ADAPTIVE PROPERTIES OF CARRIERS OF CERTAIN GENE ARRANGEMENTS IN *DROSOPHILA PSEUDO-OBSCURA*

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INTRODUCTION

RELATIVE frequencies of the different gene arrangements in *Drosophila pseudoobscura* vary in different geographic regions, often forming more or less regular geographic clines (Dobzhansky and Epling, 1944). In some localities, such as Piñon Flats and Andreas Canyon on Mount San Jacinto in California, the frequencies of certain gene arrangements in the third chromosome vary from season to season. Thus the Standard (ST) gene arrangement is most frequent during autumn and winter, decreases in frequency during spring, and increases during the hot part of the summer. The Chiricahua gene arrangement (CH) shows a cycle opposite to Standard, while Arrowhead (AR) is relatively constant in frequency (Dobzhansky, 1943).

These facts suggest that the carriers of the different gene arrangements possess different adaptive values in different environments. This inference has been confirmed in experiments in which artificial populations containing these gene arrangements were kept in population cages. At temperatures above 21° C., populations which are mixtures of Standard and Chiricahua chromosomes reach equilibria at frequencies of about 70 per cent. ST and 30 per cent. CH, regardless of what the frequencies in the original mixture might have been. At low temperatures (close to 16°) the relative frequencies of the gene arrangements present in the initial population are preserved more or less indefinitely. Wright and Dobzhansky (1946) and Dobzhansky (1947) have interpreted these results to mean that the ST/CH heterozygotes possess the highest adaptive value, while ST/ST homozygotes are less, and CH/CH least well adapted. Essentially similar phenomena are observed if the Arrowhead gene arrangement is used in competition with either Standard or Chiricahua.

The above experiments demonstrate the action of natural selection on the carriers of the different gene arrangements, but they do not permit to define either the stage of the life cycle or the precise

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mechanisms of the action of the selective process.   To be sure,
Dobzhansky (1947) has recently shown that the Hardy-Weinberg
equilibrium of the structural homo- and heterozygotes is approximately
realised among the eggs deposited in the population cages, while
among the adults developed from these eggs a very significant excess
of heterozygotes is found.   This indicates a differential mortality of
homozygotes at some time between the egg and the adult stage.
The purpose of the experiments described in the present article has
been, then, to find which physiological characteristics of the carriers
of the different gene arrangements influence differentially the adaptive
values of the latter, and thus to delimit the ecological niches to which
these gene arrangements are adapted.

MATERIAL AND TECHNIQUE

All flies used in the present experiments were descendants of
impregnated females collected at Piñon Flats, Mount San Jacinto,
in the spring of 1946.   The main attention has been concentrated on
studies of physiological properties of flies homozygotes for Standard,
Arrowhead, or Chiricahua gene arrangements, although in some
experiments heterozygotes have also been examined.   Since extreme
environmental conditions may frequently be the limiting factors in
the life of natural populations, the performance of the flies was studied
at very low and very high temperatures and humidities.   Because of
the technical difficulties, the first experiments here reported are
concerned with physiological properties of adult flies and of pupæ,
while those of larvæ and of eggs will be studied later.   Thus we have
examined the hatching percentages of the pupæ, and the longevity
of the adults at different temperatures and humidities.   Reasonably
constant humidity conditions have been obtained in desiccators with
distilled water (100 per cent. humidity), with $CaCl_2$ (0 per cent.),
or with oversaturated solutions of K_2SO_4, NaCl, and NaBr for the
relative humidities of 92, 76 and 56 per cent. respectively (after
Ludwig and Landsman, 1937).

The purpose of the experiments to be described has been to study
the properties of the carriers of ST, CH, and AR chromosomes as
classes recognised by their inversions and without regard to their
allele-content.   This distinction is important, because the chromo-
somes of wild flies very often carry recessive viability and other
modifiers, and consequently the properties of their carriers vary a
great deal from chromosome to chromosome.   The experimental flies
or pupæ were, accordingly, obtained by intercrossing at least ten
different strains with the same gene arrangement, except when
otherwise specified.   In such a way, the structural homozygotes or
heterozygotes obtained were always genic heterozygotes.   A more
detailed explanation of the relevant facts has been given by Wright
and Dobzhansky (1946).

