THE VIABILITY OF STRUCTURAL HETEROZYGOTES OF *DROSOPHILA SUBOBSCURA* AT DIFFERENT TEMPERATURES¹

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CHROMOSOMAL polymorphism is known in a wide variety of *Drosophila* species. It has been observed repeatedly that under certain conditions the structural heterozygotes are heterotic, i.e. they show a selective advantage over the structural homozygotes. The selection for heterozygous individuals is frequently dependent on environmental conditions, such as temperature fluctuation or nutritional variation. WRIGHT and DOBZHANSKY (1946) found that ST/CH heterozygotes of *D. pseudoobscura* are usually heterotic at 25°C, whereas at 16°C the ST and CH frequencies in the population remain constant. SPIESS (1950) has shown that certain structural heterozygotes of *D. persimilis* exhibit heterosis at 16°C but not at 25°C. TANTAWY (1961), on the other hand, found AR/CH heterozygotes of *D. pseudoobscura* to be superior at fluctuating temperatures. An increase of the ratio of heterozygous to homozygous individuals has sometimes been observed to occur within a single generation (DOBZHANSKY 1947; DOBZHANSKY and LEVENE 1948; SPERLICH 1958).

It was the object of this investigation to determine whether temperature as a selective force in *Drosophila subobscura* is active in the same way throughout the stages between egg and adult, or whether its action differs in the larval and the pupal stages of development.

MATERIAL AND METHODS

The investigations were carried out on a highly polymorphic strain of *D. subobscura* derived from a wild population obtained in Cambridgeshire, sent to us by DR. SPURWAY-HALDANE and inbred in our laboratory (HUBER 1952). One hundred flies of this Ca strain were crossed with the standard strain in 100 F₁ individuals examined cytologically. Of these, all were heterozygous for J₁/J_{St} and for U₁/U_{St}. For the E chromosome, 48 individuals were E₁₊₂/E_{St} and 52 were E_{St}/E_{St}. For the O chromosome, 95 individuals were found to be O₃₊₄/O_{St} and five O₃₊₄₊₈/O_{St}. The strain was therefore taken to be homozygous for J₁/J₁ and U₁/U₁. For convenience in calculations the O chromosome was taken to be homozygous for O₃₊₄, and for the E chromosome a 1:1 ratio between E₁₊₂ and E_{St} was assumed (for details of these structures see KUNZE-MÜHL and MÜLLER

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1958). To obtain heterozygous individuals for these chromosomes the strain was crossed reciprocally with the standard strain in mass cultures at 19°C. Temperature series were set up in the following way: batches, each consisting of 100 females and 100 males of the heterozygous F_1 flies, were taken from the mass cultures and when laying well were transferred to fresh culture bottles. These were kept at room temperature (about 20°C) and the flies were allowed to deposit their eggs for 24 hours. At the end of this time the flies were transferred and the bottles with eggs moved to a room kept at 23°C. The flies were again allowed to lay for 24 hours under the same conditions, were then transferred and the eggs moved to 23°C. The same process was repeated twice more, but at the end of each 24 hours the bottles of eggs belonging to these two series were removed to 19°C. In this way sets of four temperature series were obtained from the same parents kept under the same conditions, with presumably negligible variations in deposition rate. The first of these series, labelled WW, was left at 23°C throughout the development of the flies. The second series, WC, was removed from 23°C as soon as the first pupa was observed, and transferred to 19°C. The third series, CW, was treated in the reciprocal manner, so that the larvae developed at 19°C and the pupae at 23°C. The flies of the fourth series, CC, were left at 19°C throughout their development. Emerging flies were removed from the bottles, counted, and males separated from females. The males were outcrossed singly to virginal standard females. The progeny of these crosses were used to investigate the chromosome type of the male parent, the salivary glands of eight larvae from each culture bottle being stained with acetic orcein and the chromosomes examined. Where all eight larvae exhibited the heterozygous state for a particular chromosomal structure the father was taken to be an inversion homozygote; if all larvae were homozygous for the standard structure the father was taken to be homozygous for the standard arrangement. Where some larvae appeared in the homozygous and others in the heterozygous state the father was classified as an inversion heterozygote.

