CHROMOSOMAL ADAPTIVE POLYMORPHISM IN *DROSOPHILA PERSIMILIS*. I. LIFE CYCLE COMPONENTS UNDER NEAR OPTIMAL CONDITIONS¹

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AT least three principal facts concerning the adaptive functions of chromosomal variants (inversions) in *Drosophila* populations have been established notably by DOBZHANSKY (1947, 1955), DOBZHANSKY and WALLACE (1953), DOBZHANSKY and LEVENE (1951), DOBZHANSKY and PAVLOVSKY (1953), and WALLACE (1953): 1) Natural selection may build up particular genic complexes in a chromosomal variant adapting homokaryotypes for specific environments; 2) natural selection may "co-adapt" heterozygous genic complexes by acting on heterokaryotypes involving two different chromosomal arrangements from one interbreeding population so that they display greater fitness than homokaryotypes; and 3) heterokaryotypes which have been coadapted to permit survival of balanced chromosomal polymorphism often are heterotic and homeostatic for traits which contribute to their adaptedness, that is, individuals show heterosis and more constant phenotype in developmental and physiological processes throughout a wider range of environments. This last fact has been extended in LERNER's (1954) concept of the "buffering" of developmental processes (or "canalization", WADDINGTON 1942) indicated by a lowered environmental variance among heterozygotes which display heterosis.

In order to establish the nature of co-adaptation between gene complexes of two different chromosomal arrangements three procedures have been employed customarily: 1) sampling of larvae from population cages containing two or more chromosomal variants to determine relative frequencies of the variants with time, 2) counting ratios of normal to dominant marker flies in crosses of wild-dominant marker heterozygotes to determine relative viabilities and developmental rates of "wild" and dominant phenotypes, and 3) measuring various physiological components of the fly life cycle to determine the expression of the genic complexes included by the inversion.

This last method has one major advantage over the dominant marker cross technique, namely progeny of wild flies are tested without crossing to a laboratory strain, and consequently the genic milieu has a history determined by evolutionary forces and not unrelated to the genic complex included within the chromosomal variant. On the other hand, a comparison between results of these various methods employed on one pair of gene arrangements would promise far more information than the results of only one method by itself.

It was shown by the senior author (SPIESS 1950) that *Drosophila persimilis* indi-

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viduals possessing either the "Whitney" (*WT*) or "Klamath" (*KL*) inversion or both from a locality in the San Joaquin Valley of California (Jacksonville, elevation 800 ft.) are not measurably different in adaptive values at 25°C in population cages, but at 16°C heterokaryotype flies (*WT/KL*) displayed net superiority and equilibrium was established at 51% *WT*. Fecundity of females raised under crowded conditions showed that *WT/WT* flies were superior to *KL/KL* and that *WT/KL* were superior to both. A maternal effect on this heterosis was also discovered (SPIESS, KETCHEL, and KINNE 1952).

In the summer of 1953 the senior author and his family collected flies from several high elevation localities in the Yosemite Region. The locality which was chosen for detailed study, White Wolf Lodge Pond, elevation 8000 ft., is in lodgepole pine forest (*Pinus contorta*) with very little underbrush, sparse soil, and extensive bedrock outcroppings. Frequencies of karyotypes have been calculated as follows (SPIESS 1953) *WT* 87%, *KL* 7%, *MD* 3%, *ST* 2% and *SE* 1%. These frequencies agree with DOBZHANSKY's data (1948) for localities near 8000 ft. Indications are that *KL* has increased by about 5% at the expense of *ST* over the intervening five years. Collections of DOBZHANSKY in 1954 (in press) corroborate this decrease in Standard and increase in Klamath.

With fresh stocks of flies the solution of three main problems has been undertaken: 1) the relative adaptive values of gene arrangements from this locality computed by population cage technique for comparison with the same arrangements from other localities; 2) the components of the fly's life cycle displaying phenotypic differences between karyotypes: are differences evident in particular components of the life cycle or do differences between karyotypes display a general overall uniformity throughout the life cycle? 3) a possible demonstration of heterosis in particular stages of the life cycle and finding evidence of lowered environmental variance to substantiate LERNER's hypothesis of homeostasis in the heterozygote, a greater ability "to stay within the norms of canalized development".

POPULATION CAGE DATA

WT and *KL* flies were introduced into plastic population cages, and after many generations relative adaptive values of three karyotypes were calculated. (For details of culture technique and computation of adaptive values, see SPIESS, in press). Two simultaneously run cages indicate an equilibrium at 70-75% *WT* and 25-30% *KL* at 16°C (relative adaptive values: 0.28 *KL/KL*: 1.00 *WT/KL*: 0.75 *WT/WT*).

MATERIALS AND METHODS

A knowledge that two chromosomal arrangements differ in their adaptive responses to the conditions of a population cage raises the question stated above (§ 2, § 3), and in attempting to trace those developmental and physiological processes controlled by a particular karyotype it seems necessary to measure life cycle components under near-optimal conditions first and later to simulate population cage conditions by increasing population density and by introducing more than one karyotype into the culture. All experiments reported here employed cultures of low population density in comparison with population cage food dishes, only one karyo-

type was raised in any single culture, and results can be referred to as "standard" for future reference.

