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ADAPTATION OF *DROSOPHILA WILLISTONI* EXPERIMENTAL POPULATIONS TO EXTREME *p*H MEDIUM

I. CHANGES IN VIABILITY AND DEVELOPMENTAL RATE

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Summary

Nine population cages with *D. willistoni* from the centre of its distribution range (Brasilia) and another nine from the southern margin of its distribution (Eldorado, RS) have been progressively selected for adaptation to three levels of *p*H of the food medium: *low* (*p*H 2), *median* (*p*H 5) and *high* (*p*H 10) under a system of discrete generations. The degree of adaptive response was measured by viability tests at the 17th and 49th generations. The *high p*H populations, both Brasilia and Eldorado, exhibited a faster response to selection starting in the 17th generation whereas the *low p*H populations showed significant increase of viability from the 49th generation on. However, the developmental rate of *high* and *low* populations decreased, and at the 49th generation they were faster on the usual *median p*H 5 food. Selection for early adult emergence, applied during seven generations, was more effective for the *median* populations, followed by the *high* and the *low* populations. Apparently the elongation of the life cycle was initially a correlated response to selection for survival at extreme *p*H leading to a partial loss of the ability to respond to selection for faster development.

1. Introduction

Studies of the genetic responses of a population to extreme or unusual environmental conditions have given valuable information on its variability, adaptive norm, genetic organisation and coherence (Robertson, 1966). Strong directional selection is expected to change gene frequencies drastically, generating a high substitutional load (Crow and Kimura, 1963) and disturbing the co-adaptation of the gene pool of the population (Brncic, 1961). The genetic architecture of a population appears to be changed in various respects to adapt itself to extreme environmental conditions.

Since the classical work of Wagner (1944, 1949) it has been recognised that natural populations of some *Drosophila* species have specific food requirements. The extensive experimental work of Da Cunha (1951), Levine (1952) and Dobzhansky and Spassky (1954) with *D. pseudoobscura* clearly showed intraspecific food preferences related to chromosomal types and the interplay of heterotic and diversifying selection. We do not know the extent of the changes in the natural habitats of *Drosophila*, especially in decaying fruits, leaves, and fungi, the most common food source of wild species of *Drosophila*. However, some changes are certainly met by *Drosophila* during its life cycle specially in the larval stages. Yeast and bacteria are known to

change significantly the *p*H of the food medium in the laboratory (Pavan, 1952; Da Cunha, 1955). In this and subsequent papers we report the main results of experiments started in 1963, with F_1 descendants of *Drosophila willistoni* sampled in nature.

In this paper we deal with evidence of the adaptive process detected by means of partially independent changes in viability and developmental rate in population cages with extreme *p*H medium. Subsequent papers are devoted to observations on incipient sexual isolation, changes of inversion frequencies and esterases allozyme polymorphism.

2. Materials and methods

In March 1963 a sample of 300 females of *Drosophila willistoni* was obtained from a semi-isolated wood in a grassland region of the state of Rio Grande do Sul, 50 km south of Porto Alegre, Eldorado, Guaíba County. Another sample of about 500 females was obtained a few days later in a riparian forest at the margins of the river Torto, National Park Brasilia, DF. All these females were distributed singly in well yeasted culture vials and allowed to oviposit for 7 days, at 16°C. A single larva from each vial was used for cytogenetic studies, and the remainder were cultured and transferred to two population cages, one labelled Eldorado and the other Brasilia. In each of these " mother cages " with nearly 3000 individuals, 12 culture vials were introduced every morning and transferred next day to three other cages (four vials each), until 12 vials were obtained per cage (see fig. 1). After 5 days of adult emergence in these new cages, the old vials were discarded

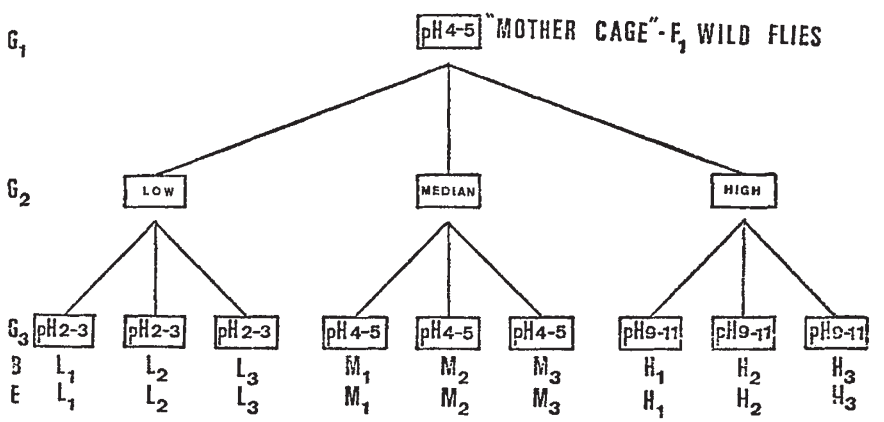

FIG. 1.—Origin of the populations studied. Eighteen cages were used. Procedures for *D. willistoni* from Brasilia were similar to those used for flies from Eldorado. *Abbreviations:* G = generations; B = *D. willistoni* from Brasilia; E = *D. willistoni* from Eldorado; L = populations selected at low *p*H food medium; M = populations selected at intermediate *p*H food medium; H = populations selected at high *p*H food medium.