INFLUENCE OF HUMIDITY ON THE VIABILITY OF THE PUPÆ

One of the environmental factors which is doubtless important in the life of *D. pseudoobscura*, and which has not been at all adequately studied, is the humidity of the air.   Ellwyn (1917) found that the relative humidity affects the survival of *D. melanogaster* pupæ, while Geisler (1942) showed that pupæ of the wild type and of certain mutants of the same species react differently to low humidities.

Pupæ homozygous for the ST, CH, or AR gene arrangements were placed in vials in desiccators kept at different relative humidities. Two series of experiments of this type were performed, and their
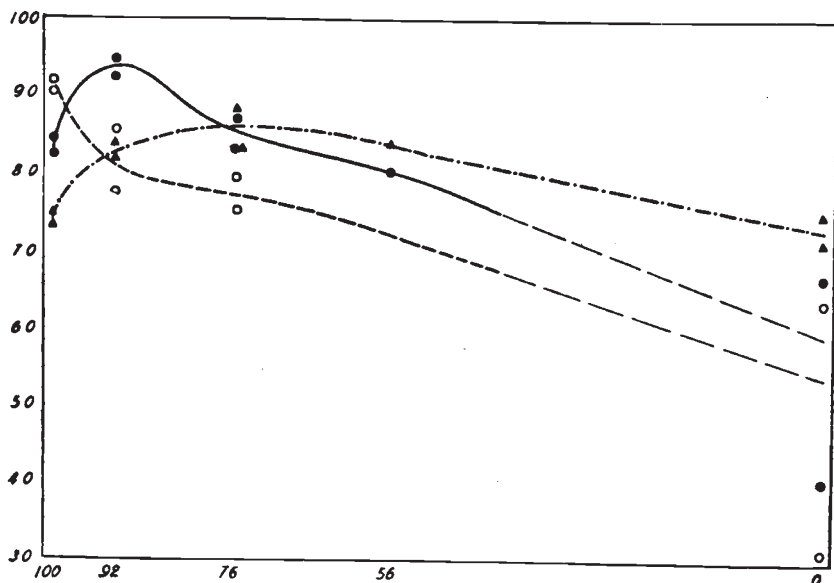

Fig. 1.—Influence of the relative humidity of the air on the hatching frequencies of pupæ of three different chromosomal types.   Ordinates : hatching percentages ; abscissæ : relative humidities.   Solid circles : ST/ST ;   open circles : CH/CH ;   triangles : RA/AR.

results have been published in detail elsewhere (Heuts, 1947*b*). Suffice it to note here that, as shown in fig. 1, CH pupæ give the highest proportion of hatchings at the 100 per cent. humidity, while ST, and especially AR, are superior to CH at low humidities.   Each point in the graph in fig. 1 is based on studying the hatching percentage in from 300 to 700 pupæ ;   in the two series of experiments pupæ of the same genotype gave rather different hatching percentages at zero per cent. humidity, but the relative positions of the points for ST, CH, and AR agree very well in both experiments.

The differential mortality of the pupæ thus favours Chiricahua homozygotes at the highest humidity, and Standard and Arrowhead at lower ones.   The superiority of Chiricahua observed is especially interesting, because, in all the population cages studied by Dobzhansky,

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Chiricahua homozygotes were found to be either inferior or, at most, equal in adaptive value to Standard and Arrowhead homozygotes. Yet the frequency of Chiricahua chromosomes increases during the spring months in the Piñon Flats population, thus indicating that in certain environments the carriers of these chromosomes are superior to the others. Whether or not the humidity is the environmental agent responsible for the changes observed in the Piñon Flats population is, of course, another question.

