THE EFFECTS OF TEMPERATURE ON CHROMOSOMAL POLYMORPHISM OF *DROSOPHILA FLAVOPILOSA* LARVAE¹

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D*ROSOPHILA flavopilosa* Frey is a neotropical species that, in Chile, is found only associated with flowers of the solanaceous plant, *Cestrum parqui* L'Her, which is common all over the central part of the country during practically every season of the year. As the whole developmental cycle takes place within these flowers, it is relatively easy to establish some basic ecological parameters, such as population density, abundance of predators, competitors, and parasites in different geographic zones (BRNCIC 1962, 1966). The natural populations of *D. flavopilosa* are polymorphic for the gene sequences in the fifth chromosome, due to the presence of inversions. Quantitative data on the distribution of the different chromosomal arrangements have shown that there are seasonal fluctuations and altitudinal gradients in their frequencies. In previous papers (BRNCIC 1966, 1967) an attempt was made to correlate these variations with population size, pupal mortality and certain ecological parameters such as climatic changes, number of parasites (two species of parasitic wasps) and competitors (one species of Thysanoptera). However, since all these components experience the same cyclic variations throughout the year, it is rather difficult to establish simple relationships. A better understanding of the mechanisms and causal connections between the variables studied may only be obtained by carrying out experiments under controlled laboratory conditions.

It is known that some climatic factors such as temperature, determine variations of the genetic make-up under experimental conditions in many species of *Drosophila* (references in DA CUNHA 1960; and DOBZHANSKY 1961). Moreover, since the classical work of WRIGHT and DOBZHANSKY (1946) in *D. pseudoobscura*, it is known that the relative fitnesses of some of the inversions found in this species are temperature sensitive in the laboratory. In the present paper, effects of temperature on the different gene arrangements of *D. flavopilosa* at the larval stage are studied, in order to determine whether this factor could be responsible for the fluctuations in chromosomal structure found in natural populations.

MATERIALS AND METHODS

Temperature sensitivity of the chromosomal polymorphism of *D. flavopilosa* was studied by

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analysis of the giant salivary gland chromosomes, prepared by the acetic-orcein squash method, in larvae bred at 16°C and 25°C.

Up to the present it has not been possible to maintain *D. flavopilosa* under experimental conditions, but the preadult forms collected in nature complete their development inside the flowers of *Cestrum parqui* brought to the laboratory (BRNCIC 1962). At room temperature (about 22°C) the developmental cycle from egg to adult takes about 21 days. At 25°C it is faster (about 18 days), and at 16°C, slower (about 25 days). Pupation takes place about three days later at 16°C than at 25°C.

The following procedure was employed in all the experiments to be discussed. Flowers of *Cestrum parqui* were brought to the laboratory and on the same day were divided into two containers, one left in a constant temperature chamber at 16°C, and the other in one at 25°C. Freshly collected flowers of *C. parqui* contain all preadult stages of the fly, but the number of small larvae greatly exceeds that of medium or large sized ones. The latter two types pupate within the first days in the laboratory. At 25°C, the very small larvae reach the prepupal stage after about 5 days and at 16°C, after about 8 days. These were the ones selected for cytological analysis. Therefore, the most conclusive cytological data come from larvae which have completed most of their development at the aforementioned temperatures, and the differences in genetic structure between the groups may be interpreted as being due to differences in viability.

The two natural populations from which flowers "parasitized" by preadults of *D. flavopilosa* were collected were, "El Tabo" (Lat. S. 33°27'—Long. W. 71°40') at sea level on the coast, and "Vizcachas" (Lat. S. 33°36'—Long. W. 70°81') located 150 km from the former in the pre-Andean region at an altitude of about 2,700 feet. In both places collections were made periodically since 1961, and information on population density, inversion frequency, number of competitors, predators and parasites, as well as climatic data have been recorded (BRNCIC 1966, 1967). Both populations differ sufficiently in their genetic structure as to consider them as well defined races: a coastal one ("El Tabo") characterized by a low frequency of *A* and a high one of *B* genetic arrangements and a pre-Andean race ("Vizcachas") with the reverse situation, i.e., a high frequency of *A* and a low one of *B*.

Five samples of flowers from "El Tabo" and six from "Vizcachas" obtained monthly from January to June, 1967, constituted the material for the present research.