and in one cage we introduced six vials with *p*H 5 food and six vials with *p*H 3 food to collect eggs and from these, two vials with *p*H 5 food and two vials with *p*H 3 food were introduced in a new cage; this same procedure was used for two more cages until we obtained three cages each with six

vials with *p*H 5 and six vials with *p*H 3. The same procedure was followed for another cage which received six vials with *p*H 5 food and six vials with *p*H 7 food. Every day two vials with *p*H 5 food and two vials with *p*H 7 food were introduced into a new cage and new bottles were introduced in the cage every day until we obtained three cages, each with six vials with *p*H 5 food and six vials with *p*H 7 food.

In the subsequent generations we introduced 12 vials in each cage and the flies were allowed to oviposit for 4 days and then discarded or maintained in the cages with new culture vials to collect larvae for cytogenetic or other experimental studies. From generation 4 the proportion of the extreme *p*H food cultures was progressively increased in the cages destined to have *p*H 2 and *p*H 10. The adults which emerged from the most extreme *p*H food vials were pooled every day for 5 or 6 days until their total reached 1600 ± 150 individuals measured, without etherisation, in a provette (4 cc). The same technique was used for the maintenance of the *median p*H 5 food population except that no change in their *p*H was effected. This is the usual *p*H of the standard laboratory food. The flies were allowed to oviposit for 4 days and then discarded or maintained in the cages with new culture vials to collect larvae for cytogenetic or other experimental studies.

The populations are designated by the initials B = Brasilia and E = Eldorado, followed by l = low *p*H 2-3, m = median *p*H 4-5, h = high *p*H 9-11 and numbered in order: Bl_1, Bl_2, Bl_3, Bm_1, Bm_2, Bm_3, Bh_1, Bh_2, Bh_3, El_1, El_2, El_3, Em_1, etc.

The composition of the culture medium is critical in these experiments. We used one developed by Marques *et al.* (1966) for the *p*H 4-5 or *median* population: corn meal 825 g, full wheat flour 375 g, soy bean flour 50 g, brown sugar 775 g, Moldex or Nipagin 50 g, table salt (NaCl, KCl, KI, etc.) 7·5 g, HCl 0·3 M 57·5 ml, tap water 7000 ml. To raise the *p*H in the *high* cages, hydrochloric acid was replaced by NaOH, glycine, and after the 14th generation by $Na_3PO_4 . 12H_2O$ as a better buffer. Even with this change the *p*H of the food dropped within 7 days from *p*H 11 down to 8 or 7 and sometimes 5 due to bacterial and yeast contaminants. However, the *p*H of the first 3 days of oviposition was maintained more constant. By increasing the 6 M HCl to 35 ml in the above recipe we obtained *p*H 2. However, we had to increase by 200 g the wheat flour to maintain the food consistency. This *low p*H did not change. In all cultures we used a suspension of boiled baker's yeast in 0·2 M NaOH for the *high p*H, in 6 M HCl for the *low p*H, and in tap water for the *median p*H populations.

3. Results

At the eighth generation in 3 successive days, 11 different culture vials with *p*H 2, *p*H 3 . . . to *p*H 12 were exposed for 1 day in each population. A total of 99 test tubes was used for each level of *p*H in Brasilia and in Eldorado, and about 15,000 to 20,000 flies counted for each level, totalling 39,771 flies for the nine Brasilia and 59,701 for the nine Eldorado populations. However, no significant divergence in viability was observed among them when a t′ test was applied at *p*H 5 (median-low = 0·56; median-high = 0·36).

Nine and 41 generations later, we collected eggs in all populations (by the usual technique of exposing strips of food on pieces of card) and distributed 50 eggs per test-tube with different *p*H. For each population four

TABLE 1

Average egg to adult viability of three Drosophila willistoni *experimental populations from Brasilia and three from Eldorado after 17 and 49 generations in food of different* pH. *For the t′ test of generation 49 the arc sin of the percentage of adult/egg per culture was used. The standard errors are given and the t′ test significant with* $\alpha = 0{\cdot}05$* *and* $\alpha = 0{\cdot}01$**