INFLUENCE OF HUMIDITY ON THE VIABILITY OF THE ADULTS

The influence of humidity on the longevity of *Drosophila* flies has been studied by several authors. Most experiments have been concerned with the longevity in the absence of food ; such experiments are technically simpler than those involving feeding of the flies. Furthermore, a starving adult insect is an energetically closed system, and its longevity becomes a measure of the rates of loss of the reserve materials, including water. Kalmus (1941) found that mutants which increase the body pigmentation in several species of *Drosophila* show greater longevities at low humidities and at a temperature close to 25° C. than do mutants with lighter body pigmentation. The length of life is correlated with the rate of loss of the body weight. In another experiment (Kalmus, 1945) the ebony mutant of *D. melanogaster* proved to be superior in competition with the wild type at low humidities and temperatures. This agrees with the findings of Greiff (1940), who found that ebony lives longer than the wild type at 79·3 per cent. of relative humidity and 25° C. Lilleland (1938) compared the longevity of *D. pseudoobscura* and *D. persimilis*, and found the former species to live longer than the latter, especially at low humidities. Strains of the same species coming from different localities proved to be often significantly different in their reactions.

The material for the experiments to be described was prepared as follows : Stender jars with food were inserted in population cages with flies homozygous for ST, CH, and AR chromosomes respectively. The eggs deposited within twenty-four hours were distributed in a number of regular culture bottles with food, so that overpopulation was avoided. The development, as well as the oviposition, took place at 19° C. As soon as pupæ appeared in the bottles, they were extracted by means of a needle, only light, and hence very young, pupæ being taken. Pupæ coated with the food medium were discarded. The pupæ to be used were placed on pieces of filter paper in sterilised vials covered with cheese cloth held in place by a rubber band. The development of the pupæ took place in a constant temperature room at 25° C., and under desired humidity conditions, namely, between 70 and 75 per cent. in one, and at 100 per cent. in another, experiment. Hatching flies were collected at six-hour intervals, and placed in test tubes 10 c.c. in volume, five flies per tube,

sexes being separated.   The test tubes were numbered and lined against the walls of the desiccators, so that the flies in the vials could be seen through the glass of the desiccator without opening it ; the vials were held in place by white cardboard, which made the observation easier.   The counts of the surviving flies were made at six-hour intervals, at 6 a.m., 12 noon, 6 p.m., and 12 midnight.

The results of the first experiment, in which the pupæ developed at 70 to 75 per cent. humidity, are reported in table 1 and fig. 2.   It

TABLE 1

Mean longevity (in hours) at different relative humidities of adult flies hatched from pupæ which developed at 70 to 75 per cent. of relative humidity

Humidity	Karyotype	N	Longevity	Significance
0 per cent.	CH/CH	70	$33 \cdot 17 \pm 0 \cdot 40$	} $P < 0 \cdot 01$
0 ,,	AR/AR	70	$35 \cdot 48 \pm 0 \cdot 45$	
0 ,,	ST/ST	70	$33 \cdot 76 \pm 0 \cdot 68$	} $P = 0 \cdot 04$
76 ,,	CH/CH	70	$53 \cdot 66 \pm 1 \cdot 00$	} } $P < 0 \cdot 01$
76 ,,	AR/AR	70	$61 \cdot 72 \pm 1 \cdot 07$	$P < 0 \cdot 01$
76 ,,	ST/ST	70	$61 \cdot 63 \pm 1 \cdot 22$	
100 ,,	CH/CH	70	$60 \cdot 34 \pm 0 \cdot 87$	} } $P < 0 \cdot 01$
100 ,,	AR/AR	70	$67 \cdot 56 \pm 1 \cdot 09$	$P < 0 \cdot 01$
100 ,,	CH/CH	70	$66 \cdot 85 \pm 1 \cdot 04$	

TABLE 2

Mean longevity (in hours) at different relative humidities of adult flies hatched from pupæ which developed at 100 per cent. of relative humidity