RESULTS

The description and frequencies of the different gene arrangements found in *D. flavopilosa* populations have been given in previous papers (BRNCIC 1962, 1966). Both in the populations of "El Tabo" and of "Vizcachas" there are four inversions derived from the "Standard" gene order in the right arm of the fifth chromosome (described as *A*, *B*, *C* and *D* inversions). Until now these inversions have been clearly identified only in the heterozygous state. The structural heterozygotes, *A/Standard* and *B/Standard*, are always present in relatively high frequencies while the others are rare. For this reason, in Table 1, which summarizes the data on the frequencies of the different gene arrangements found in all samples analysed, the *A* and *B* heterozygotes are listed separately, while all the other gene orders in the heterozygous state, such as *C/Standard*, *D/Standard* as well as the extremely rare combinations, *A/B*, *A/C*, *A/D*, *B/C*, *B/D* and *D/C*, are included in a common group. All the structural homozygotes are also grouped under a single column.

It can be seen in Table 1 that there are some interesting differences in the chromosomal structure of the larvae bred at 16°C and at 25°C. This is especially clear with respect to the frequencies of *A* and *B* heterokaryotypes. The *A* heterokaryotypes are more frequent at 25°C than at 16°C in four out of five samples

TABLE 1

Frequencies (in percent) of homozygotes and heterozygotes for the gene arrangements in the V-R chromosome in larval samples of D. flavopilosa that developed at 16°C and 25°C

Locality	Date of collection	Temperature (in °C)	Number	Heterokaryotypes			Homokaryotypes
				A	B	Others	
EL TABO	Jan. 25	16°	50	10.00	36.00	4.00	50.00
		25°	50	12.00	30.00	10.00	48.00
	Mar. 26	16°	50	10.00	46.00	6.00	38.00
		25°	50	26.00	20.00	8.00	46.00
	Apr. 18	16°	100	10.00	31.00	7.00	52.00
		25°	100	22.00	20.00	2.00	56.00
	May 18	16°	100	9.00	40.00	3.00	48.00
		25°	100	7.00	32.00	7.00	54.00
	June 6	16°	100	7.00	44.00	1.00	48.00
		25°	100	18.00	29.00	4.00	49.00
	Total	16°	400	9.00	39.00	4.00	48.00
		25°	400	16.50	26.50	5.50	51.50
VIZCACHAS	Feb. 2	16°	50	44.00	8.00	4.00	44.00
		25°	50	54.00	6.00	4.00	36.00
	Mar. 20	16°	50	40.00	10.00	2.00	48.00
		25°	50	56.00	8.00	0.00	36.00
	Apr. 9	16°	50	30.00	16.00	4.00	50.00
		25°	50	40.00	16.00	0.00	44.00
	Apr. 21	16°	100	34.00	16.00	2.00	48.00
		25°	100	42.00	10.00	2.00	46.00
	May 11	16°	100	29.00	10.00	3.00	58.00
		25°	100	34.00	14.00	2.00	50.00
	June 23	16°	50	28.00	34.00	4.00	34.00
		25°	50	40.00	20.00	4.00	36.00
	Total	16°	400	33.50	15.00	3.00	48.50
		25°	400	42.75	12.25	2.00	43.00

from "El Tabo" and in the six samples from "Vizcachas." In contrast, *B* heterokaryotypes are more frequent at 16°C in all the samples from "El Tabo" and in four of the six samples from "Vizcachas."

For statistical analysis of the data summarized in Table 1, contingency tables (2×4) were formed, taking all the chromosomal arrangements into account, i.e., heterozygotes for *inversion A*, *inversion B*, other heterozygotes, and the homozygotes at both temperatures for each collection. The χ^2 homogeneity test (2×2 tables with Yates' correction) was also performed in order to investigate whether the differences in the total number of structural homo- and heterozygotes between both temperatures are statistically significant. The same analysis was finally made for only the *A* and *B* heterokaryotypes. In general, the χ^2 values are relatively low. Nevertheless, in the March and April samples from "El Tabo," significant differences are observed on comparing all the chromosomal arrangements at both temperatures ($P = 0.01-0.02$). This finding can be related to the