Test vials' pH	Generation of testings	Brasilia						Eldorado					
		Low	t′	*Median*	t′	*High*	t′	*Low*	t′	*Median*	t′	*High*	t′
*p*H2	17th	—	—	—	—	3·25 ± 3·86	—	26·75 ± 3·56	—	—	—	9·66 ± 3·34	—
	49th	33·01 ± 3·46	2·05	22·85 ± 2·03	—	—	—	33·85 ± 3·46	2·68**	20·44 ± 3·00	—	—	—
*p*H3	17th	2·00 ± 1·82	—	29·00 ± 16·54	—	22·59 ± 10·13	—	44·50 ± 2·82	—	30·92 ± 3·77	—	24·41 ± 2·94	—
	49th	31·07 ± 3·12	1·73	23·79 ± 2·14	—	—	—	40·14 ± 2·75	7·73**	16·03 ± 2·43	—	—	—
*p*H4	17th	40·37 ± 4·74	—	41·42 ± 3·78	—	32·83 ± 9·95	—	42·83 ± 2·69	—	37·75 ± 2·55	—	35·25 ± 2·62	—
*p*H5	17th	41·12 ± 5·19	—	42·91 ± 4·21	—	40·50 ± 2·66	—	40·42 ± 2·25	—	41·00 ± 2·32	—	27·83 ± 2·61	—
	49th	21·20 ± 3·02	3·20**	36·52 ± 2·57	2·36*	27·24 ± 2·47	—	33·87 ± 3·47	3·21**	50·00 ± 2·75	8·29**	29·86 ± 2·26	—
*p*H6	17th	23·25 ± 3·76	—	41·17 ± 2·99	—	40·67 ± 3·40	—	42·00 ± 2·13	—	37·58 ± 2·27	—	38·33 ± 2·20	—
*p*H7	17th	21·62 ± 2·15	—	41·08 ± 3·06	—	38·25 ± 5·63	—	40·92 ± 2·38	—	36·92 ± 2·49	—	36·83 ± 2·43	—
*p*H8	17th	22·62 ± 10·21	—	30·50 ± 7·91	—	37·17 ± 2·69	—	37·58 ± 2·99	—	33·83 ± 2·36	—	36·66 ± 2·67	—
*p*H9	17th	24·62 ± 6·19	—	31·75 ± 9·30	—	36·67 ± 4·46	—	39·08 ± 2·96	—	34·83 ± 2·76	—	34·50 ± 2·86	—
*p*H10	17th	17·87 ± 8·52	—	22·25 ± 7·30	—	36·17 ± 4·77	—	27·08 ± 3·58	—	26·25 ± 3·01	—	21·83 ± 3·08	—
	49th	—	—	21·43 ± 2·42	4·79**	47·10 ± 3·94	—	—	—	21·17 ± 2·67	7·70**	47·74 ± 3·07	—
*p*H11	17th	24·87 ± 4·48	—	17·25 ± 3·90	—	37·08 ± 5·97	—	30·08 ± 2·97	—	28·58 ± 3·24	—	25·25 ± 3·16	—
	49th	—	—	19·96 ± 2·32	4·29**	38·76 ± 3·32	—	—	—	16·48 ± 2·74	3·99**	37·57 ± 3·71	—
*p*H12	17th	24·00 ± 11·23	—	10·92 ± 5·96	—	35·08 ± 8·05	—	23·75 ± 2·72	—	24·92 ± 3·01	—	26·66 ± 2·53	—
	49th	—	—	—	—	30·42 ± 2·42	—	—	—	—	—	12·16 ± 1·87	—

test-tubes were used for each *p*H from 2 to 12 in the 17th generation, but in the 49th generation the test was made only in the extreme *p*H (the *low* populations were tested on *p*H 5, 2 and 3; the *high* populations on *p*H 5, 10 11 and 12 and the control population on *p*H 2, 3, 5, 10, 11 and 12). A summary of the results can be seen in table 1 and fig. 2 shows the results for generation 49.

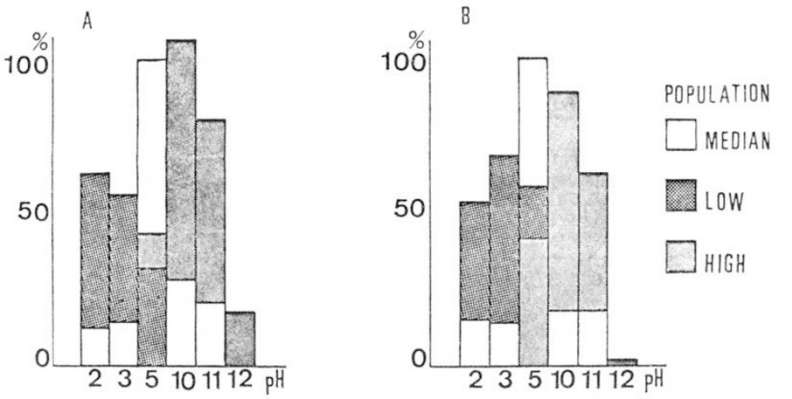

FIG. 2.—Averaged viability of *D. willistoni* populations from Brasilia (A) and Eldorado (B) selected during 49 generations in foods of different *p*H's and tested in different *p*H medium. The average viability in the *p*H 5 bottles was taken as control (100 per cent viability).