Humidity	Karyotype	N	Longevity	Significance
0 per cent.	CH/CH	50	$39 \cdot 12 \pm 0 \cdot 70$	...
0 ,,	ST/ST	50	$39 \cdot 72 \pm 0 \cdot 79$	...
0 ,,	AR/AR	50	$40 \cdot 44 \pm 1 \cdot 03$	...
76 ,,	CH/CH	50	$65 \cdot 88 \pm 1 \cdot 29$	
76 ,,	AR/AR	50	$62 \cdot 48 \pm 1 \cdot 86$	} $P = 0 \cdot 035$
76 ,,	ST/ST	50	$62 \cdot 04 \pm 2 \cdot 26$	...
100 ,,	CH/CH	60	$66 \cdot 30 \pm 1 \cdot 37$	
100 ,,	AR/AR	60	$62 \cdot 50 \pm 1 \cdot 35$	} $P = 0 \cdot 05$
100 ,,	ST/ST	60	$64 \cdot 30 \pm 1 \cdot 25$	...

can be seen that at 100 and 76 per cent. humidities the longevity of Standard and Arrowhead flies is about equal, while Chiricahua flies live fewer hours than the others.   At zero humidity the longest lived flies are Arrowhead, while Standard and Chiricahua do not differ significantly from each other.   It should be noted that these results are obtained with flies hatched from pupæ which developed at 70 to 75 per cent. humidity ;   as shown above, this humidity is more favourable for the development of Arrowhead and Standard pupæ than it is for Chiricahua pupæ (fig. 1).   However, at 100 per cent.

humidity, a greater proportion of Chiricahua than of Standard and Arrowhead pupæ give rise to adult flies. Accordingly, the experiment on the longevity of the adults at different humidities was repeated, using adults hatched from pupæ developed at 100 per cent. humidity. All other conditions in the two experiments were alike. The results are reported in table 2 and in fig. 2.

In the second experiment the highest longevity at 100 per cent. humidity has been found in Chiricahua flies (66·3 hours). Standard flies do not differ significantly from Chiricahua, while Arrowhead
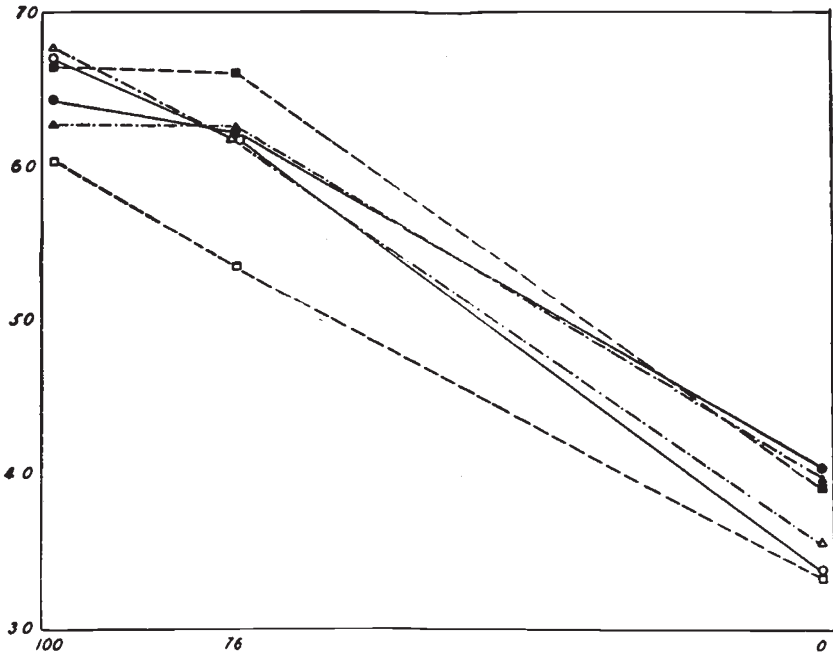

FIG. 2.—The average longevity of adults of three different inversion types in three different humidities. Circles : ST/ST ; squares : CH/CH ; triangles : AR/AR. Solid symbols : adults coming from pupæ developed in 100 per cent. humidity ; open symbols : adults coming from pupæ developed in 70 to 75 per cent. humidity.