Four strains of *WT* and four of *KL* were chosen at random from an original sample of nearly fifty wild strains, each strain consisting of the descendants of a single wild female caught at White Wolf Pond, elevation 8000 ft. All *KL* strains were derived from *WT/KL* wild females by sib mating for about five generations in order to produce homokaryotype *KL*. *WT* strains were subjected to only two or three initial generations of sib mating. Flies to be tested were usually F_1 individuals from strain crosses. Some tests were performed on original strain flies for the sake of comparison with strain cross F_1 's. Four strain crosses were made for each karyotype: $WT_1 \times WT_2$, 2×3 , 3×4 , 4×1 : Series A-D; $KL_1 \times KL_2$, etc.: Series E-H; $WT_1 \times KL_1$, etc.: Series I-L; and $KL_1 \times WT_1$ etc.: Series M-P.

This plan of mating was chosen to represent a population of karyotypes approximating a "wild" genotype and at the same time provide a basis for calculation of uniformity between genotypic combinations resulting from each cross. Does the hypothetical gene complex organized as a supra-genic unit with a particular adaptive value become expressed unalterably in all combinations of that unit? Does the heterotic genotype demonstrate lowered environmental variance also? Others (MOOS 1955; HEUTS 1948; WALLACE 1948; and LEVINE 1952) who have studied life cycle components of *D. pseudoobscura* possessing certain gene arrangements either do not record variance between strain crosses or they synthesize an F_1 population by crossing several strains at random in an experimental cage so that variance between crosses and within crosses cannot be distinguished.

Adult females to be tested for fecundity were raised as follows: stock parents (strain #1 ♀♀ × strain #2 ♂♂) were mated in several replicate bottles about six to ten pairs per bottle and provided with yeasted food on plastic spoons. Spoons on which F_1 eggs had been laid were withdrawn, counted for eggs, and inserted into the food medium of ordinary culture bottles; no more than 100 eggs were put into any culture. Adult females from at least three and no more than five of these culture bottles were isolated and tested for egg-laying capacity.

For tests of egg hatchability, survival of larvae, and rate of development, matings were made between strains in the same manner. Eggs collected from the spoons were washed first in 70% ethyl alcohol, then in sterile water to cut down contamination, and finally planted on a nutrient agar surface in 9 cm petri dishes. Eggs were planted evenly in numbers of 50-100 per petri dish; aggregation of eggs into crowded groups lowers the survival percentage considerably (GRUCCI 1954).

Cream of wheat-molasses food medium inoculated with Fleischmann's Bakers Yeast was used for cultures of strains. The test medium for developmental stages was devised from nutrient agar of near transparency so that larvae could be identified at all stages (SCHUELLEIN 1954). All experiments were carried out at 15°C.

OBSERVATIONS

Egg-laying capacity

Females to be tested were mated to sibling males in glass vials one pair per vial, and food to which lampblack had been added was introduced on plastic spoons. Every two days spoons were changed and eggs counted. Records were kept only for

TABLE 1

Fecundity of females in average number of eggs per day in terms of ten day periods and overall average for the first 30 days of egg-laying. N = no. ♀♀. C. V. = Coefficient of variability within series

	Karyotype	Mean (Eggs/day)	N	σ^2 (Between series' means)	$\bar{\sigma}^2$ (Within series)	C.V.
1-10 days	WT ₁ /WT ₂	20.28	78	2.89	14.66	0.182
	KL ₁ /KL ₂	18.56	72	0.87	14.74	0.207
	WT ₁ /KL ₁	20.34	92	1.96	11.60	0.168
	KL ₁ /WT ₁	18.56	79	14.34	10.48	0.174
11-20 days	WT ₁ /WT ₂	17.58	77	15.29	12.02	
	KL ₁ /KL ₂	17.61	59	0.59	11.89	
	WT ₁ /KL ₁	20.25	82	4.33	14.81	
	KL ₁ /WT ₁	19.28	70	4.14	14.70	
21-30 days	WT ₁ /WT ₂	17.37	61	2.96	10.73	
	KL ₁ /KL ₂	18.29	38	2.32	14.56	
	WT ₁ /KL ₁	19.68	71	1.27	13.42	
	KL ₁ /WT ₁	19.39	60	7.03	20.65	
Average for 30 day period	WT ₁ /WT ₂	18.46	61	1.98	6.07	0.133
	KL ₁ /KL ₂	18.40	38	0.46	5.48	0.127
	WT ₁ /KL ₁	20.08	71	0.51	5.72	0.119
	KL ₁ /WT ₁	19.23	60	7.89	7.28	0.140

the first thirty days; in previous work it has been shown that *D. persimilis*'s average rate of production remains quite constant for more than sixty days and that any differences which exist between karyotype performances may be recognized from the start, although daily production may be very uneven (SPIESS, KETCHEL, and KINNE 1952). It has also been shown that the particular culture conditions in which females were raised had no measurable bearing upon the maternal effect in heterokaryotypes: as long as conditions of crowding among developing larvae were of the same magnitude it made no difference whether flies came from several population cage food cups or from culture bottles. Every attempt was made in the present experiments to keep culture and testing conditions uniform.

