RATE OF DEVELOPMENT AND INVERSION POLYMORPHISM IN *DROSOPHILA PAVANI* AT TWO TEMPERATURES[1]

MYRIAM BUDNIK, S. KOREF-SANTIBAÑEZ AND D. BRNCIC

Departamento de Genetica y Biologia, Facultad de Medicina y Facultad de Ciencias, Universidad de Chile

Received October 6, 1970

DROSOPHILA PAVANI Brncic 1957 is a species of the mesophragmatica group, endemic to Chile, chromosomally polymorphic for inversions in the right and left arms of the fourth chromosome (BRNCIC 1957, 1961). The chromosomal polymorphism is of a "rigid" or "stable" character (DOBZHANSKY 1962), as the frequency of heterozygotes for the gene arrangements in the fourth chromosome is always around fifty percent in all the populations studied, both in nature and in the laboratory under different environmental and breeding conditions. Whether heterokaryotype superiority (chromosomal heterosis) plays a role in the stability of this polymorphism may be estimated by the analysis of certain components of fitness, such as longevity, viability, fecundity, speed of development (which are usually considered as components of the intrinsic rate of increase), developmental homeostasis, and some behavioral traits such as competitive ability and mating behavior. One or several of these may play a significant role (reviews in SPERLICH 1967, SPIESS 1968).

Many observations on *D. pavani* indicate that chromosomal polymorphism is maintained by the adaptive advantage of the structural heterozygotes. In fact, experimental evidence shows that inversion heterozygotes for the fourth chromosome genetic arrangements are superior with respect to some of the above mentioned components of fitness such as longevity (BRNCIC and DEL SOLAR 1961), mating activity (BRNCIC and KOREF-SANTIBAÑEZ 1964; KOREF-SANTIBAÑEZ and BRNCIC 1965), and rate of development (BRNCIC *et al.* 1968).

Since the pioneer work of WRIGHT and DOBZHANSKY (1946) it is known that in several species of Drosophila different environmental conditions, such as changes in temperature, can modify the adaptive advantage of different chromosomal arrangements (reviews in SPERLICH 1967 and SPIESS 1968). In spite of the rigid character of the polymorphism in *D. pavani*, the following experiments were designed in order to investigate whether the frequency of heterokaryotypes in the latter species can be modified when one of the components of fitness, rate of development, is altered by the effect of temperature.

Besides extending the information on the differences between the frequency of homo- and heterokaryotypes in relation to developmental speed and temperature,

[1] This work was financed in part by grants from the Faculty of Medicine and Grant No. 41/67 of CONICYT (Comisión Nacional de Investigación Científica y Tecnológica) and by the Multinational Genetics Program of the Organization of American States.

the characteristics of this biological parameter under different temperature conditions in *D. pavani* are discussed.

EXPERIMENTS AND RESULTS

In the experiments to be described, a strain of *D. pavani* was employed which originated from a large number of females collected in the locality of Bellavista near Santiago in 1961. The breeding system by which the stock was maintained has been described in detail by BRNCIC and KOREF-SANTIBAÑEZ (1964). At the time the experiments were performed, the frequency of the heterokaryotypes in the left arm of chromosome IV was around 55%, and around 48% in the right arm.

The experiments were performed independently at 16°C and 25°C, and the same method for egg collection described by BRNCIC *et al.* (1968) was used.

In the first series of experiments (which was made up of six replicates), the eggs were collected, placed in numbers of 50 in vials containing food medium, and left to develop in a constant temperature room at 16°C. In the second series of experiments (also six replicates), the eggs were collected and sown in the same manner, but incubated at a constant temperature of 25°C.

At 16°C, from 33,340 eggs, 19,447 imagoes emerged; the first flies, both male and female, eclosed 29 days after the eggs were collected, and the last did so after 65 days. At 25°C, 13,280 eggs gave 6,601 adults, which emerged between 14 and 29 days after the eggs had been collected.