flies seem to die in a shorter time (62·5 hours). At 76 per cent. humidity, Chiricahua flies live longer than Arrowhead and Standard, which do not differ from each other. At 0 per cent. humidity no significant differences are apparent. A comparison of the survival rates of pupæ and of adults at different humidities (figs. 1 and 2) shows that these rates are clearly interrelated. Indeed, at 100 per cent. humidity Chiricahua pupæ are superior to the others, and the flies hatched from these pupæ are relatively more resistant in their adult life than pupæ of the same genotype developed at lower humidities. A carry-over from the larvæ to the adult was observed also by Alpatov and Pearl (1929), who found that *D. melanogaster* adults vary in longevity depending upon the temperature at which the larvæ had developed.

LONGEVITY OF ADULT FLIES IN RELATION TO TEMPERATURE

Variations in response to temperature are well known to exist in species of *Drosophila*. Thus Timofeeff-Ressovsky (1934) found that the bobbed mutant in *D. funebris* is more viable than the wild type at high temperatures, but at low ones the conditions are reversed. Populations of the same species which live in different parts of the distribution area react differently to temperature (Timofeeff-Ressovsky, 1935). Dubinin and Tiniakov (1946*a*) placed adult flies homozygous and heterozygous for certain inversions at temperatures fluctuating between −2° and +3° C. After two and a half months, flies homozygous for the Standard gene arrangement were found to have survived better than those homozygous or heterozygous for the inversions. These findings can be correlated with the cyclic changes in the frequencies of the inversions observed by Dubinin and Tiniakov (1946*b*) in populations of *D. funebris* at different seasons. The results of the experiments of Wright and Dobzhansky (1946) and of Dobzhansky (1947) on gene arrangements of *D. pseudoobscura* have already been mentioned. We have examined the longevity of homozygotes and heterozygotes for ST and CH chromosomes at two extreme temperatures, one between 0° and 4° C. and the other between 28° and 30° C., approximately corresponding to the temperatures encountered by the flies in their natural habitats during the hibernation and during the hot part of the summer respectively.

The flies to be used in the following experiments were raised in population cages at 19° C. Flies were extracted from the cages at approximately forty-eight-hour intervals. Seventy-five individuals were placed together in culture bottles with regular amounts of the culture medium. The sexes were not separated. Counts of the surviving flies were made at weekly intervals. In the high temperature series the survivors were transferred to fresh bottles every week. The humidity conditions are not exactly known, but in bottles with food a high relative humidity normally prevails. In the low temperature series, condensation water was frequently present in the bottles, indicating humidity close to the saturation point.

The experiments at 28° to 30° were started with 375 flies of each of the three karyotypes, ST/ST, ST/CH, and CH/CH. The results obtained are shown in table 3. The average longevities, in days, are as follows :—

$$ST/ST \quad . \quad . \quad . \quad . \quad . \quad 26 \cdot 39 \pm 0 \cdot 25$$
$$ST/CH \quad . \quad . \quad . \quad . \quad . \quad 23 \cdot 45 \pm 0 \cdot 28$$
$$CH/CH \quad . \quad . \quad . \quad . \quad . \quad 21 \cdot 63 \pm 0 \cdot 14$$

Standard homozygotes are clearly superior to the heterozygotes, and these are in turn superior to the CH/CH homozygotes. This finding differs from those of Dobzhansky and Wright quoted above, who found the adaptive value of the heterozygotes to be higher than

those of either homozygote ; however, these authors were concerned
with over-all selective advantages and disadvantages displayed by the
different karyotypes in population cages kept at temperatures close to
25°, so that the experiments are by no means comparable.   In our

TABLE 3

Numbers of surviving adult flies kept at 28° to 30°

Weeks	ST/CH	ST/ST	CH/CH
1	368	372	373
2	351	361	363
3	116	270	47
4	49	35	0
5	0	0	...

experiments the superiority of Standard homozygotes is attested not
only by their greater longevity but also by their greater fertility.
In all the bottles kept at 28° to 30°, eggs were deposited by the flies.
However, only very few larvæ appeared in the bottles with Chiricahua
homozygotes, and none of them pupated.   Therefore CH chromosomes
may be said to be lethal when homozygous at 28° to 30°.   The
Standard homozygotes produced, during the four weeks which the
experiment lasted, 714 F_1 and F_2 offspring.   The heterozygous flies
produced at the same time only 65 F_1 flies.   Of these, 20 flies were
outcrossed at room temperature to normal flies homozygous for ST
chromosomes.   All but two crosses proved to be sterile ; the larvæ
from the two fertile crosses were examined cytologically and found to
be ST homozygotes.