Data are given in eggs per day per female in ten day intervals and the average of the thirty day period in table 1. If the original strains had been highly inbred and were reasonably homozygous, we could state that most of the between series means was genetic and most of the variance within series (replicate crosses of strains) was environmental, assuming sampling error to be a component of variance equally distributed to each. The original strains have not been consistently inbred, however; replications of strain crosses will not give genetically uniform results, nevertheless it is likely that over the time these strains have been maintained in the laboratory some homozygosity must be attained because in chromosome analysis for the first four or five generations sib mating had been carried out, but since that time the strains have been mass mated. These variances are relative to the amount of genetic uniformity within strains but the component between means indicates a measure of

TABLE 2

Percent hatchability of eggs, i.e. number of first instar larvae per 100 eggs

Karyotype	<i>m</i> (%)	No. petri dishes	σ^2 (Between series means)	$\bar{\sigma}^2$ (Within series)	C.V.
<i>WT</i> ₁ / <i>WT</i> ₂	86.4	42	11.76	45.87	0.078
<i>KL</i> ₁ / <i>KL</i> ₂	87.3	30	2.75	66.48	0.093
<i>WT</i> ₁ / <i>KL</i> ₁	94.0	26	7.76	9.11	0.034
<i>KL</i> ₁ / <i>WT</i> ₁ (omitting M series)	90.4	24	19.77	20.47	0.053
<i>WT</i> ₁ / <i>WT</i> ₁	72.6	19	641.27	67.28	0.105
<i>KL</i> ₁ / <i>KL</i> ₁	78.5	17	190.7	97.86	0.126
<i>WT</i> ₁ / <i>WT</i> ₂ (transformed to $\sin^2 \theta$)	68.93	—	9.60	36.87	0.088
<i>KL</i> ₁ / <i>KL</i> ₂ (transformed to $\sin^2 \theta$)	70.03	—	1.94	48.61	0.099
<i>WT</i> ₁ / <i>KL</i> ₁ (transformed to $\sin^2 \theta$)	76.90	—	17.42	15.38	0.051

genetic differences between strain crosses and therefore the genetic uniformity within any karyotype while that component within series indicates how the karyotype as a unit performs in environments which differ by relatively minor factors.

Among the data for ten day intervals no differences between means are statistically significant. Most strain crosses give uniform results (σ^2 between means) except *KL/WT* in the initial ten days and *WT/WT* in the second ten days. *KL/KL* is exceedingly uniform throughout.

The 30-day average has consistently lower variance both within and between series. This fact suggests the 30-day average to be a more reliable measure of fecundity than the short 10-day periods.

Heterosis for *WT/KL* is not marked if indeed present at all. In the 30-day average *WT/KL* differs from *WT/WT* by 1.62 eggs per day ($t = 2.05$, $d.f. = 6$, $P = 0.08$) and from *KL/KL* by 1.68 eggs per day ($t = 3.41$, $d.f. = 6$, $P = 0.014$). Of course the latter is much more uniform genetically ($\sigma^2 = 0.46$) than the former ($\sigma^2 = 1.98$). Homeostasis is also not apparent to any degree.

Coefficients of variability have been calculated for the first period of ten days and for the 30-day average. LERNER (1954) has followed the method of GUSTAFSSON (1946) in calculating the coefficient of variability for numerous experiments in which heterozygotes and homozygotes could be compared, but he states "... when the means and the variances are correlated, the coefficient of variation is not an entirely independent criterion". The coefficient of variability does not seem to be reliable as a measure of lowered variability unless the mean increases with an accompanying decrease in variance. It happens that the data in table 1 will give a low C.V. for heterokaryotype (*WT/KL*) but none of the C.V.'s are significantly different from any other because variances and means are so close.

A maternal effect in the heterokaryotype is not obvious from the data in table 1. Table 3 gives a breakdown of 30-day average data contributed by each series cross to show reciprocal differences in fecundity among heterokaryotype females. Series I and M, J and N, and so forth are reciprocals. Note three crosses are lower and one higher when *KL* is the mother. Two differences are highly significant and one is nearly significant. Apparently whatever uniformity of phenotype might be gained by heterozygosity in the heterokaryotype using a *WT* mother is lost by using a *KL*

TABLE 3

Series averages demonstrating reciprocal differences in the heterokaryotype for 30 day average fecundity and for hatchability of eggs

