a period equivalent to six discrete generations, by testing trapped flies, most of which were wild type in appearance, for heterozygosis.

4. The frequency estimated was very close to what would be deduced on the assumption that elimination of the recessive type before maturity was complete.

My thanks are due to Dr Slater and Mr Pearson of Dartington Hall, Totnes, for their assistance. I am also indebted to Prof. J. B. S. Haldane and Prof. Lancelot Hogben for advice and criticism. I also wish to thank Capt. C. Diver for assistance in collecting *Drosophila* at Studland Bay. The expenses of collecting at Studland Bay and at Slough in 1936 were defrayed by a grant from the Royal Society. The collections at Dartington Hall in the summer of 1935 were undertaken when I was in receipt of a personal grant from the Rockefeller Foundation.

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Note added in proof. A paper by Olenov *et al.* (1937) was overlooked. These workers released numbers of the mutant *Bar* into areas, mainly cellars, where *Drosophila melanogaster* was already present. They conclude that *Bar* is eliminated very rapidly, i.e. $k = 1$ approx.

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