GENETIC MODIFICATION OF A TEMPERATURE SENSITIVE LETHAL IN *DROSOPHILA WILLISTONI*

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THE SURVIVAL value of a genetic factor is conditioned by its phenotypic manifestation in all environments encountered by the species, as well as by its performance in combination with the rest of the genotype of the species. The hereditary norm of reaction can be analyzed by observing a given factor under various environmental conditions and in different genotypes. DOBZHANSKY and SPASSKY (1944) studied the expression of characters associated with the second and fourth chromosomes of *Drosophila pseudoobscura* by changing the environment as well as the residual genotype. They found one chromosomal type which had an almost normal viability at 16½°C, was semilethal at 21°C and lethal at 25½°C. Various degrees of viability of this chromosome were obtained at 21° by altering the genetic background. Another factor on the fourth chromosome exhibited temperature-conditioned visible characters which could be paralleled by changes in genetic residues. Viability influences of five systems of geographic modifiers were tested for a number of homozygotes for second and third chromosomes derived from natural populations. Some modifiers caused larger viability changes than others, but the influence of a particular geographic modifying system was not beneficial to all homozygotes nor deleterious to all.

A temperature-sensitive lethal factor (*2WB*), the viability effect of which can also be modified by alterations in the genotype, has been discovered in *Drosophila willistoni*. The recessive factor *2WB* on the second chromosome was extracted from the Goyaz (Brazil) population of *D. willistoni*. At 25°C this factor is a lethal. The *2WB* homozygotes are viable below 25°C and can be distinguished by their wing and eye abnormalities. The wings of the newly emerged flies are blistered and vesiculated, but in older flies they appear shrunken and slightly darker in color than normal wings. The wings are held in the unfolded position (divergent). The description of *balloon wings* in *D. melanogaster* fits this lethal more closely than any other described mutant (BRIDGES and BREHME 1944). Irregular curvature of the eyes also occurs in the *2WB* homozygotes. A pure culture of the abnormal flies has been maintained at 18°C.

The balanced lethal stock of *2WB* was outcrossed to a wild strain, "Standard"; the *2WB/+* flies obtained in the F₁ were mated and their offspring raised at 25°C. Among the progeny of the F₂ there were flies with shrunken wings which had survived the lethal temperature. Repetition of this experiment revealed discrepancies in the percent survival of the shrunken flies in the F₂. These preliminary observations raised several questions. One problem concerns the nature of the factors which are

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responsible for the modification of the *2WB* norm. Are these results due to the presence of genetic modifiers affecting the viability of the *2WB/2WB* zygotes or is the appearance of the shrunk flies at the higher temperatures a result of crossing over between a lethal factor and a factor for shrunk wings? Furthermore, what is the geographic distribution of these viability factors in Brazilian populations of *D. willistoni* with respect to the population which gave rise to *2WB*? Since the genetic variability and biology of numerous Brazilian populations of *D. willistoni* have been so extensively examined (BURLA *et al.* 1949; SPASSKY and DOBZHANSKY 1950; PAVAN *et al.* 1951) it was considered desirable to study the geographic distribution of the modifiers of the *2WB* factor. Such analyses may provide information about the evolutionary significance of the concealed genetic variability found in natural populations.

METHODS

In order to determine whether the shrunk flies are the result of crossing over between the lethal factor and a shrunk factor, or whether the change in the vi-

FIGURE 1. Scheme of replicate crosses to measure the amount of heterogeneity in the Belem wild strain and the effect of inbreeding.

ability of the shrunken flies is due to the presence of genetic modifiers in the wild strain, the principle of inbreeding was employed. It was argued that inbreeding a wild strain of flies for a number of generations and testing by replicate crosses for its reaction with the *2WB* norm after each inbred generation would distinguish between the two alternatives. If inbreeding increases the genetic homogeneity within the wild strain, then the variability of replicate crosses of the wild strain to the *2WB* stock should decrease with each subsequent generation of inbreeding. Such results would indicate that genetic modifiers of the wild strain must be responsible for the viability changes of *2WB*. Recombination, however, should show no regular trend of increasing homogeneity.

The factor *2WB* had been maintained in the laboratory in a balanced lethal system for three years before the present experiment was begun. To further insure the homogeneity in the *2WB* stock, it was inbred for eight generations by single pair matings. Then the crosses outlined in figure 1 were made. A single male (F_0 , C_1) from the Belem wild strain was crossed to a group of *2WB*/Balancer females. The F_1 *2WB*/+ flies were mated and placed in egg collection chambers. From this cross 600 F_2 larvae were collected and raised in creamers (20 larvae per creamer) on cream of wheat-molasses medium (SPASSKY 1943). The method of egg and larval collection was that previously described by RIZKI (1952) for *D. willistoni*. Five replicates of this series of crosses were made (C_1 - C_5), and 600 larvae were obtained from each. The larvae were raised at a constant temperature of $26 \pm 0.25^\circ\text{C}$. Every precaution was taken to equalize the environmental conditions throughout the experiment from creamer to creamer. Mortalities were also taken into account by running normal controls under the same conditions. The pupae in each creamer were counted and the adults were classified according to sex and wing type (normal and shrunken). This scheme was repeated for the subsequent inbred generations of the Belem wild strain; each successive generation of Belem wild strain used was the result of a single pair mating of the preceding generation.