		Mean (Eggs/day)	N	σ_m	Mean (% hatch- ability)	N	σ_m
F ₁	WT ₁ × WT ₂						
	A	19.44	19	0.514	86.5	9	2.12
	B	17.00	20	0.635	83.6	8	2.55
	C	17.54	7	0.973	91.2	13	1.76
	D	19.87	15	0.552	84.3	12	1.87
F ₁	KL ₁ × KL ₂						
	E	18.26	14	0.581	87.4	9	2.85
	F	18.31	11	0.602	86.9	7	2.04
	G	19.34	7	0.719	85.5	7	3.78
	H	17.71	6	1.27	89.5	7	2.21
F ₁	WT ♀ ♀ × KL ♂ ♂						
	I	20.19	16	0.314	91.7	4	0.687
	J	20.58	21	0.457	95.8	7	1.61
	K	20.50	19	0.553	97.0	6	0.808
	L	19.04	15	0.814	91.6	9	0.934
F ₁	KL ♀ ♀ × WT ♂ ♂						
	M	18.33	11	1.05	78.5	7	2.16
	N	23.30	14	0.539	89.7	7	1.27
	O	16.84	18	0.601	95.2	8	0.878
	P	18.47	17	0.620	86.4	9	2.21
			<i>t</i>	<i>P</i>		<i>t</i>	<i>P</i>
		I-M	1.87	0.062	I-M	4.45	0.0017
		J-N	3.82	0.0006	J-N	2.75	0.016
		K-O	4.49	0.0001	K-O	1.46	0.16
		L-P	0.528	0.63	L-P	2.17	0.043

mother. Specific combining ability factors producing greater or less compatibility between egg cytoplasmic determiners and the father's genotype might be concerned in the causation of this maternal effect. Why this large variance should be evidenced by *KL/WT* flies and not by *KL/KL* is hard to explain on any other basis.

It is of interest to compare fecundity of these flies with those from Jacksonville raised in substantially the same conditions (SPIESS 1954). *WT/WT* females from the near sea level locality average 20 eggs per day, which is not significantly different from the data included here for this karyotype. Evidently *D. persimilis* from 8000 ft. and from 800 ft. are alike in fecundity under these laboratory conditions.

Hatchability and survival

As eggs hatched, a daily check on numbers of larvae was made with a tabulation grid designed to fit the petri dish. When larvae reached the third instar, a piece of

TABLE 4
Percent eclosion of adults per 100 eggs

Karyotype	$m(\%)$	No. cultures	σ^2 (Between series means)	$\bar{\sigma}^2$ (Within series)	C.V.
WT_1/WT_2	84.6	18	4.77	17.37	0.049
KL_1/KL_2	80.8	17	14.86	13.95	0.046
WT_1/KL_1	89.4	20	2.56	13.59	0.041
KL_1/WT_1 (omitting M)	88.6	14	1.12	12.25	0.040
WT_1/WT_1	60.1	17	657.0	14.10	0.062
KL_1/KL_1	70.4	14	353.6	71.96	0.120

paper toweling was placed on the agar surface. Most larvae pupated upon the toweling, and stray pupae were transferred to it.

Table 2 shows relative hatchability of eggs. WT/WT and KL/KL eggs hatch about equally when their heterozygosity is increased by strain-crossing. Individual strains of both karyotypes average much lower but are extremely heterogeneous: the individual WT strains averaged 52.4, 94.5, 98.0, and 45.6% hatchability while the KL strains averaged 70.5, 63.1, 90.9, and 89.4%. It is apparent no true mean can be calculated from these data for these individual strains, and at first glance the F_1 results in table 3 might appear to indicate the presence of dominant hatchability alleles in certain strains. That such an hypothesis is erroneous becomes clear when the F_1 series crosses are known: see table 4. Series A is the F_1 of 52.4×45.6 above, B is 52.4×94.5 , etc., series E is the F_1 of 70.5×63.1 above, F is 63.1×89.4 , etc. Variances within individual strains are of the same order of magnitude as those of their F_1 's but are slightly larger in the single strains. The great increase in uniformity of means and the decrease in the environmental component of variance in homokaryotype F_1 's indicate a homeostatic heterozygosity among both WT and KL strain cross progeny.

Heterokaryotype WT/KL displays a uniformly higher hatchability percent than any other karyotype with a markedly low average variance within series (difference between WT/WT and WT/KL is 7.6%, $t = 3.44$, $d.f. = 6$, $P = 0.013$; difference between KL/KL and WT/KL is 6.7%, $t = 3.53$, $d.f. = 6$, $P = 0.012$). There is evidently greater viability and "buffering" to be obtained by heterozygosity for loci in the heterokaryotype than for loci in the homokaryotype. Evidently heterokaryotypes are superior to homokaryotypes. This must also imply a greater amount of heterozygosity in the heterokaryotype since the two inversions are nearly independent systems.

Using percentages close to 100% for calculation of variance may be open to criticism; therefore the data used in calculating the top three rows of table 2 were transformed to $\sin^2 \theta$. Variance within series for WT/KL is still smallest: $F = 2.40$ comparing WT_1/WT_2 to WT_1/KL_1 ($d.f. = 38$ and 22 , respectively) gives a probability of 0.02. Therefore it appears well-founded that the environmental component of variance in the heterokaryotype from WT mothers is significantly less than in either homokaryotype.

