

DIVERSIFYING SELECTION AND ECOTYPIC VARIATION
IN EXPERIMENTAL POPULATIONS OF DROSOPHILA MELANOGASTER

A Thesis

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For many years population genetics was an immensely rich and powerful theory with virtually no suitable facts on which to operate. It was like a complex and exquisite machine, designed to process a raw material that no one had succeeded in mining. Occasionally some unusually clever or lucky prospector would come upon a natural outcrop of high grade ore, and part of the machinery would be started up to prove to its backers that it really would work. But for the most part the machine was left to the engineers, forever tinkering, forever making improvements, in anticipation of the day when it would be called upon to carry out full production.

Quite suddenly the situation has changed. The mother-lode has been tapped and facts in profusion have been poured into the hoppers of this theory machine. And from the other end has issued—nothing.

R. C. Lewontin (1974. p189)

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ABSTRACT

Two populations of Drosophila melanogaster were established in the laboratory and each was offered alternative habitats in the form of different foods. At the end of fourteen months, experiments were run to determine whether or not there was any response to this diversifying selection. The results lend support to the supposition that environmental heterogeneity may lead to greater phenotypic diversity.

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INTRODUCTION

Mather (1955) defined disruptive or diversifying selection as the differential survival of more than one phenotypic optimum within a population; he further suggested that at least two outcomes are possible depending upon the conditions of the selection. Isolation could arise if the following three conditions are met: 1) selection is separate on two functionally independent optima, 2) the groups are sufficiently distinct from one another for the different selective forces to be effective, and 3) the environmental differences giving rise to the optimal phenotypes persist. If these conditions are not met, a polymorphism may result, especially if an interdependence of related optima exists.

Thoday (1972) has suggested that such disruptive selection might be expected to occur in two cases: where heterogeneity of selection is intrinsic to the biology of the population itself (e. g., sexual dimorphism, heterostyly, sex-limited polymorphism and any form of genetic facilitation); secondly, where heterogeneity of selection arises from environmental heterogeneity in space, either due to mosaicism of different ecological niches which the

population may occupy, or due to a linear variation of some relevant environmental factor (such as may be the cause of some phenotype-frequency clines).

Some of the most interesting experimental results were reported by Thoday and Gibson (1962), who used a scheme of disruptive selection for increased and decreased sternopleural chaeta number in Drosophila melanogaster. The flies in their study produced fewer and fewer hybrids per generation, despite the fact that the selected flies had the opportunity for random mating. This evidence gave experimental support to the theoretical supposition that isolation could arise without allopatry. One of the major problems with this work is that, although a number of experiments by others (Scharloo et al., 1967; Chabora, 1968; Barker and Cummins, 1969) have selected for the same trait as did Thoday and Gibson with very similar experimental designs, the results have not been repeatable. Beardmore and Baldawi (as cited by Thoday, 1972) have obtained results similar to those of Thoday and Gibson, providing some additional evidence for isolation arising from disruptive selection. Thoday and Gibson (1970) suggested that the major reason for the failure of many of the experimenters to duplicate their results is the lack of appropriate genetic variation in the original stocks. Their Southacre stock was made up of the progeny of four wild female Drosophila melanogaster found in the same garbage can.

Most of the other experiments were done with laboratory stocks which Thoday and Gibson suggested would not have the genetic variation necessary (an exception being the work of Chabora (1968) who employed both natural and laboratory stocks of D. melanogaster). Beardmore and Baldawi collected their stocks from nature and their positive results seem to indicate that initial variation is a very real point to be considered.

While many of the experiments above purport to be analogous to niche selection with habitat choice for ovipositing females, very little work seems to have been done directly on actual habitat choice. Pimentel et al., (1967), however, selected for two different habitat preferences in a population of Musca domestica. Although they found a significant correlation between the separate sub-populations and their sites of oviposition, they could not draw any conclusions as to whether there was any greater success on the appropriate medium than on the alternative medium. The three authors failed to test for any mating preference between the sub-populations, claiming a lack of manpower.

Saitta (1973), using a population of D. melanogaster which contained a mutator gene on the third chromosome and whose males had been X-rayed at 1000 r, offered three different substrates to the flies in the same cage. Flies were collected from the three different substrates after twenty and forty generations and productivity of each type of fly on each type of medium was tested. Two of the three types

of fly did significantly better on their respective media than on the other two media, and all three types of fly had an increased productivity on all foods when compared with the controls.