The egg-adult viability of *median* Brasilia and Eldorado populations is higher at *p*H 4 to 7, declining more sharply toward a low *p*H than in the opposite direction. It is clear that the populations of Brasilia and Eldorado living on low *p*H 2 to 3 are still very badly adapted to *p*H 3, if compared with the *median* populations (*p*H 5 level), despite the fact that Eldorado was far more viable at low *p*H than Brasilia (table 1). The selective progress to adaptation to alkaline medium was apparently faster than toward the extremely acid medium. It is obvious from the table that *D. willistoni* shows a tolerance for high *p*H, the viability curves being bimodal. The viability increase of the Brasilia populations living at the high level *p*H took place without a significant reduction in their ability to survive on the standard *p*H 5 as can be seen in fig. 2. A significant $t' = 3{\cdot}93$ ($P = 0{\cdot}01$), was obtained between the average viability per vial of the *median* populations and the ones living at *p*H 10, tested in *p*H 5.

At the 49th generation a total of 3600 eggs was collected in the three *median p*H 5 populations of Brasilia and an equal number in the *median p*H 5 populations of Eldorado. These eggs were distributed in four culture vials with *p*H 2, *p*H 3, *p*H 10, *p*H 11, *p*H 12 and *p*H 5 in a total of 24 tubes. The populations with *p*H 3 food were tested in 12 vials, *p*H 2, *p*H 3, and *p*H 5, four vials each, receiving 50 eggs each as in all the others. For the *high* populations the test tubes had *p*H 5, 10, 11 and 12. The comparisons have been made directly between the average viability of the *median p*H 5 populations and the extreme ones. A test showed that all differences are significant (table 1) except for *p*H 3 and 2 Brasilia populations.

(i) *Developmental rate*

The observed progress in egg-adult viability was not followed by an increase in the developmental rate. The opposite occurred, the populations that exhibited good viability in the *p*H 10 at the 49th generation, proved to have a faster development at *p*H 5 despite their lower viability on that medium. The same can be said about the Brasilia and Eldorado populations adapted to *low* *p*H level (all the comparisons were significant at 0·05 for a Kolmogorov-Smirnoff test). This fact is of special interest. The selective effect that improved viability at extreme *p*H levels did not increase the speed of development. If the survival at extreme *p*H was attained at the expense of the ontogenetic rate it should be difficult or even impossible to increase the latter while maintaining a high level of viability. To test this we carried out selection for fast developmental rate from the 94th to the 102nd generation. Two populations for each level of *p*H were randomly derived from the original Eldorado and Brasilia populations. To start with, each cage received 900 individuals which were allowed to oviposit for 5 days. The first 900 individuals which eclosed in each cage were used as parents for the next generation. All flies were counted every day for 9 or 10 days from the 1st day of eclosion or until no more flies eclosed. The cumulative percentage of eclosion of selected populations of Brasilia and Eldorado were compared. These unselected populations for extreme *p*H media had, on the average, a better response to selection for developmental rate than the other populations. However, the high *p*H populations both from Eldorado and Brasilia, responded to a greater extent to selection for developmental rate than the lower *p*H populations. No significant changes in the egg to adult viabilities were detected in these populations.

4. Discussion

Waddington (1959) demonstrated that, when *D. melanogaster* larvae were grown for about 20 generations on medium with high salt content, they showed increased adjustment to this substrate. In a careful study, Robertson (1960*a*, *b*) analysed selection responses for extreme body size and developmental time on different diets on defined media. Among other significant results he found a positive association between body size and the duration of growth period as a response to minor and specific nutritional variation, while the reverse (negative) association was observed with variation in the diet. Our results showed a negative association between fitness to extreme *p*H medium and developmental time. Despite the fact that we did not measure these responses with the precision used by Robertson in his experiments, we can see that our results are in the same direction. As expected theoretically by Robertson (1955), Hiraizumi (1961) found a negative correlation between the fitness components, female fertility and developmental rate. This held only when developmental rate was very high but was reversed (became positively correlated) at lower developmental rates or when less fit, lethal bearing genotypes were studied (Hiraizumi and Crow, 1960). Hiraizumi (1961) estimated that a 5 per cent reduction in the rate of development involved at least a 15 per cent increase in fertility. Lewontin (1966) suggested, on theoretical grounds, that small absolute changes of 10 per cent in developmental rate are roughly equivalent to large increases of fertility