The experiments at the " hibernation " temperature 0° to 4° C.
were started with 675 flies of each karyotype.   The results are
summarised in table 4 and fig. 4.   The average duration of life,
in weeks, for each of the three karyotypes is as follows :—

ST/ST   .   .   .   .   . $15 \cdot 71 \pm 0 \cdot 14$
ST/CH   .   .   .   .   . $17 \cdot 24 \pm 0 \cdot 18$
CH/CH   .   .   .   .   . $14 \cdot 05 \pm 0 \cdot 16$

In all three types the duration of life at low temperature is
surprisingly high in comparison with *D. funebris*, in which, according
to Dubinin and Tiniakov (1946*a*), very few survivors are left after
about ten weeks of hibernation.   If our data are indicative, *D. pseudo-
obscura* withstands much longer periods of hibernation without much
risk of extinction.   The most long-lived type is clearly the ST/CH
heterozygote, the Standard homozygote is next, while the Chiricahua
homozygote is relatively short-lived.   How great a selective action
may result from these differences in longevity is clear from inspection
of table 4 ; after fifteen weeks of hibernation less than half of the

initial number of Chiricahua flies are alive, contrasted with almost three-quarters of the heterozygotes. After eighteen weeks the number of surviving heterozygotes is almost twice that of Standard, and more than three times that of Chiricahua homozygotes. After twenty weeks the heterozygotes are four to five times more frequent than the homozygotes.

TABLE 4

Numbers of surviving adult flies kept at 0° to 4°

Weeks	ST/CH	ST/ST	CH/CH
1	669	675	670
2	666	675	658
3	660	670	642
4	654	669	632
5	649	666	621
6	643	663	616
7	637	659	607
8	633	654	597
9	624	638	575
10	613	628	539
11	586	602	492
12	568	547	453
13	547	495	401
14	526	435	344
15	495	368	294
16	443	296	237
17	367	210	145
18	294	156	90
19	253	108	69
20	169	40	34
21	105	24	23
22	54	16	15
23	29	9	7
24	13	1	0
25	8	0	0
26	4	0	0
27	3	0	0
28	2	0	0
29	2	0	0
30	2*	0	0

* The experiment was finished at this time ; in the calculation of the mean longevity (see page 70) the surviving flies were considered as having died during the 31st week.