TABLE 2

χ^2 analysis of the influence of temperature, date of collection and the interaction of both factors, on the frequency of all the chromosomal arrangements analysed, i.e., heterozygotes for inversion A, inversion B, other heterozygotes, and homozygotes (2×4 tables); homokaryotypes and all heterokaryotypes together (2×2 tables); and A and B heterokaryotypes (2×2 tables)

	All chromosomal arrangements			Homokaryotypes and heterokaryotypes			A and B heterokaryotypes		
	χ^2	df	P	χ^2	df	P	χ^2	df	P
<i>EL TABO</i>									
Temperature	19.805	3	< 0.001	0.720	1	0.25–0.50	17.319	1	< 0.001
Date	18.688	12	0.05–0.10	4.100	4	0.25–0.50	10.632	4	0.025–0.050
Interaction	15.803	12	0.10–0.25	0.240	4	0.90–0.99	7.678	4	0.10–0.25
Total	54.296	27	0.001–0.005	5.060	9	0.75–0.90	35.629	9	< 0.001
<i>VIZCACHAS</i>									
Temperature	7.721	3	0.05–0.07	2.438	1	0.10–0.20	3.981	1	0.04–0.05
Date	37.079	15	0.001–0.005	12.140	5	0.03–0.05	19.676	5	0.001–0.002
Interaction	15.731	15	0.35–0.45	1.402	5	0.20–0.30	3.124	5	0.60–0.70
Total	60.531	33	0.001–0.005	15.980	11	0.10–0.20	26.781	11	0.004–0.006

differences in the frequency of the A and B heterokaryotypes in the same, as well as in the June sample from "El Tabo," which are also statistically significant ($P = 0.01$ – 0.02). In contrast, the χ^2 values obtained on comparing the chromosomal structure of the total number of larvae cultured at 16°C and 25°C are very high in both populations (Table 2). Considering all the chromosomal arrangements investigated, the probabilities are below 0.001 for "El Tabo" and 0.05–0.07 for "Vizcachas." If only the total number of A and B heterokaryotypes are considered, the probabilities are below 0.001 for "El Tabo" and between 0.04 and 0.05 for "Vizcachas." It is interesting to point out that, taking into account each sample, as well as considering the sum of them, the total frequency of homokaryotypes is surprisingly similar at 16°C and at 25°C .

There are also variations in the frequencies of the gene arrangements observed at the different dates at which the samples were obtained. In general, these differences are in accord with the seasonal fluctuations found in the corresponding natural populations during the last six years (references in BRNCIC 1966, 1967). Table 2 gives the data of the relative influence on the chromosomal frequencies of the temperature and the date of collection. In "El Tabo" it can be observed that the temperature seems to be more relevant than the date of collection, and the total number of homo- and heterozygotes for inversions does not seem to be significantly modified throughout the six month period of collections. In contrast, in "Vizcachas" the date of collection seems to be of importance in the changes of the chromosomal structure, and it is also the most relevant factor in determining the total heterogeneity of the samples included in Table 2. It is interesting

to observe that both in "El Tabo" and "Vizcachas" there is no significant interaction between the effects of temperature and the date of collection.

DISCUSSION

The analysis of the aforementioned results leads to the conclusion that the temperature at which larvae develop represents an important factor for the viability of the *A* and *B* heterokaryotypes in *D. flavopilosa*. *A* heterokaryotypes seem to be favored at 25°C, while *B* heterokaryotypes seem advantageous at 16°C. These data are in accord with what is observed in the natural populations of the species, where the *A* inversion is more abundant during the summer months, while the *B* inversion tends to increase during winter (BRNCIC 1966, 1967). As a result of the increase of *A* concomitant to the decrease of *B* at 25°C, and the reverse tendency at 16°C, the total heterozygosity for inversions is maintained at a more or less constant level. The data also agree with the altitudinal gradients found in the distribution of the inversions observed in natural populations. "El Tabo" at sea level has a lower mean temperature than "Vizcachas" during summer and autumn when the samples for the experiments were collected. The "El Tabo" population has a much lower frequency of *A* heterokaryotypes than "Vizcachas", and the reverse occurs for the *B* inversion. In *D. flavopilosa* as in most polymorphic species of *Drosophila*, the different inversions may have become coadapted to each other in the course of evolution. Therefore, changes in the environment would determine modifications of the entire genetic make-up of the population and not only of a single inversion.