An interesting behavioral polymorphism was reported by DeSouza et al., working with a population of irradiated D. willistoni. They found that at a relative humidity of 90%, after about one year, there were flies that pupated inside the food cups and a second morph that pupated on the floor of the cage. By doing the appropriate crosses, they attributed this behavioral difference to the action of a single locus. This niche expansion allowed a greater population density.

These habitat selection experiments include a behavioral response by the individuals, which would seem to be one factor which must be included in this type of disruptive selection experiment. There have been few reports in the literature of disruptive selection on behavioral traits. Grant and Mettler (1969) attempted to disrupt a population on the basis of the "escape" behavior of D. melanogaster, and Coyne and Grant (1972) succeeded in producing isolation under disruptive selection (the major modification over the work of Grant and Mettler was the separation of the selected females after mating and the complete lack of migration between the selected extremes save for heterogamic matings).

Concern with disruptive selection in nature suggests that an experiment might be designed which incorporates two distinct habitats offered as alternatives to a population of organisms which are then free to choose which habitat they will occupy. These habitats should be sufficiently stringent to provide a selective premium in choosing the habitat in which there is greater success. This was the major impetus for the series of experiments described herein, in which two normally suboptimal food types were offered in addition to the normal food of the population of D. melanogaster used. By complete selection against the larvae on the normal food, the two suboptimal environments became the optimal environments.

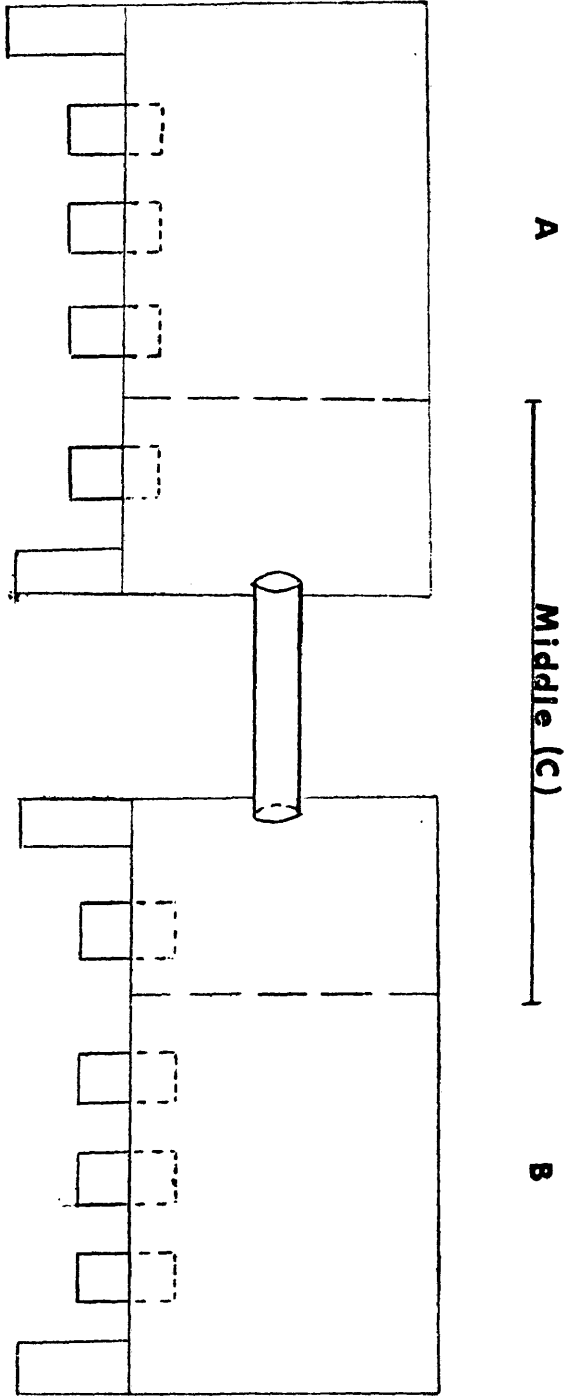
MATERIALS AND METHODS

The Drosophila melanogaster employed in this study were derived from a population established in 1971 from collections made by B. Grant across Virginia and the East Coast of the United States.