The reciprocal cross (KL/WT) however does not produce either such uniformity

or high viability. Table 3 gives a breakdown of individual series crosses to show the maternal effect in the heterokaryotype. The "M" series is especially striking in low viability compared with its reciprocal "I"; the original strains of *WT* and *KL* average 52.4% and 70.5%, respectively. Series M data have been omitted from the mean calculations of tables 2 and 4 because of an exceptionally low hatchability value. This series was repeated seven times and gave consistently low egg viability results. Presumably there must be specific incompatibility between the two parent genotypes when *KL* is the mother which is not generally found among all the other crosses. Inclusion of M data would give an exceptionally low average for the *KL/WT* karyotype, which would be misleading in the reported average for that karyotype. Reciprocal series J-N and L-P are also significantly different though not so strikingly. In all cases *KL* mothers seem to lower the average viability of their offspring.

Unfortunately, observation of late larval stages in petri dishes is made difficult by the vigorous wandering of the creatures. Third instar larvae often leave the petri dish so that survival percentage can no longer be calculated although data for rate of development can still be obtained for those which do not escape. The problem was partially solved by paraffining the edge of the dish so that the cover would fit more snugly. Data given in table 4, however, were obtained completely on flies raised in half-pint bottles containing the same nutrient agar medium which had been used in the hatchability and rate of development experiments. These data serve as a check on percentages of survival obtained from petri dishes and are not significantly different from them.

Percent eclosion is a partial function of percent hatchability. The ratio of % eclosed/% hatched subtracted from 100% is a measure of larval and pupal mortality. The average mortality of *KL/KL* (7.5%) is the greatest for any of the karyotypes, none of which are significantly different. The high variance between means for *KL/KL* eclosion results from the mortality percent of one series cross, the other three average much more closely at a higher figure: E = 1.9%, F = 12.1%, G = 6.1%, H = 9.5%. The mortality of the E series is nearly equal to that of *WT/WT* 2.1%.

Superior eclosion values among the heterokaryotypes (omitting M) stems from their superior hatchability. Mortality is 4.8% for *WT/KL* and 2.0% for *KL/WT* (omitting M). Mortality for the M series is 7.0% and is within the range of the other heterokaryotype series. The M series had the very lowest egg viability but is not significantly low in postembryonic viability. Therefore it can be seen that differences initiated by viability of eggs are not radically altered by larval and pupal mortality.

The maternal effect at hatching is less marked at eclosion. There are no significant differences between reciprocals for eclosion values except I-M, but this difference was initiated at hatching. Relative survival of larvae and pupae do not display maternal effect.

While the original strains of *WT* and *KL* average the same mortality of larvae and pupae, inspection of individual strain data demonstrates the diversity of the strains and in some cases the lowered viability of nearly all with respect to their F_1 's. *WT* strains showed 14.7%, 48.5%, 31.3%, and 0% while *KL* strains showed 16.1%, 20.8%, 1.9% and 6.8% mortality of larvae and pupae. Apparently heterosis and

TABLE 5

Percent survival of adult females from start of egg-laying experiment to the end of 30 days

Karyotype	N	% Survivors		
		10 days	20 days	30 days
<i>WT</i> ₁ / <i>WT</i> ₂	92	88.0	82.6	69.6
<i>KL</i> ₁ / <i>KL</i> ₂	92	87.0	71.8	58.7
<i>WT</i> ₁ / <i>KL</i> ₁	111	90.9	82.7	71.8
<i>KL</i> ₁ / <i>WT</i> ₁	106	86.8	70.8	59.5

uniformity are both gained by crossing strains: among *WT* crosses the 14.7% $\times$ 48.5% gave 2.2% mortality while among *KL* crosses the 16.1% $\times$ 20.8% gave 2.0% mortality.

It is reasonably clear that karyotype differences are mostly determined by egg hatchability where "buffering" is quite marked in the heterokaryotype, and that all karyotypes survive about equally in larva and pupa. This result agrees with figures obtained on pupal survival among flies from Jacksonville, California (SPIESS, YANKOPOULOS, and HUTCHINSON 1952).

Percent of adult female survival was calculated for those flies which were used in the egg-laying experiment (see table 5). *WT/KL* and *WT/WT* survive about equally well, while *KL/WT* and *KL/KL* are decidedly less viable. This result differs somewhat from results obtained on flies from low elevation (SPIESS, KETCHEL, and KINNE 1952), where no maternal effect on survival could be demonstrated, and both *WT* and *KL* flies were less viable than *WT/KL*. However the two experiments are not strictly comparable because the Jacksonville flies were cultured under crowded conditions, a factor which might have considerable bearing upon the viability of survivors.

Rate of development

Table 6 shows average number of days in each of the post-embryonic stages of development and the total time from egg to adult.