Chromosomal polymorphism in certain *Drosophila* species is very sensitive to relatively small environmental changes. WRIGHT and DOBZHANSKY (1946) showed, under laboratory conditions, that in *D. pseudoobscura* the adaptive values of the inversions depended, among other factors, on temperature. At 25°C, the inversion heterozygotes were superior but at 16°C heterozygotes and homozygotes were equal. In *D. persimilis*, SPIESS (1950) has demonstrated a reverse situation. In *D. funebris* (DUBININ and TINIAKOV 1947) and in *D. robusta* (LEVITAN 1951) the different gene arrangements were also temperature sensitive. None of these studies indicates the stage of the life cycle at which temperature affects the adaptive value of the karyotypes. Only HARTMANN-GOLDSTEIN and SPERLICH (1963) found that in *D. subobscura* high temperature favored the structural heterozygotes for certain inversions during the preadult stage.

The finding that in *D. flavopilosa* the inversion polymorphism can be modified by breeding the larvae at different temperatures contributes to the understanding of the genetic structure in relation to the population dynamics of this species. The flexible character of the polymorphism in *D. flavopilosa* has led to the conclusion that the different chromosomal types possess specific selective values in relation to some environmental factors. In this species, for reasons inherent to its ecology, it was possible to establish certain correlations between the cyclic changes of the frequencies of some gene orders, the fluctuations of population size, and the incidence of parasites and predators. All these changes are also related to the

seasonal variations of climate (BRNCIC 1966, 1967). From the observations reported in this paper it may be concluded that among all biological and physical components of the environment, temperature seems to be a critical selective factor for maintaining the balance of karyotypes in populations of this species.

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SUMMARY

Data are reported on the effects of temperature on the frequencies of different gene arrangements of *Drosophila flavopilosa* Frey at the larval stage, in order to determine whether this factor could be responsible for the seasonal fluctuations in the chromosomal structure found in natural populations. The results lead to the conclusion that the temperature at which the larvae develop is an important factor for the viability of certain heterokaryotypes in the two populations of *D. flavopilosa* employed. *A* heterokaryotypes seem to be favored at 25°C, while *B* heterokaryotypes seem advantageous at 16°C. These data agree with what is observed in the corresponding natural populations, where *A* heterozygotes are more abundant during the summer months, while *B* heterozygotes tend to increase during winter. As a result of the increase of *A* concomitant to the decrease of *B* at 25°C, and the reverse tendency at 16°C, the total heterozygosity for inversions is maintained at a more or less constant level.

LITERATURE CITED

- BRNCIC, D., 1962 Chromosomal structure of populations of *Drosophila flavopilosa* studied in larvae collected in their natural breeding sites. *Chromosoma* **13**: 183-195. — 1966 Ecological and cytogenetic studies of *Drosophila flavopilosa*, a neotropical species living in Cestrum flowers. *Evolution* **20**: 16-29. — 1967 Chromosomal polymorphism in an ecologically restricted species of *Drosophila* living in Chile. *Ciencia e Cultural (Brazil)* **19**: 45-53.
- DA CUNHA, A. B., 1960 Chromosomal variation and adaptation in insects. *Annual Rev. Entomol.* **5**: 85-110.
- DUBININ, N. P. and G. G. TINIAKOV, 1947 Natural selection in experiments with population inversions. *J. Genet.* **48**: 11-15.
- DOBZHANSKY, TH., 1961 On the dynamics of chromosomal polymorphism in *Drosophila*. *Symp. Rev. Entomol. Soc. (London)* **1**: 30-42.
- HARTMANN-GOLDSTEIN, I. and D. SPERLICH, 1963 The viability of structural heterozygotes of *Drosophila subobscura* at different temperatures. *Genetics* **48**: 863-869.
- LEVITAN, M., 1951 Experiments on chromosomal variability in *Drosophila robusta*. *Genetics* **36**: 285-305.
- SPIESS, E., 1950 Experimental populations of *Drosophila persimilis* from an altitudinal transect of Sierra Nevada. *Evolution* **4**: 14-33.
- WRIGHT, S. and TH. DOBZHANSKY, 1946 Genetics of natural populations. XII. Experimental reproduction of some of the changes by natural selection in certain populations of *Drosophila pseudoobscura*. *Genetics* **31**: 125-156.