RESULTS AND DISCUSSION

According to the Hardy-Weinberg formula one would expect the following ratios of the structural combinations in each of the four temperature series (in view of the small numbers of individuals examined, the homozygous states *inv/inv* and *st/st* were added for purposes of calculation): 50 percent homozygotes: 50 percent heterozygotes for chromosomes J, U and O, and 62.5 percent homozygotes: 37.5 percent heterozygotes for the E chromosome. Observations showed deviations from these expected ratios, listed in Table 1. This table shows that the heterozygotes predominated in 11 cases out of 16, were less than expected in four cases and in one case were equal to the expected ratio. The greatest of these deviations occurred in the WW series. The significance of the observed deviations in each experimental series was calculated by means of the chi-square method for chromosomes J, U and O. The observed figures for chromosome E suggest that this chromosome may behave similarly to the other chromosomes, but as it was

TABLE 1

Numbers of individuals, homozygous and heterozygous for each of four chromosomes in the four temperature series

	I		U		E		O		n	Temperature series
	Hom.	Het.	Hom.	Het.	Hom.	Het.	Hom.	Het.		
Expected no. individuals	12.5	12.5	12.5	12.5	15.6	9.4	12.5	12.5		
Observed no. individuals	9	16	2	23	1	24	1	24	25	WW
X ²	1.96		17.6				21.2			
	P = 0.20-0.10		P < 0.001				P < 0.001			
Expected no. individuals	26	26	26	26	32.5	19.5	26	26		
Observed no. individuals	36	16	27	25	21	31	28	24	52	WC
X ²	7.7		0.1				0.3			
	P < 0.01		P = 0.80-0.70				P = 0.70-0.50			
Expected no. individuals	20	20	20	20	25	15	20	20		
Observed no. individuals	22	18	10	30	8	32	14	26	40	CW
X ²	0.4		10.0				3.6			
	P = 0.70-0.50		P < 0.01				P = 0.10-0.05			
Expected no. individuals	16	16	16	16	20	12	16	16		
Observed no. individuals	16	16	10	22	7	25	15	17	32	CC
X ²	0		4.5				0.1			
	P = 1.0000		P = 0.05-0.01				P = 0.80-0.70			

I, U, E, and O signify chromosomes. W = warm, C = cool (see text for temperatures).

not homozygous results obtained from it are less reliable and will not be further considered. Calculations for chromosomes J, U and O (Table 1) show that deviations from the expected in favor of heterozygotes were significant in series WW for the U and O chromosomes and in the CW series for the U chromosome. Weakly significant results were found in the CC series for the U chromosome. On the other hand, in the series WC a significant deviation in favor of the homozygotes (J_1/J_1) was seen in the case of chromosome J, the distribution of frequencies being 53.8 percent J_1/J_1 ; 30.8 percent J_1/J_{st} ; 15.4 percent J_{st}/J_{st} .

Stage of life cycle during which selection occurs: To get a more concise formulation of the extent of heterosis in these experiments the number of heterozygous states of chromosomes J, U and O was added for each series and compared with all homozygous states in that series. These calculations, found in Table 2, show that there was a 34.0 percent excess of heterozygotes in series WW, and 11.7 percent excess in CW, a 7.3 percent excess in CC and an 8.3 percent deficit of heterozygotes in WC. Thus the deviations in favor of heterozygotes were greatest where individuals underwent their pupal development at 23°C. MAYNARD SMITH and S. MAYNARD SMITH (1954) observed that the optimal temperature for *D.*

TABLE 2
Numbers of homozygous and heterozygous chromosomes for each of the four temperature series

	Series WW (n=75)		Series WC (n=156)		Series CW (n=120)		Series CC (n=96)	
	Homozygous	Heterozygous	Homozygous	Heterozygous	Homozygous	Heterozygous	Homozygous	Heterozygous
Observed no. chromosomes	12 (16.0%)	63 (84.0%)	91 (58.3%)	65 (41.7%)	46 (38.3%)	74 (61.7%)	41 (42.7%)	55 (57.3%)
Expected no. chromosomes	37.5 (50%)	37.5 (50%)	78 (50%)	78 (50%)	60 (50%)	60 (50%)	48 (50%)	48 (50%)
Difference (% obs. — % exp.)	—34.0	+34.0	+8.3	—8.3	—11.7	+11.7	—7.3	+7.3
	$X^2 = 34.68$		$X^2 = 4.33$		$X^2 = 6.53$		$X^2 = 2.04$	
	$P < 0.001$		$P = 0.05-0.02$		$P = 0.02-0.01$		$P = 0.2-0.1$	