In both first and second instars *WT/WT* is faster than *KL/KL* with *WT/KL* intermediate. No maternal effect on the heterokaryotype can be detected. There is no heterosis in the heterokaryotype and no substantial lowering of average variance within series. Apparently the *WT/WT* gene complex develops faster than the other karyotypes and has the lowest environmental component of variance. Original strain flies take as long a time as *KL/KL* to go through these instars and in general display a larger average variance within series, showing that the environment has more influence upon them than upon their *F*₁'s.

It is in the third instar, the most prominent of the three larval stages, that marked heterosis for *WT/KL* is expressed. Also *WT/WT* is still faster than *KL/KL*. Most interesting are the low variances both within series and between series means for the fastest karyotypes. *F*₁ *WT/WT* has much lower variances than single strains of *WT* showing not only genetic uniformity but homeostasis brought about by the heterozygosity increase. This effect is not evidenced by the *F*₁ *KL/KL* which takes longest

TABLE 6

Rate of development in post-embryonic stages (three larval instars, pupa, and total time from hatching to eclosion)

Karyotype	Life cycle period	Mean days	No. cultures	σ^2 (Between series means)	Av. σ^2 (Within series)
WT_1/WT_2	Larval Instar 1	2.9	32	0.515	0.097
	2	2.5	32	0.084	0.092
	3	11.0	32	0.063	0.512
	Pupa	12.9	32	0.153	0.151
	Total	29.3	32	0.396	2.570
KL_1/KL_2	Larval Instar 1	4.1	25	0.140	0.140
	2	3.4	23	0.022	0.107
	3	13.1	23	2.005	1.666
	Pupa	14.4	23	0.327	0.673
	Total	35.0	23	5.522	3.607
WT_1/KL_1	Larval Instar 1	3.4	25	0.388	0.170
	2	2.8	24	0.075	0.066
	3	8.2	24	0.413	0.565
	Pupa	12.6	23	1.732	0.239
	Total	27.0	23	3.978	0.442
KL_1/WT_1	Larval Instar 1	3.5	25	0.083	0.165
	2	2.7	25	0.038	0.119
	3	9.8	25	0.261	0.588
	Pupa	13.2	25	0.164	1.709
	Total	28.2	25	0.730	1.884
WT_1/WT_1	Larval Instar 1	4.6	17	0.915	0.309
	2	3.5	17	0.620	0.255
	3	10.5	16	3.352	1.668
	Pupa	14.7	16	0.879	0.504
	Total	33.3	16	19.538	5.762
KL_1/KL_1	Larval Instar 1	4.3	17	0.143	0.498
	2	3.3	17	0.067	0.108
	3	12.1	17	0.876	1.121
	Pupa	14.5	17	0.008	0.409
	Total	34.2	17	5.489	4.382

to develop and no lowering of variance with respect to its parental strains. These facts would tend to indicate that heterozygosity in the residual genome has little heterotic effect on rate of development, since KL F_1 's and WT F_1 's must have equal heterozygosity except on chromosome III.

WT/WT pupae develop faster than KL/KL by about one and one half days while WT/KL are equal to WT/WT . In this period WT seems to have a dominant effect in the heterokaryotype, and its low environmental variance component is repeated in pupae from WT mothers. There is no significant maternal effect in rate of pupal development of heterokaryotypes although variance within series is relatively large in pupae from KL mothers. Both variance components are greater for KL/KL F_1 's

than their parental strains, while the reverse is true for *WT/WT*. This pattern is similar to that in the third instar with regard to the relative effect of residual genome heterozygosity upon increase of developmental rate. Apparently the *WT* chromosome must differ from *KL* in possession of heterotic loci governing rate of post-embryonic development, and loci on non-homologous chromosomes do not directly affect this trait in any degree when heterozygous.

The total days from hatching to eclosion can serve as a measure of net rate of development for these karyotypes. Like the percent eclosion figures given above, this measure represents several parameters, but many interesting net effects bearing on the behavior of these karyotypes are obvious. *WT/WT* is faster than *KL/KL* by about six days and shows greater genetic and environmental uniformity. *WT/KL* has the most rapid rate of all and the lowest environmental component of variance. There is no marked reciprocal effect. Heterosis and lowered variances in all *F*₁'s except *KL/KL* show that the final effect is due to increased heterozygosity for loci on *WT* vs. *KL* arrangements of chromosome III in contrast to the effect observed in percent survival which can be accounted for by heterozygosity throughout the genome.

More evidence that the difference in rate of development between *WT* and *KL* is little affected directly by the residual genome is given in some data heretofore unpublished: the orange recessive marker and the orange-Blade-Scute-purple marker stock for chromosome III (see DOBZHANSKY and SPASSKY 1953) were backcrossed for five generations to a strain of *WT* from White Wolf Pond. Then by following the technique outlined in the above reference *WT* and *KL* chromosomes were made homozygous on a common residual genome derived from one wild strain. Normal flies should average 33.3% of the total when counted with dominant marker (Blade-Scute) in the analysis ratio. Exactly the same strain numbers, now outcrossed to the marker stocks, were crossed to simulate series crosses comparable to those studied above. Chromosome III was genetically comparable with that of the wild strain crosses tested in the above experiments, but the residual genome was far more uniform since it was derived from one wild strain. Results in terms of percent normals (non-Blade) in five day intervals of counting are given below:

			1st 5 days	N	2nd 5 days	N	3rd 5 days		Aver- age	N	
$\frac{or\ Bl\ Sc\ pr}{WT_1}$	$\times$	$\frac{or\ Bl\ Sc\ Pr}{WT_2}$	—	35.8%	830	35.5%	265	23.9%	167	34.6%	1262
$\frac{or\ Bl\ Sc\ pr}{KL_1}$	$\times$	$\frac{or\ Bl\ Sc\ pr}{KL_2}$	—	33.3%	603	30.4%	171	33.6%	152	32.8%	926

Even on a comparatively uniform genetic background the *WT* arrangement controls faster development than *KL*, but total survival is not significantly greater for one homokaryotype than the other as can be seen from the average percent.

DISCUSSION

Net adaptive value

Granted that there are other factors in the fly life cycle which have yet to be tested, it is of interest to compare the net fitness of the three karyotypes obtained by the methods above with adaptive values calculated from population cage data. The effects

TABLE 7

Relative adaptive values (W) of karyotypes determined from life cycle components of flies cultured under near-optimal conditions at 15°C

Life-cycle component	WT/WT	WT/KL	KL/WT	KL/KL
Females surviving (30 days) (rate per day)	0.999	1.000	0.996	0.995
Egg-laying capacity (daily average for 30 days)	0.914	1.000	0.957	0.911
Rate of development (egg to adult)	0.924	1.000	0.954	0.773
Adults eclosing per 100 eggs	0.946	1.000	0.991	0.904
Net <i>W</i>	0.799	1.000	0.900	0.634
Net <i>W</i> (at equilibrium compensated for maternal effect)	0.811	1.00		0.643

of crowding, extreme competition, and the presence of more than one karyotype in a population cage are at least three principal factors which may strongly influence the net fitness values. Some preliminary tests indicate that crowding of larvae tremendously intensifies the heterotic effect in survival.

Table 7 summarizes all factors tested in this project in terms of net relative adaptive values for each life cycle component. The four principal factors, adult female survival, 30-day average egg-laying capacity of females, rate of development, and percent adults eclosing, have been transformed into a rate per day relative to *WT/KL* (the so-called "fly-day" unit of WALLACE 1948). These transformations require further explanation. Rate of female survival was reckoned on the basis of a linear relation between time and percent survival. The data of table 5 follow reasonably a linear relation so that the mortality percent can be divided by 30 and subtracted from 100 to obtain survival per day. Egg-laying is already averaged per day per female. Rate of development was calculated by taking the reciprocal of the total days values in table 6. Adults eclosing can be considered in terms of relative "fly-days" if the time it takes for eclosion of all flies is equal for these karyotypes. This time ranged from six to fifteen days in various cultures and no karyotypes differed significantly in total time. Each proportion of fly-days in table 7 can then be multiplied with the corresponding proportion of the next factor to arrive at a net adaptive value (*W*) for the four main factors tested.

The final values given in table 7 are estimated on the basis of chromosomal frequencies in the population at equilibrium as determined from population cage results. Granted the conditions are not strictly comparable in the cage with these near-optimal "standard" conditions, the only point to be emphasized is this: the less frequent chromosome will be less likely to occur in a mother's genome. When the frequency of *KL* drops to 30% only 15% of all *WT/KL* individuals will have *KL/KL* mothers. In nature where *KL* is even less frequent (7% at White Wolf Pond) *KL/KL* individuals will exist very rarely indeed and nearly all *WT/KL* individuals will have either *WT/WT* or *WT/KL* mothers. Heterokaryotypes with *KL* mothers have consistently lower values than those with *WT* mothers.

By comparing these relative adaptive values with those obtained by population cage methods (above), it can readily be seen that both homokaryotype values are overestimated under these "standard" conditions, although the relative values of *WT/WT* and *WT/KL* are exceedingly close. Either population cage conditions might

intensify these values or factors in the life cycle not yet tested might alter the net results.

A comparison of these facts with those obtained for karyotypes of Standard and Chiricahua chromosomes in *D. pseudoobscura* (LEVINE 1952; MOOS 1955) is worth noting. LEVINE found that when flies of that species from Pinon Flats were raised under near-optimal conditions no differences could be demonstrated in terms of egg viability, egg hatching rate, larval viability, or larval growth rate, although many different food yeasts, temperatures, and relative humidities were employed. Pupae and adults did display slight differences between certain karyotypes. Moos measured similar life cycle components on the same karyotypes from Pinon Flats but raised them under competitive conditions for direct comparison with population cage calculations of net adaptive values. In contrast with LEVINE's results Moos found not only substantial differences between karyotype performances but also showed that net adaptive values from population cage competition were reasonably close to values obtained by averaging the life cycle components which she had chosen to measure.