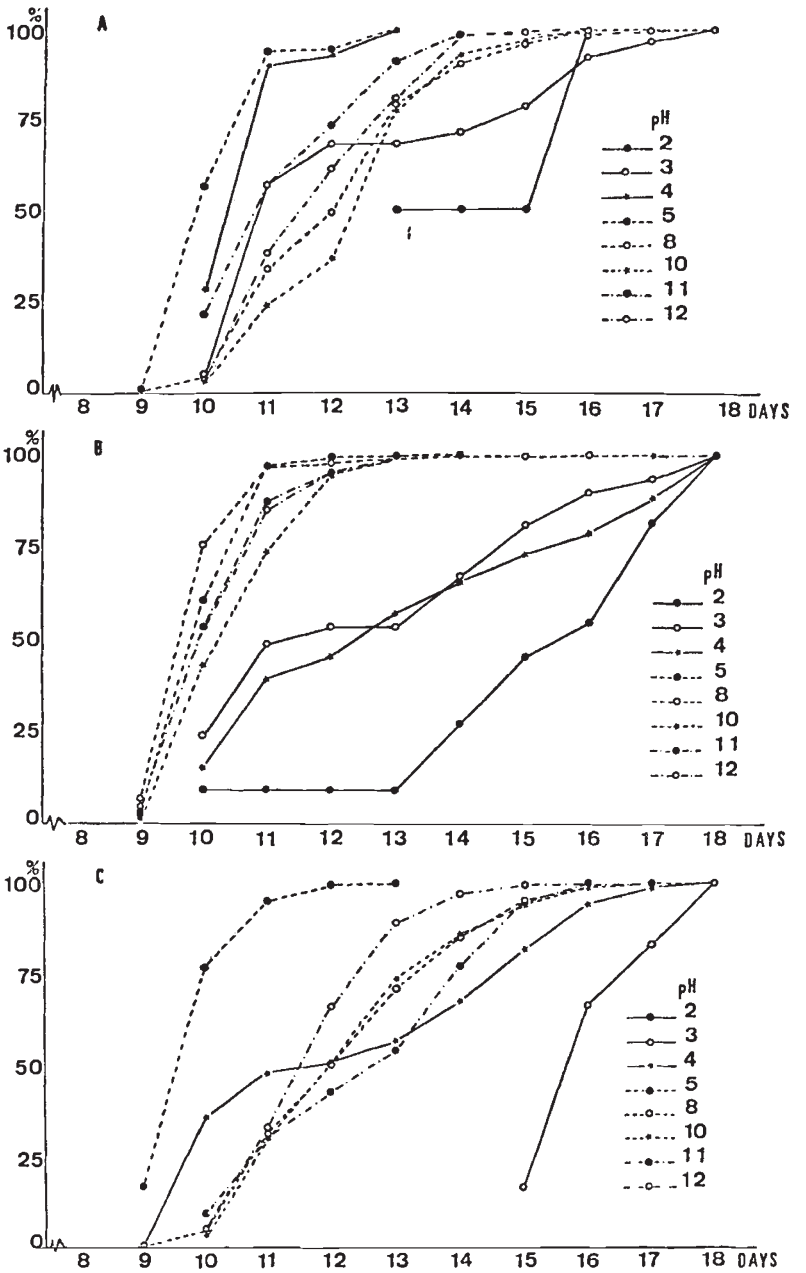

FIG. 3.—Developmental rate. Averaged results of accumulated daily eclosion rates of 12 vials (600 eggs) for the tested *p*H's of three *D. willistoni* from Brasilia (A = Bm_1, Bm_2, Bm_3) (B = Bh_1, Bh_2, Bh_3) (C = Bl_1, Bl_2, Bl_3) maintained during 17 generations in foods of different *p*H's and tested in different *p*H media.