TABLE 3
Numbers of homozygous and heterozygous chromosomes compared for series WC and CW

	Homozygous	Heterozygous
WC	91	65
CW	46	74
	137	139
	$X^2 = 10.85$	
	$P < 0.001$	

subobscura is 69°F (20.5°C) and that the maximum temperature at which females survive and lay eggs is 75°F (24°C). Thus the 23°C used in these experiments represents a value close to the maximum, whereas the 19°C is near the optimal temperature. The greater selection in favor of heterozygotes observed at the higher temperature is therefore probably because this is a less favorable condition. Similarly one might expect greater selection at temperatures which are close to the minimum at which individuals are still viable, but such temperatures were not used in the present experiments. RUDERER (1959) finds an advantage of structural heterozygotes for the X chromosome at 14°C.

When considering the series WW and WC, one observes that in the former, where both larvae and pupae were exposed to high temperatures, the heterozygous structures predominated, whereas in the latter series, in which only the larvae were exposed to the high temperature, homozygous structures predominated. In series CC and CW the greater deviation occurred in the latter, where pupae were kept at the unfavorable temperature. Comparing series WC and CW, the statistically significant difference between these series shown in Table 3 indicates a difference in sensitivity to environment between pupae and larvae. One may conclude that high temperatures during the pupal stage favor heterozygotes in a northern strain of *D. subobscura*. It seems likely that high temperatures would favor homozygotes in populations from southern areas.

As shown in Table 4, the results of those series in which pupae developed at high temperatures were compared by the chi-square method with those where pupal development was at low temperatures, and a significant difference was found. Similar calculations for larvae showed no significant difference. Since the selective advantage of heterozygotes appears to be greater at unfavorable temperatures during the pupal than the larval phase, the deviation should be

TABLE 4

Numbers of homozygous and heterozygous chromosomes compared for series where pupae developed at high and low temperatures

	No. of homozygotes	No. of heterozygotes	Homogeneity χ^2	P
High temperature during pupal development WW + CW	58	137	23.05	$\ll 0.001$
Low temperature during pupal development CC + WC	132	120		
High temperature during larval development WW + WC	103	128	0.85	0.50-0.30
Low temperature during larval development CW + CC	87	129		
With temperature change WC + CW	137	139	15.02	< 0.001
Without temperature change WW + CC	53	118		

most marked in the WW series, somewhat less marked in CW, and WC and CC should show little or no selection effect in favor of the heterozygotes. Observations showed this sequence to hold for series WW and CW, and the deviation was least in CC; but in WC selection was found to favor the homozygotes.

To account for this it may be suggested that a temperature change during development increases the selective advantage of the homozygotes. On this basis selection in the four series would be as follows:

Selective advantage of heterozygotes	WW	CW	CC	WC
Primary influence: pupal development at 23°C	+	+	-	-
Secondary influence: absence of temperature change	+	-	+	-

Assuming the primary influence to be greater than the secondary, there would be greatest selection in favor of heterozygotes in the WW series, more in CW than in CC, and least in the WC series.

In Table 4, the series with and without a temperature change were compared by the chi-square method, and the difference was found to be significant. In the series with a temperature change the expected 1:1 ratio of homozygotes to heterozygotes was found, whereas in those series with a constant temperature there was a large excess of heterozygotes. This is at variance with the findings of TANTAWY referred to above, and may be due to the fact that in the present experiments the two strains used were from different geographical localities.

SUMMARY

A northern strain of *D. subobscura*, differing from the standard arrangement in four autosomal structures, was crossed with the standard strain. Temperatures during larval and pupal stages were varied in different series of the F₂ individuals. One series was kept at 19°C, and one at 23°C throughout development, while two other series were kept at the one temperature during larval, and at the other during pupal development. High temperatures, particularly during the pupal stage, appear to favor heterozygotes. Under the combined conditions of low temperature during the pupal stage and a temperature change during development, a significant excess of homozygotes was observed. It is suggested that a temperature change during development favors the homozygotes.

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