Table 1 shows that the mean developmental rate (R.D.) from egg to adult was 40.2 ± 5.5 days at 16°C, and only 18.8 ± 2.7 days at 25°C. It is interesting to note that females emerged earlier than males at both temperatures. At 16°C, in five of the six experiments, Student's t-test gave significant differences between male and female developmental speed, while at 25°C, the differences were statistically not significant.

TABLE 1

Mean ($\bar{x}$) *rate of development in days from egg to adult of* D. pavani *males and females at 16°C and 25°C in six experiments* (a to f), *and statistical analysis of the observed differences* (t-*test*)

Experiment	16°C				25°C			
	$\bar{x}$ males	$\bar{x}$ females	t	P	$\bar{x}$ males	$\bar{x}$ females	t	P
a	38.54	37.14	6.31	<0.001	18.43	18.22	1.29	0.1–0.2
b	39.01	38.22	3.81	<0.001	19.33	19.26	0.47	0.5–0.9
c	43.93	43.64	2.21	0.03–0.04	19.37	19.37	—	—
d	37.69	37.20	0.26	0.80–0.90	19.22	19.10	0.61	0.5–0.9
e	39.45	38.64	3.12	0.001–0.003	19.09	19.08	0.29	0.5–0.9
f	38.75	37.93	9.06	<0.001	19.09	19.08	0.75	> 0.9
Total Number of ♂	9557				3253			
Total Number of ♀		9850				3348		
Mean R.D.	40.2 ± 5.5				18.8 ± 2.7			

According to their developmental rates, the flies were arbitrarily divided into two groups: those of "fast" development (those emerged from 29 to 35 days at 16°C, and 14 to 16 days at 25°C), and those of "slow" development (44 to 65 days at 16°C, and 21 to 29 days at 25°C). In order to analyze the chromosomal constitutions of the individuals classified into these groups, males and females were crossed individually to imagoes of the opposite sex of the sibling species *D. gaucha* which is homozygous for the "Standard" gene arrangements on all the chromosomes. The salivary glands from eight F_1 larvae from each individual pair were prepared by means of the lactic-acetic-orcein rapid squash method, and examined in order to determine whether the progenitor was a homo- or heterozygote. From the 16°C series, 397 "fast" and 375 "slow" individuals were examined, while 238 "fast" and 154 "slow" were studied from the 25°C group.

Table 2 summarizes the frequencies of structural homozygotes (*St/St* and *In/In*) and heterozygotes (*St/In*) in both the left and right arms of the fourth chromosome in the different experimental groups. The numbers of males and females of each genotype was approximately equal. On analyzing the deviations

TABLE 2

Observed (O) and Expected (E) frequencies (in percent) of heterozygotes (St/In) *and homozygotes for the "Standard"* (St/St) *and "Inverted"* (In/In) *chromosomal sequences in the right and left arms of the fourth chromosome among the flies of fast and slow development (R.D.) at 16°C and 25°C*

Chi-square values were calculated according to the deviations from the expected Hardy-Weinberg ratios

Temperature °C	R.D.	Number		Chrom 4-R			Chrom 4-L		
				St/St	*St/In*	*In/In*	*St/St*	*St/In*	*In/In*
16°	fast	395	O	11.39	70.12	18.48	11.64	67.34	21.01
			E	21.57	49.75	28.66	20.53	49.56	29.89
					$\chi^2 = 16.77$†			$\chi^2 = 12.85$†	
16°	slow	375	O	30.93	45.33	23.73	31.20	43.20	25.60
			E	28.73	49.74	21.53	27.87	49.84	22.27
					$\chi^2 = 0.73$			$\chi^2 = 1.75$	
25°	fast	238	O	26.47	60.08	13.14	18.90	59.66	21.42
			E	31.93	49.14	18.90	23.75	49.75	26.26
					$\chi^2 = 4.93$*			$\chi^2 = 3.77$	
25°	slow	154	O	48.05	44.15	7.79	36.36	49.35	14.28
			E	49.16	41.89	8.92	37.24	47.56	15.17
					$\chi^2 = 0.28$			$\chi^2 = 0.14$	
Total at 16°		770	O	20.90	58.05	21.03	21.16	55.58	23.24
			E	24.93	49.98	25.07	23.96	49.97	26.06
					$\chi^2 = 2.61$			$\chi^2 = 1.24$	
Total at 25°		392	O	34.94	53.94	11.22	25.76	55.61	18.62
			E	38.25	47.18	14.54	28.69	49.74	21.55
					$\chi^2 = 1.66$			$\chi^2 = 1.39$	

* P (2 df) 0.05–0.10.
† P (2 df) 0.001.