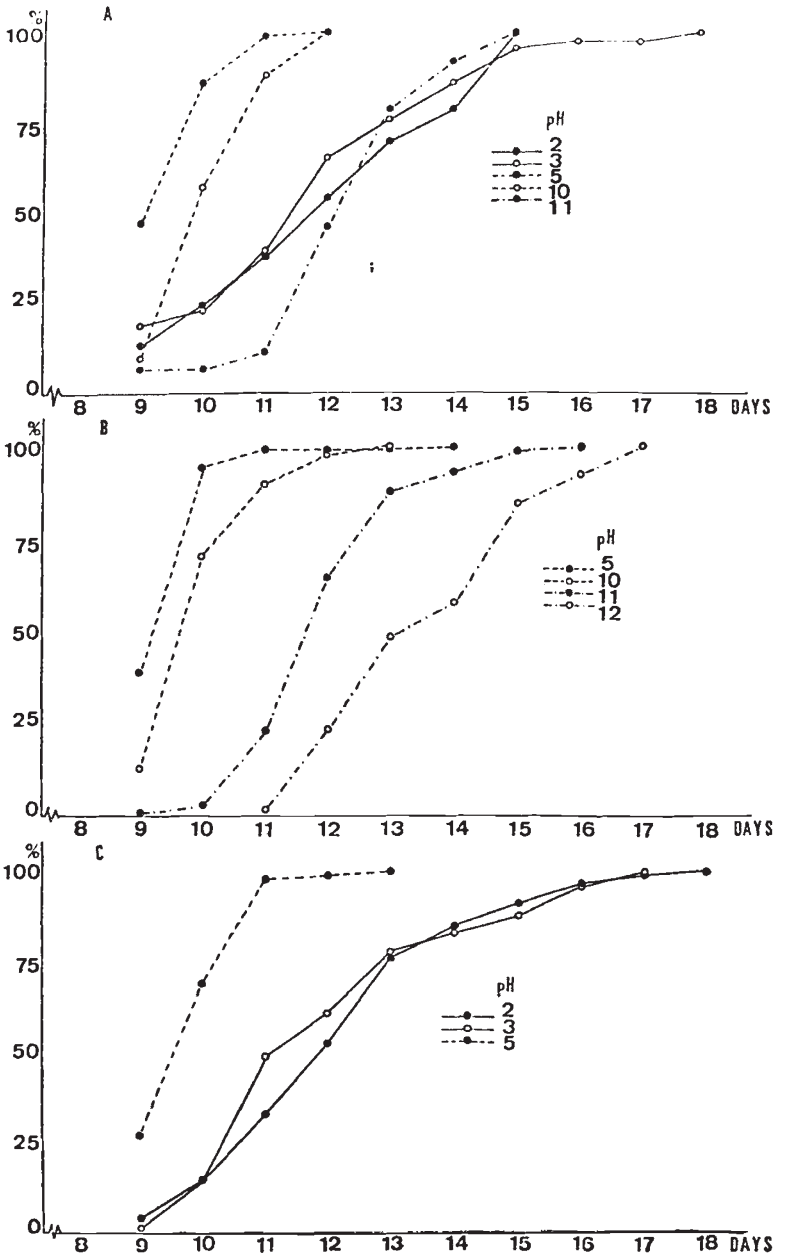

FIG. 4.—Developmental rate. Averaged results of accumulated daily eclosion rates of 12 vials (600 eggs) for the tested *p*H's of three *D. willistoni* populations from Brasilia (A = Bm_1, Bm_2, Bm_3) (B = Bh_1, Bh_2, Bh_3) (C = Bl_1, Bl_2, Bl_3) maintained during 49 generations in foods of different *p*H's and tested in different *p*H media.