Mr Bruce Wallace has performed a longevity experiment with the same three chromosomal types, using, however, a different experimental procedure, and has generously permitted quotation of the following data secured by him. Flies were raised at room temperature in usual culture bottles. Several strains of each chromosome were intercrossed. When the flies hatched, the sexes were separated, and groups of about 100 flies of a given chromosomal constitution and sex were placed in empty half-pint bottles. A paper spoon with the food medium was inserted in each bottle three times a week for about twenty-four hours. During the intervals between the feedings the flies were starving. The bottles with the flies were
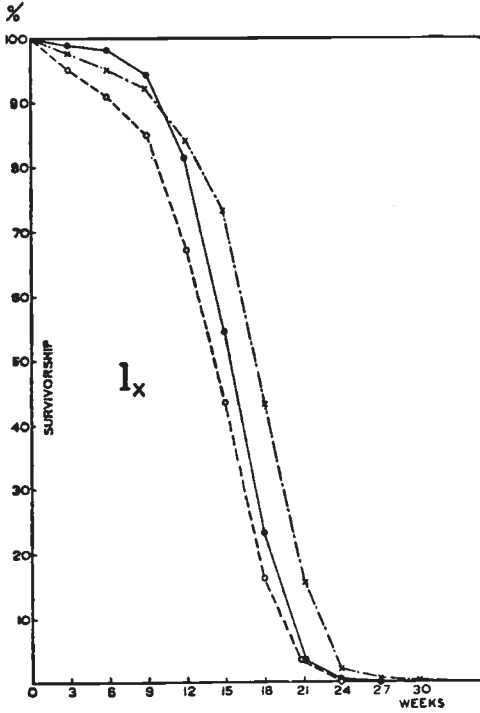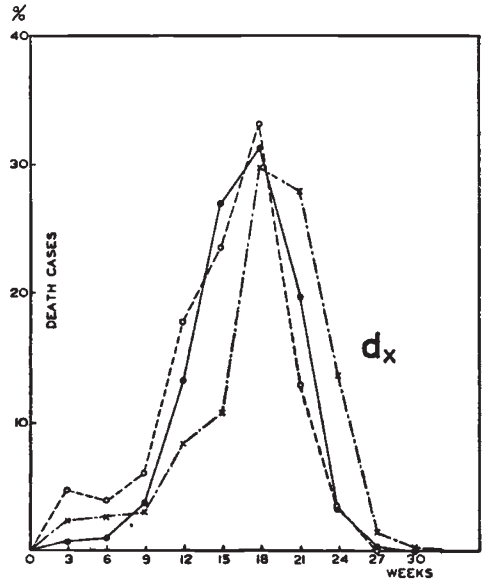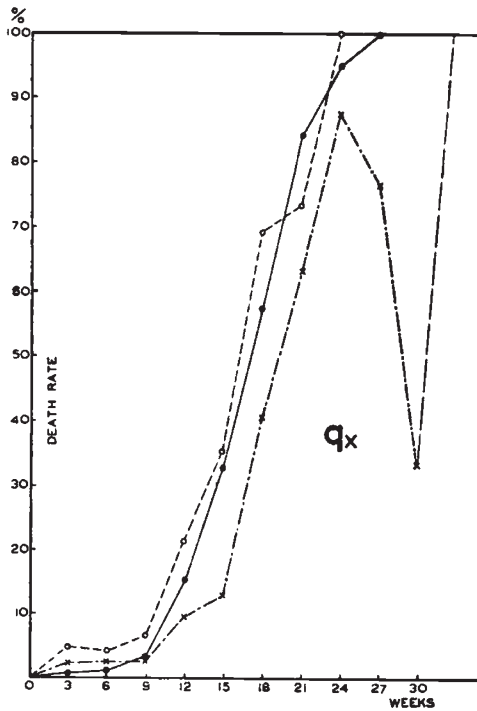
FIG. 3*a*.

FIG. 3*b*.

FIG. 3*c*.

FIG. 3.—Life table curves of the three chromosomal types at 4°, from table IV : (*a*) survivorship (lx) ; (*b*) death curve (dx) ; (*c*) death-rate (qx). Solid circles : ST/ST ; open circles : CH/CH ; crosses : ST/CH.

kept in an incubator at 25° C. The average duration of life under these conditions proved to be as follows (in days) :—

ST/ST .	.	.	.	.	21·82±0·33
ST/CH .	.	.	.	.	21·43±0·33
CH/CH .	.	.	.	.	18·62±0·36

The longevity of homozygous Standard flies is the same as in heterozygotes, while homozygous Chiricahua live significantly fewer days. It may be noted that in our experiments at 28° to 30°, Standard homozygotes live longer than do the heterozygotes ; at 0° to 4° the opposite relationship holds, since the heterozygotes are clearly longer-lived than the Standard homozygotes.   In Mr Wallace's experiment carried out at 25°, the heterozygotes and the Standard homozygotes are alike in longevity.   Although these three series of experiment were carried out under conditions diverse enough to make their results not strictly comparable, it is tempting to infer that the heterozygotes are intermediate in longevity between the two homozygotes at high temperatures, but gradually become equal to the better homozygote, and finally superior to both homozygotes, as the temperature becomes lower.