TABLE 3

Observed (O) and Expected (E) number of homozygous and heterozygous individuals for the gene arrangements in the right and left arms of the fourth chromosome in the groups of fast *and* slow *development, at 16°C and at 25°C*

Temperature °C	R.D.	Number	Chromosome 4-R Homokaryotypes O	E	Heterokaryotypes O	E	Chromosome 4-L Homokaryotypes O	E	Heterokaryotypes O	E
16°	fast	395	118	132.91	277	262.08	129	140.40	266	254.59
25°	fast	238	95	80.08	143	157.91	96	84.59	142	153.40
Chi-square				*6.69*				*4.09*		
P (df 1)				*0.01–0.001*				*0.05–0.02*		
16°	slow	375	205	206.28	170	168.71	213	206.28	162	168.71
25°	slow	154	86	84.71	68	69.28	78	84.71	76	69.28
Chi-square				*0.03*				*1.65*		
P (df 1)				*0.95*				*0.20*		

from Hardy-Weinberg equilibrium ratios in the four groups it should be pointed out that at 16°C there was a significant deviation from the expected value in the "fast" group, and a smaller one at 25°C. This did not occur in the group with "slow" development, nor when the total number of individuals analyzed at each temperature was considered. This result reflects the fact that the number of heterozygotes among the rapidly developing individuals was greatly increased over the expected frequency at both temperatures, but this tendency was much lower at 25°C. Nevertheless, it is important to keep in mind that the higher chi-square values at 16°C are determined also by the larger number of flies analyzed in this group.

Table 3 shows the results of a chi-square homogeneity test (2×2 tables) of the variations in the number of homo- and heterokaryotypes at 16°C and 25°C, in the groups of flies of "fast" and "slow" development. The deviations from the expected values are significant for both fourth chromosome arrangements in the "fast" group. It is concluded that, in the experiments here reported, temperature represents an important factor in determining the excess of structural heterozygotes among the faster developing flies.

DISCUSSION

The above mentioned experiments provide further evidence for the stability of inversion polymorphism in *D. pavani*, since they indicate that heterokaryotypes have a greater developmental speed both at 16°C and at 25°C.

Nevertheless, the response is not equal at both temperatures; at the lower one, there is a significantly greater number of heterozygotes for inversions in the fourth chromosome among the rapidly developing flies than at 25°C. In *D. subobscura*, MAYNARD-SMITH *et al.* (1954, 1955) and HARTMANN-GOLDSTEIN and SPERLICH (1963) have shown that, besides modifying eclosion time, higher temperatures seem to increase heterozygote advantage. In *D. pseudoobscura*,

DOBZHANSKY, LEWONTIN and PAVLOVSKY (1964) and AYALA (1965, 1966) have also shown that the response of heterokaryotypes is greater at 25°C. BRNCIC (1968), on the other hand, finds that in *D. flavopilosa*, different chromosomal arrangements have different selective values in relation to temperature. All these findings point once more to the fact that the genetic make-up of each population or species is the result of its interaction with the ecological background to which it has become adapted, responding differently to the same environmental modifications.