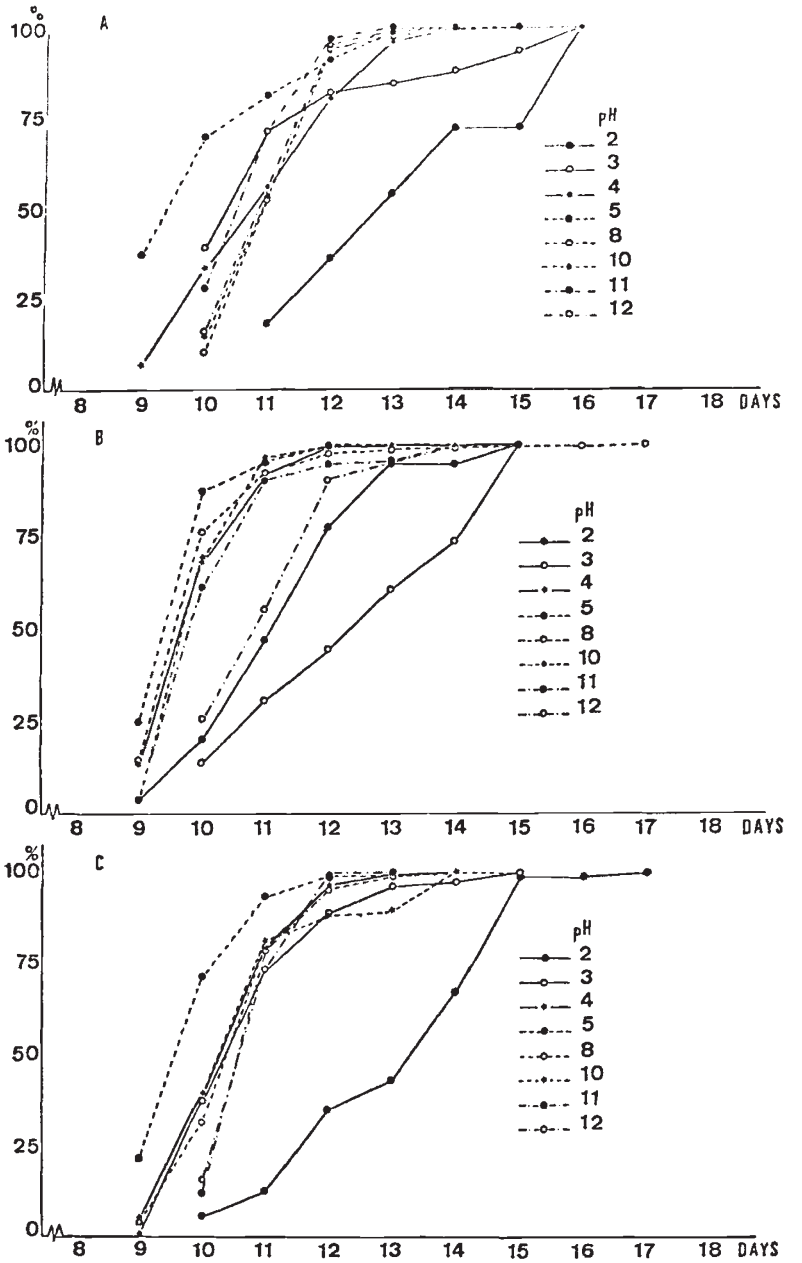

FIG. 5.—Developmental rate. Averaged results of accumulated daily eclosion rates of 12 vials (600 eggs) for the tested *p*H's of three *D. willistoni* populations from Eldorado (A = Em_1, Em_2, Em_3) (B = Eh_1, Eh_2, Eh_3) (C = El_1, El_2, El_3) maintained during 17 generations in foods of different *p*H's and tested in different *p*H media.

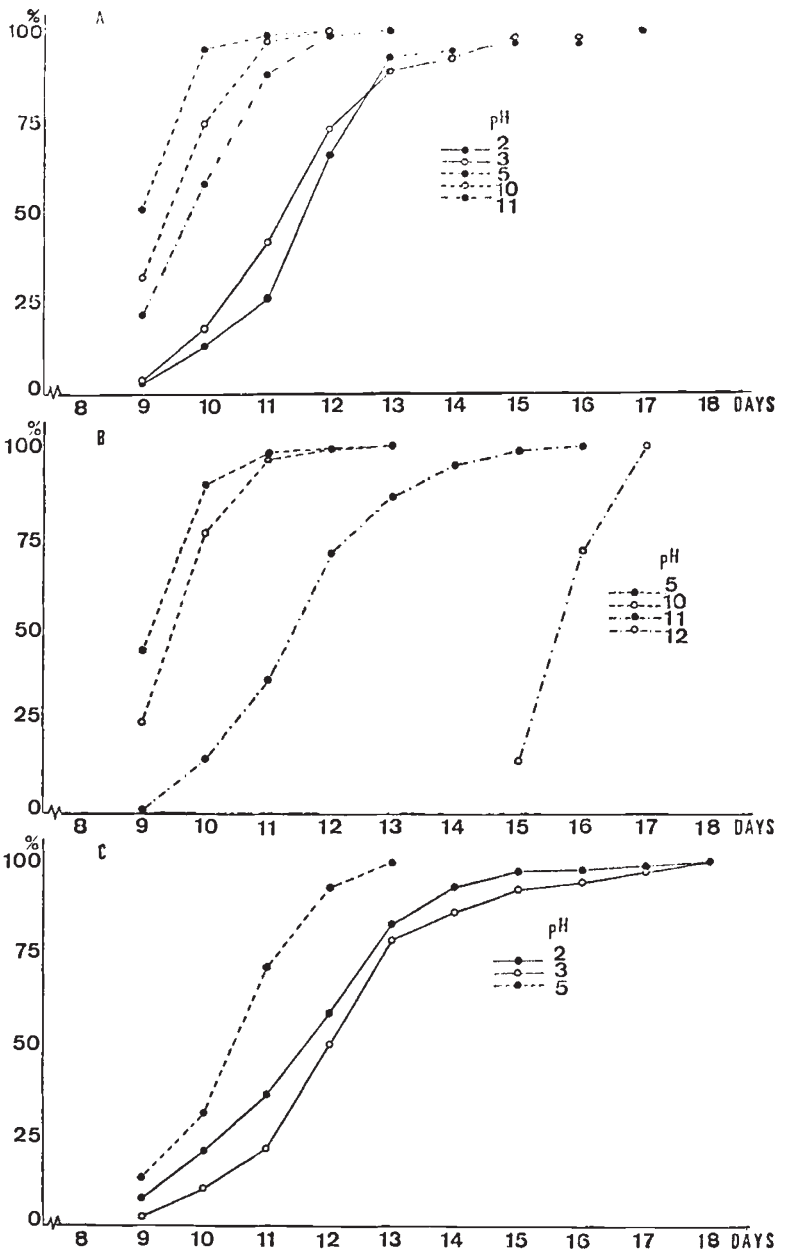

FIG. 6.—Developmental rate. Averaged results of accumulated daily eclosion rates of 12 vials (600 eggs) for the tested *p*H's of three *D. willistoni* populations from Eldorado (A = Em_1, Em_2, Em_3) (B = Eh_1, Eh_2, Eh_3) (C = El_1, El_2, El_3) maintained during 49 generations in foods of different *p*H's and tested in different *p*H media.

of the order of 100 per cent. Consequently we would theoretically expect very little genetic variance for developmental time in wild populations of *Drosophila*, which was not confirmed by the experimental results in this study and the one by Spiess and Spiess (1966).