DISCUSSION

The experiments described in this article show that *D. pseudoobscura* carrying different gene arrangements are clearly different in their physiological properties. Thus some of the physiological characteristics of the fly are for the first time shown to be correlated with its chromosomal structure. Furthermore, the physiological properties involved are important in the adaptation of the insect to its natural environments. Indeed, both temperature and humidity requirements are probably limiting factors in many potential habitats in the distribution area of the species. Flies with Standard, Arrowhead, and Chiricahua chromosomes each possess temperature and humidity preferences which make them adapted to different ecological niches, each of the chromosomal types being superior to the other under some, and inferior under other conditions.

The question immediately arises, what bearing have the physiological differences observed between carriers of different gene arrangements on the cyclic changes observed in the Piñon Flats population by Dobzhansky (1943), and on changes taking place in the artificial populations kept in population cages (Wright and Dobzhansky, 1946 ; Dobzhansky, 1947). In agreement with the findings of these authors, our observations prove that one of the stages of the life cycle of the fly at which a selective process may take place lies in the pupal stage. Since our experiments did not involve larvæ, no conclusions can be drawn regarding the possible differential viability in the larval stage. The differential viability of the pupæ at different humidity conditions is more important than it seems at

first sight, since the adults hatched from the pupæ kept at optimal conditions show a greater longevity than those hatched from pupæ which developed in unfavourable environment. It is possible, although not proven, that other physiological functions of the adult fly, for example its fecundity, may be likewise affected by conditions during the pupal stage. In any case, the fact that each chromosomal type has an ecological niche in which it is superior to other types agrees very well with the observation that in the natural habitat the seasonal changes are cyclic and reversible.

Whether or not the conditions during the pupal life would influence the longevity of the adult in the presence of food remains to be determined. This seems, however, likely to be the case, because the physiological differences between the chromosomal types suggest shifts in the water balance regulatory system of the insect concerned. The findings at 100 per cent. humidity may indicate differences in permeability of the epidermal tissues, or differences in active water excretion abilities, between the carriers of the different karyotypes. If so, the increase in the frequency of Chiricahua in the Piñon Flats population observed every spring may be connected with the relatively high humidity prevailing during this season, and with the dryness prevailing during the summer months.

The known geographic distribution of the gene arrangements in *D. pseudoobscura* also agrees with the finding that Arrowhead is superior to the other chromosomal types at low, and Chiricahua at high, humidities. In the driest part of the distribution area of the species, namely in Arizona, Utah, and western New Mexico, populations are found in which Arrowhead is the predominant, or even the only one, gene arrangement. Chiricahua, and other members of the Santa Cruz phylad of gene arrangements, is relatively more common on the Pacific Coast (Dobzhansky and Epling, 1944).

It may be concluded that the water concentration in the environment of the fly, defined as it is by temperature and humidity, plays an important rôle in the evolution of the species *D. pseudoobscura*, just as it does, by way of temperature and osmotic pressure in the case of the fish *Gasterosteus aculeatus* according to Heuts (1947a).

SUMMARY

1. The relative humidity during the pupal stage at 25° C. affects differentially the carriers of different gene arrangements in *D. pseudoobscura*. Pupæ homozygous for the Arrowhead gene arrangement give higher hatching percentages at low humidities, and Chiricahua homozygotes at high humidities. Standard homozygotes are intermediate.

2. The humidity conditions during the pupal stage affect differentially the longevity of the adult flies hatching from these pupæ of a given chromosomal type.

3. The duration of life in the presence of food at high temperatures (20° to 30° C.) is highest in flies homozygous for Standard chromosomes, lowest in Chiricahua homozygotes, and intermediate in heterozygotes. Larvæ homozygous for Chiricahua chromosomes are inviable at this temperature, while those homozygous for Standard survive.

4. The duration of life in the presence of food at low temperatures (0° to 4° C.) is higher in Standard/Chiricahua heterozygotes than in either homozygote.

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