Thus, in *D. pavani*, which lives mostly in temperate climatic conditions, and which is present only during part of the year, from spring to autumn, the rigid polymorphism may be the expression of the heterotic properties of the heterokaryotypes in the environments in which the species lives. The greater selective advantage of the heterozygotes in their developmental rate is expressed better at lower temperatures, although both in nature as well as in the laboratory, higher temperatures may also allow a greater number of heterozygotes among the flies that develop faster than among those that do so more slowly. In *D. pavani* selective forces seem to operate chiefly during the pupal period. As was indicated previously (BRNCIC *et al.* 1968), at 16°C there was no difference in the frequency of heterokaryotypes between larvae that pupated first and those that did so later, although these differences were present on the emergence of adults. At higher temperatures, although the heterokaryotypes did develop faster than the corresponding homokaryotypes, the differences were not significant. This might be correlated to the fact that the developmental period is greatly reduced. In nature, during winter, when *D. pavani* does not seem to reproduce, a few adults and pupae are probably the only stages of development that persist, thus allowing the action of selective forces that favor the emergence of heterozygotes first, which will rapidly occupy the available ecological niches.

At this point, it may be noted that, considering all the individuals examined, both of rapid and slow development, the frequencies of the "Standard" and of the "Inversion" homo- and heterozygotes in the fourth chromosome are in equilibrium. However, selective agents are operating, as may be observed among the rapidly developing individuals where there is an excess of heterozygotes in relation to the expected value according to Hardy-Weinberg equilibrium. BIRCH (1955) indicated that the "Hardy-Weinberg equilibrium may be maintained, even under heavy selection, provided there are selective differences between the genotypes". This may be the case in *D. pavani* where the excess of heterozygotes among the rapidly developing flies may be compensated by a greater number of homozygotes among the last individuals to emerge. A natural population is made up of both types of individuals, but, as has already been mentioned (BRNCIC *et al.* 1968), the fast ones will probably contribute a greater number of offspring, will occupy the available ecological niche more rapidly, thus causing, together with the other fitness parameters, such as longevity (BRNCIC and DEL SOLAR 1961), mating activity (BRNCIC and KOREF-SANTIBAÑEZ 1964; KOREF-SANTIBAÑEZ and BRNCIC 1965), the maintenance of the high number of heterozygotes.

On analyzing the time *D. pavani* took to develop from egg to adult, it was

observed that a temperature rise of 9°C reduced the period by more than one half. At 16°C, the females developed significantly more rapidly than the males, but at 25°C, this difference was not so noteworthy. Several authors, among them BONNIER (1960), BAKKER (1961) and DAVID and CLAVEL (1967) in *D. melanogaster*, and OHBA (1967) in *D. pseudoobscura*, have found that females eclose before males. Others, such as MAYNARD SMITH and MAYNARD SMITH (1954) in *D. subobscura*, have shown that males develop more rapidly than females. This indicates that the developmental rate of each sex is also a species specific character, determined by factors that influence the growth rate of the larvae.

The authors wish to thank Mrs. HERTHA FENNER, Mr. ENRIQUE ZÁRATE and Miss PATRICIA ITURRA for their valuable technical aid. They also wish to acknowledge the advice given by Mr. MORITZ BENADO in the statistical analysis, and the computing facilities provided by Dr. DAVID YUDILEVICH.

SUMMARY

The present work is a continuation of a series of studies on the physiological properties of heterokaryotypes in the neotropical species, *D. pavani*, that exhibits "stable" polymorphism for gene arrangements due to the presence of inversions. The frequency of heterokaryotypes in relation to rate of development was analyzed at two different temperatures. Eggs from a heterogeneous strain of *D. pavani* were placed in equal numbers in vials containing food medium; one group was incubated at 16°C, another at 25°C. Adults were collected every 24 hr, until all emerged. At every temperature two groups were established, according to their emergence after the eggs were collected: those of "fast" development (29 to 35 days at 16°C, 14 to 16 days at 25°C), and those of "slow" development (44 to 65 days at 16°C, and 21 to 29 days at 25°C). The chromosomal analysis of these groups indicated that the frequency of heterokaryotypes in the left and right arms of the fourth chromosome was higher among the rapidly developing flies than among the slow ones, but this difference was significantly smaller among those flies that were raised at 25°C. It was also found that among the rapidly-developing individuals at both temperatures, there was a significant deviation of the frequency of the different genotypes from the expected Hardy-Weinberg ratios; the population as a whole was in equilibrium. The rate of development of males and females at both temperatures is also discussed.