The χ^2 test for heterogeneity was applied to the data. At the outset of the experiment it was decided to discontinue inbreeding the Belem wild strain when the χ^2 value between replicates for a given generation changed from significant to non-significant and remained so for the next generation; otherwise the inbreeding should be continued for at least six generations to note any trend.

In addition to the Belem wild strain several males of each of the following wild strains were crossed to *2WB*/Balancer females and the percent shrunken flies in the F_2 from each cross was determined: Iguassu, Palma, Imperatriz, Marajo, Rio Negro, Bolivia and Içana.

RESULTS

The data from the inbreeding experiments of the Belem wild strain are presented in table 1. The χ^2 test reveals considerable heterogeneity among the Belem males (C_1 - C_5) of the F_0 and F_1 inbred strain with regard to modification of *2WB*. But the nonsignificant χ^2 value obtained by testing the five males of the F_2 inbred generation indicates homogeneity which is retained in the F_3 generation. The trend toward homogeneity in the modification of *2WB* by inbreeding the wild strain is also apparent in the variance of the ratio of normal to shrunken wing per generation (table 2).

TABLE 1

Measurement of heterogeneity of the Belem strain

		Normal		Shrunken		Total	$\chi^2_{1d.f.}$	P
		♂♂	♀♀	♂♂	♀♀			
F ₀	C1	160	156	83	33	432	40.30	<0.0001
	C2	155	151	77	48	431		
	C3	135	156	77	43	411		
	C4	141	142	79	79	441		
	C5	181	173	61	37	452		
	Total	772	778	377	240	2167		
F ₁	C1	106	168	42	51	367	56.66	<0.0001
	C2	197	184	64	37	482		
	C3	192	142	51	52	437		
	C4	209	177	27	34	447		
	C5	187	175	59	64	485		
	Total	891	846	243	238	2218		
F ₂	C1	192	194	63	74	523	18.37	≈0.12
	C2	173	177	75	76	501		
	C3	172	162	51	50	435		
	C4	153	173	67	63	456		
	C5	148	175	87	75	485		
	Total	838	881	343	338	2400		
F ₃	C1	217	178	31	33	459	13.30	≈0.35
	C2	184	155	36	34	409		
	C3	202	164	29	25	420		
	C4	167	181	19	25	392		
	C5	167	160	29	31	387		
	Total	937	838	144	148	2067		
Repeat F ₁	C1	195	180	24	32	431	50.10	<0.0001
	C2	189	156	50	27	422		
	C3	192	189	15	16	412		
	C4	199	185	15	16	415		
	C5	199	173	19	12	403		
	Total	974	883	123	103	2083		

TABLE 2

The Trend of Increasing Homogeneity of 2WB Modification by Inbreeding the Belem Strain as Shown By the Variance, S²

	$\bar{X}_c$	S ²	N
F ₀	0.715	0.002531	5
F ₁	0.783	0.003759	5
Repeat F ₁	0.890	0.002175	5
F ₂	0.716	0.001475	5
F ₃	0.859	0.000305	5

This homogeneity in the modification of 2WB obtained by inbreeding implies the presence of genetic modifiers of 2WB in the Belem strain.

The crosses (C₁-C₅) of the F₁ generation were repeated by raising a new line from the F₀ (mass culture) by single pair mating. The χ^2 value shows heterogeneity among the five males as in the sample of the original F₁. The presence of heterogeneity in the original Belem strain is confirmed. (The difference in ratios of normal to shrunken flies in the original F₁ and the repeat F₁ is not clear on the basis of the present experiment. It may be due to the heterogeneity and the initial number of modifiers in the F₀. Also compare the ratio of normal and mutant individuals in the repeat F₁ with F₃.)

Additional evidence that the shrunken flies are carrying the "lethal" 2WB factor has been obtained from data on zygotic mortalities. Mortality of the Belem wild strain was determined under the same experimental conditions as the mortality occurring in the cross 2WB x F₀ Belem. The total mortality of the Belem wild strain was 18% as compared with an average mortality of 25% from the cross of the Belem strain to 2WB. This difference of 7% does not account for the presence of a recessive lethal factor which has an expected mortality of 25%.

Furthermore an average of 0.29 of the progeny from the cross 2WB x F₀ generation of the Belem wild strain had shrunken wings. Theoretically such a high proportion of flies with shrunken wings would not be expected as a result of crossing over. If the two factors are completely separable and independent from one another then the expected ratio of normal to shrunken flies is 5:1. Any linkage between the factors would decrease the proportion of shrunken flies.