D. persimilis from the Yosemite region raised under near-optimal conditions does display karyotype differences in life cycle components. Whether this fact establishes another physiological contrast with its sibling species or whether it reflects some difference in the ecology and population density of the two in nature cannot as yet be answered. CARSON (personal communication) has stated that the density of these species in breeding sites is an enigma not yet solved.

Heterosis and homeostasis

The heterokaryotype *WT/KL* occurs at a frequency of 0.12 in the locality tested. This represents a low frequency relative to *WT/WT*; nevertheless *KL* has maintained itself in approximately the same proportion in the Yosemite since the region was first collected in 1940. Undoubtedly some or all of these factors could contribute to the balanced condition of these chromosomes in nature: Heterosis is expressed as superiority of heterokaryotype over either homokaryotype under these experimental conditions markedly in some components of the life cycle, namely egg hatchability and rate of third larval instar development, and less markedly in survival of adult females and thirty day average fecundity of females. A lowering of the environmental variance component is demonstrable also especially in egg hatchability and in rate of post-embryonic development. These heterotic effects in the heterokaryotype are above any significant increases of strain cross F_1 homokaryotypes over their parental strains. Maintenance of equilibrium in population cage experiments may well be founded upon life cycle components such as those tested but their intensities may be altered if culture conditions for testing were made to simulate the cage.

WT, the more common karyotype in nature, displays heterosis in rate of development when made heterozygous by strain crossing but *KL* does not. In no other factors tested was there any marked difference between these homokaryotypes. Certainly in such large populations as *D. persimilis* attains in summer at high elevations, heterozygosity at most loci is maintained by the breeding structure. The *WT* arrangement seems to possess heterotic loci not found in the *KL* arrangement which increase rate of development. Loci in the residual genome are just as heterozygous in *KL F_1*'s;

apparently then loci determining faster rate of development are on chromosome III to a large extent and act in heterozygotic state either between *WT* arrangements or between *WT* and *KL* arrangements, the latter giving a superior rate.

On the other hand in survival of embryonic and post-embryonic stages both homokaryotypes display equal net survival but are heterotic with respect to their more homozygous parents. Superimposed upon an apparent total genome heterosis, then, is the increased superiority of the heterokaryotype in terms of egg hatchability and adult eclosion. In this case it can be seen that the inversion contains a complex of many genes selected for the totality of expression in heterozygous condition as a supra-genic unit.

Without doubt homokaryotype *WT* must have adaptive superiority over other homokaryotypes occurring in the White Wolf locality. It is too early to speculate about the main causes for the super-abundance of that arrangement and its gradient in relative frequency from low to high elevation, but the results reported above can partially account for the maintenance of a relatively rare arrangement (*KL*) for many generations in the locality.

SUMMARY

1. To test for adaptive differences between karyotype combinations of the Whitney (*WT*) and Klamath (*KL*) arrangements of chromosome III from *D. persimilis* native to an 8000 ft. locality in Yosemite National Park, four strains of each arrangement were tested as well as their strain cross F_1 's in homokaryotype and heterokaryotype under near optimal conditions at 15°C for the following life cycle components: 30 day egg-laying capacity, % hatchability, % adults eclosing, rate of development from egg to adult, and survival of adult females.

2. No differences are significant between *WT/WT* and *KL/KL* for egg-laying capacity, hatchability of eggs and net survival of post-embryonic stages.

3. *WT/WT* is superior to *KL/KL* in rate of development in all post-embryonic stages; the former reaches adulthood six days ahead of the latter on the average. *WT* is the most common arrangement in the native locality tested, and this factor may play a major role in maintaining the relative frequencies of these karyotypes in nature.

4. Heterosis is expressed in the heterokaryotype in some components of the life cycle, notably in % hatchability of eggs and in rate of third larval instar development, and less markedly in survival and fecundity of adult females. The advantage gained by high hatchability contributes substantially to high eclosion since mortality of larvae and pupae is about equal for all karyotypes.

5. Karyotypes which express traits which might be termed adaptively superior often show an accompanying lowering of variance in the environmental component, substantiating the hypothesis of developmental homeostasis. This fact is distinctly evident in *WT/KL*'s egg hatchability and total days from hatching to eclosion. When homokaryotypes F_1 's are compared with their parental strains heterosis and uniformity occur in *WT/WT* and *KL/KL* for egg hatchability and eclosion, while only in *WT/WT* for rate of post-embryonic stages of development. These results indicate that loci on chromosome III determine faster rate of development when heterozygous

in the *WT/WT* or *WT/KL* karyotype while heterozygous loci throughout the entire genome probably increase viability of eggs and post-embryonic stages.

6. Reciprocal effects in the heterokaryotype are most marked in egg-laying capacity, survival of adult females, and hatchability of eggs; there is no measurable maternal effect on rate of development.

7. A comparison of "net adaptive values" obtained by measuring life cycle components with those obtained by population cage technique shows an overestimation of homokaryotype values with respect to the heterokaryotype, although under these near-optimal conditions the relative values of *WT/WT* and *WT/KL* are exceedingly close. Either population cage conditions or factors in the life cycle not yet tested might alter the net result.

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