Natural populations of *Drosophila* have proved to have an unsuspected wealth of genetic variability which enable them to develop resistance to extremes of temperature, desiccation, salt concentrations and unusual compounds, like PTC, EDTA, insecticides, etc. (Parsons, 1973). The possibility of selecting populations previously adjusted for viability, for faster development, on extreme *p*H food suggests an independent genetic basis for these characteristics, despite the fact that the initial response to selection was an increase of viability which was negatively correlated with developmental rate. This correlation was not reversed nor entirely suppressed by the second phase of the selection experiment. The normal *median p*H 5 populations always gave better responses to selection for developmental rate than the extreme *p*H populations.

Asymmetrical response to selection in extreme conditions is a common result in *Drosophila* and other organisms. The only condition to be fulfilled is that natural selection favours one of the extremes, and, consequently natural populations are at or close to a " plateau ". Conversely, if we find that experimental selection on low *p*H medium is slower than on high *p*H medium, either in central or marginal populations of *D. willistoni*, we may conclude that natural food sources are usually acid. However, the significance of such faster adaptive response to very alkaline food obviously depends on the existence of readily available reserve of genetic variability.

We are far from understanding the molecular mechanisms of adaptation to extreme *p*H medium. We believe that changing the *p*H optimum of many enzymes directly responsible for the metabolism of food may be involved in this adaptive process.

5. References

BRNCIC, D. 1961. Integration of the genotype in geographic populations of *Drosophila pavani*. *Evolution*, *15*, 92-97.

CROW, J. F., AND KIMURA, M. 1963. The theory of genetic load. *Proc. XI Intl. Cong. Genet., Genetics Today*, *I*, 495.

DA CUNHA, A. B. 1951. Modification of the adaptative values of chromosomal types in *D. pseudoobscura* by nutritional variables. *Evolution*, *5*, 395-404.

DA CUNHA, A. B. 1955. Contribuição ao estudo da adaptação das populações de *Drosophila* a diferentes levedos. *Boletim 220. Biologia Geral no. 10. Fac. Fil. Ciências e Letras USP.*

DOBZHANSKY, TH., AND SPASSKY, N. 1954. Environmental modification of heterosis in *D. pseudoobscura*. *Proc. Nat. Acad. Sci. (US)*, *40*, 407-415.

HIRAIZUMI, Y. 1961. Negative correlation between rate of development and female fertility in *D. melanogaster*. *Genetics*, *46*, 615-624.

HIRAIZUMI, Y., AND CROW, J. F. 1960. Heterozygous effects on viability, fertility, rate of development, and longevity of *Drosophila* chromosomes that are lethal when homozygous. *Genetics*, *45*, 1071-1073.

LEVINE, R. P. 1952. Adaptative responses of some third chromosome types of *D. pseudoobscura*. *Evolution*, *6*, 216-233.

LEWONTIN, R. C. 1966. Hybridization as a source of variation for adaptation to new environments. *Evolution*, *20*, 315-336.

MARQUES, E. K., NAPP, M., WINGE, H., AND CORDEIRO, A. R. 1966. A cornmeal, soy bean flour, wheat germ medium for *Drosophila*. *Drosophila Information Service*, *41*, 187.

PARSONS, P. A. 1973. *Behavioural and Ecological Genetics*. A study in *Drosophila*. Clarendon Press, Oxford.

PAVAN, C. 1952. Relações entre populações naturais de *Drosophila* e o meio ambiente. Tese para provimento de cátedra USP.

ROBERTSON, F. W. 1955. Selection response and the properties of genetic variation. *Cold Spr. Harb. Symp. Quant. Biol., 20,* 166-177.

ROBERTSON, F. W. 1960*a*. The ecological genetics of growth in *Drosophila*. 1. Body size and development time on different diets. *Genet. Res., 1,* 288-304.

ROBERTSON, F. W. 1960*b*. The ecological genetics of growth in *Drosophila*. 3. Growth and competitive ability of strains selected on different diets. *Genet. Res., 1,* 333-350.

ROBERTSON, F. W. 1966. The ecological genetics of growth in *Drosophila*. 8. Adaptation to a new diet. *Genet. Res., 8,* 165-179.

SPIESS, E. B., AND SPIESS, L. D. 1966. Selection for rate of development and gene arrangement frequencies in *Drosophila persimilis*. II. Fitness properties at equilibrium. *Genetics, 53,* 695-708.

WADDINGTON, C. H. 1959. Canalization of development and genetic assimilation of acquired characters. *Nature, 183,* 1654-1655.

WAGNER, R. P. 1944. Studies in the genetics of *Drosophila*. III. The nutrition of *D. mulleri* and *D. aldrichi*. Growth of the larvae on a cactus extract and the microorganisms found in cactus. *Univ. Texas Publ., 4445,* 104-128.

WAGNER, R. P. 1949. Studies in the genetics of *Drosophila*. VI. Nutritional differences in the mulleri group. *Univ. Texas Publ., 4920,* 39-41.