TABLE 3

Percentage of shrunken flies obtained by crossing 2WB to various wild strains of D. willistoni

Wild strain	No. larvae	No. adults	Fraction shrunken	
			p	σ
Iguassu	580	400	0.14 $\pm$ 0.017	
Palma	580	352	0.09 $\pm$ 0.015	
Imperatriz	580	292	0.08 $\pm$ 0.016	
Marajo	580	411	0.08 $\pm$ 0.013	
Rio Negro	600	412	0.05 $\pm$ 0.011	
Bolivia	600	416	0.07 $\pm$ 0.013	
Içana	600	381	0.06 $\pm$ 0.012	

In addition to the Belem wild strain seven other wild strains of *D. willistoni* were crossed to the *2WB* stock and the offspring raised at the lethal temperature. Shrunk flies were obtained in the F_2 generation from all of these crosses ranging in proportion from 0.05 to 0.14. The data are summarized in table 3. The cross with the Iguassu wild strain gave 0.14 shrunk flies; this value is significantly different from that of the Belem wild strain which gave the highest proportion of shrunk flies, 0.29. The t value for the comparison of Palma and Rio Negro indicates significance. Since the results from the Rio Negro population are not significantly different from those of Marajo and Imperatriz which in turn are not different from Palma, then it may be concluded that the difference between Palma and Rio Negro is not highly significant.

DISCUSSION

Environmental conditions can alter the viability of the *2WB* factor. At 25°C this factor is a lethal, but at lower temperatures viable phenotypes result from the same genotype. Changes in the residual genotype also can produce viability changes of the *2WB* factor, and depending upon the modifying system, the lethal factor *2WB* may become a semilethal or viable. Most of the wild strains tested showed modification to the level of semilethality. It should be stressed that in these experiments on genetic modification every precaution was taken to reduce environmental fluctuations between crosses to a minimum. The inbreeding experiments were designed to determine whether the modification of *2WB* by a wild strain is the result of recombination of genetic factors or whether genetic modifiers of *2WB* exist in the wild strain. The observed results are best explained by the occurrence of genetic modifiers.

The time of death of the lethal homozygotes of *2WB* is very close to the stage of emergence of the imagines from the puparium. There is an upset in the hatching movement and time of emergence. The wings of the lethals begin expanding while the fly is still in the puparium and become filled with haemolymph. This haemolymph in the wings forms blisters which often rupture while the fly is enclosed in the pupal case. The excessive loss of haemolymph and desiccation, or the entanglement of the imago in the puparium, or both factors may be considered the final cause of the lethality. It is conceivable that a slight change in the timing or rate of action of the factors involved in emergence induced either by alterations in the environment or residual genotype can shift the individual zygote into the lethal or the viable class.

Of the eight wild strains of *D. willistoni* which were crossed to the *2WB* stock, Belem and Iguassu gave higher proportions of shrunk flies than all of the others. The highest proportion of shrunk wings, 0.29, resulted from the cross to the Belem strain, 0.14 were found in the Iguassu cross, and values ranging from 0.05–0.09 occurred in the other strains.

The *2WB* factor originated in the *D. willistoni* population of Goyaz (Brazil). No correlation was found between the distance of the wild strains from the point of origin of the lethal factor and the amount of modification of *2WB*. The distribution of the genetic modifiers is geographically random. There is also no correlation between the mean number of inversion heterozygotes per fly in different populations and the degree of modification of the *2WB* norm (DOBZHANSKY *et al.* 1950; CUNHA *et al.* 1950). However, there does seem to be a possible correlation between the fre-

quencies of lethals and semilethals in different populations of *willistoni* and the degree of modification of viability of *2WB* in the present experiment. PAVAN *et al.* (1951) in an extensive study of concealed genetic variability in Brazilian populations of *D. willistoni* found that, "it seems that the populations of central and northern Brazil, except that of Para carry more lethals and semilethals than those in the south" (Para = Belem). Of the southern populations studied by Pavan, Iguassu had a low frequency of lethals and semilethals and gave high modification of *2WB*. Belem is in northern Brazil, but is the exceptional case in Pavan's study by having lower frequencies of lethals and semilethals; in the present experiment Belem gave high modification of *2WB*.

SUMMARY

1. The recessive factor *2WB* is a lethal at 25°C whereas at lower temperatures the *2WB* homozygotes are viable and are recognizable by their shrunk wings. Shrunk flies are also obtained among the progeny of crosses of the *2WB* stock to various wild strains of *D. willistoni* raised at 25°C.

2. It has been demonstrated that the survival of the "lethal" homozygotes of *2WB* in combination with the Belem wild genotype is due to the action of viability modifiers present in the Belem strain.

3. Eight wild strains of *D. willistoni* were crossed to the *2WB* stock and the percent of shrunk flies in the F₂ determined. The wild strains from Belem and Iguassu gave significantly higher modification of the *2WB* norm than the other tested strains, producing respectively 29% and 14% shrunk flies. Values ranging from 5%–9% shrunk flies resulted from the crosses of the other wild strains to *2WB*.

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