LITERATURE CITED

AYALA, F., 1965 Relative fitness of populations of *Drosophila serrata* and *Drosophila birchii*. Genetics **51**: 527–544. ——, 1966 Evolution of fitness. I. Improvement in the productivity and size of irradiated populations of *Drosophila serrata* and *Drosophila birchii*. Genetics **53**: 883–895.

BAKKER, K., 1961 An analysis of factors which determine success in competition for food among larvae of *Drosophila melanogaster*. Arch. Néerland. Zool. **14**: 200–281.

BIRCH, L. C., 1955 Selection in *Drosophila pseudoobscura* in relation to crowding. Evolution **9**: 389–399.

BRNCIC, D., 1957 Chromosomal polymorphism in natural populations of *Drosophila pavani*. Chromosoma **8**: 699–708. ——, 1961 Non-random association of inversions in *Drosophila pavani*. Genetics **46**: 401–406. ——, 1968 The effects of temperature on chromosomal polymorphism of *Drosophila flavopilosa* larvae. Genetics **59**: 427–432. ——, 1969 Long term changes in chromosomally polymorphic laboratory stocks of *Drosophila pavani*. Evolution **23**: 502–508.

BRNCIC, D. and E. DEL SOLAR, 1961 Life cycle and expression of heterosis in inversion heterozygotes in *Drosophila funebris* and *Drosophila pavani*. Am. Naturalist **95**: 211–216.

BRNCIC, D. and S. KOREF-SANTIBAÑEZ, 1964 Mating activity of homo and heterokaryotypes in *Drosophila pavani*. Genetics **49**: 585–591.

BRNCIC, D., S. KOREF-SANTIBAÑEZ, M. BUDNIK and M. LAMBOROT, 1968 Rate of development and polymorphism in *Drosophila pavani*. Genetics **61**: 471–478.

BONNIER, G., 1960 Experiments on hybrid superiority in *Drosophila melanogaster*. II. Rate of development from egg hatching to eclosion. Genetics **46**: 86–91.

DAVID, J. and M. F. CLAVEL, 1967 Influence de la température d'élévage sur la mortalité larvo-nymphale et la durée de dévéloppement de la Drosophile. Naturaliste Can. **94**: 209–219.

DOBZHANSKY, TH., 1962 Rigid *vs.* flexible chromosomal polymorphisms in Drosophila. Am. Naturalist **96**: 321–328.

DOBZHANSKY, TH., R. C. LEWONTIN and O. PAVLOVSKY, 1964 The capacity for increase in chromosomally polymorphic and monomorphic populations of *Drosophila pseudoobscura*. Heredity **19**: 594–614.

HARTMANN-GOLDSTEIN, I. and D. SPERLICH, 1963 The viability of structural heterozygotes of *Drosophila subobscura* at different temperatures. Genetics **48**: 863–869.

KOREF-SANTIBAÑEZ and D. BRNCIC, 1965 Mating activity and chromosomal polymorphism in *Drosophila pavani* females. Genetics **52**: 453.

MAYNARD SMITH, J. and S. MAYNARD SMITH, 1954 Genetics and cytology in *Drosophila subobscura*. VIII. Heterozygosity, viability and rate of development. J. Genet. **52**: 152–164.

MAYNARD SMITH, J., J. M. CLARKE and M. J. HOLLINGSWORTH, 1955 The expression of hybrid vigour in *Drosophila subobscura*. Proc. Royal Soc. London B **144**: 159–171.

OHBA, SH., 1967 Chromosomal polymorphism and capacity for increase under optimal conditions. Heredity **22**: 169–185.

SPIESS, E. B., 1968 Experimental population Genetics. Ann. Rev. Genet. **2**: 165–208.

SPERLICH, D., 1967 Populationsgenetik (Teil I. Drosophila). Fortschritte der Zoologie. **18**: 223–278.

WRIGHT, S. and TH. DOBZHANSKY, 1946 Genetics of natural populations. XII. Experimental reproduction of some of the changes caused by natural selection in certain populations of *Drosophila pseudoobscura*. Genetics **31**: 125–156.