The Population Cages

The cages used for the experimental populations consisted of two plastic shoeboxes, each 30.5cm long, 9cm high and 16.5cm wide, connected end-to-end by a 10cm plastic tube with a diameter of 3cm (Figure 1). The bottom of each shoebox had eight holes fitted with a rubber gasket into which food cups were inserted. On the inside of each shoebox, a baffle was placed, five inches from the end into which the plastic tube opened. This baffle had five 0.5cm holes punched in it to allow the flies to move freely from one area of the cage to the next (the purpose of the baffles was to prevent bolting of the flies from one end of the cage to the other, if disturbed). These cages were on eight 10cm plastic legs in a soapwater moat to prevent devastation of the populations by the omnipresent ants. The room in which the cages were kept was on a repeating twelve hour (10AM, 10 PM; EST) light-dark cycle.

Figure 1. The population cage. The dashed lines represent the baffles.



Three of these cages were set up, each having four regions. The outside regions, which were used for the extreme habitats, each had five holes for food cups. At the end of five weeks, all five insert holes were filled so when the sixth food cup was placed in this region, it replaced the first cup, when the seventh food cup was placed in the cage, it replaced the second food cup and so on. The two inner regions were treated as one central zone, with one food cup in each region. These food cups were removed every four days and replaced with fresh ones to prevent the eclosion of any flies which were oviposited on this medium.

A commercial *Drosophila* medium (Carolina Biological Supply Company) was prepared with distilled water and inoculated with yeast. Food prepared in this fashion was placed in the central regions of the cage. The end regions were supplied with similar food except the distilled water was replaced with a salt solution in one end and the other end was adjusted for a pH difference.

The conditions of the three setups were as follows: NaCl-High pH cage; Originally, a NaCl concentration of 0.5M (in distilled water) was mixed with the medium (giving a pH of 5.1). The High pH medium was mixed with a Tris-HCl buffer (Dawson et al., 1959, p205) providing a pH of 7.3 after inoculation with yeast. After about four weeks, there appeared to be much greater success in the pH end of the cage than in the salt end of the cage (judging from the number

of flies present) and therefore, the NaCl concentration was lowered to 0.2M while the pH was raised to 7.6. This seemed to provide a better balance and these conditions were maintained for four months, at which point the salt concentration was raised once more to 0.5M and the pH was raised to 7.8. Five months after the cage was established, the flies were transferred to clean cages having the same three food habitats. The experimental populations were exposed to the food alternatives for a total period of fourteen months.

KCl-Low pH cage; This cage was set up and cleaned in the same fashion as the preceding one. Instead of NaCl, however, a 0.5M concentration of KCl was used as one food alternative (pH 5.35) and a pH of 3.8 for the other food choice by use of a sodium citrate-citric acid buffer (Dawson et al., 1959, p156). This pH was lowered to 3.3 three months after the cage was established and to 3.0 two months after that.

Experimental Tests

Migration; In order to determine whether the baffles had any inhibitory effect on the movement of the flies, a cage was set up, as those described above, but with normal food only. One hundred Control flies were put in each end and allowed to remain for one week. In order to differentiate between the two groups, those in one end were marked by subtle wing clipping. At the end of one week, the two halves of the cage were separated and plugged; the flies

were then anesthetized with CO₂, removed and viewed under a dissecting microscope in order to determine their origin.

Developmental rate and survival on different media;

This series of tests was designed to determine whether flies from one habitat (food) do better in that same habitat than in the alternative habitat and whether or not they differ significantly in performance from the control flies: e. g., High pH flies raised on High pH medium, High pH flies raised on NaCl medium and High pH flies raised on a Control medium compared with NaCl flies raised on these three media and compared with Control flies raised on these three media. In order to obtain eggs for these tests, the medium appropriate to the habitat (High pH medium for the High pH end of the cage etc.) was mixed in a food cup, dyed blue, the surface smoothed, and the food cup placed in the cage for a period of twenty-four hours. When the cup was removed, it was placed under a dissecting microscope and the eggs were carefully transferred to shell vials (9.5cm X 2.5cm, 30 eggs per vial) containing the medium to be tested. The flies which eclosed were removed and the resulting data recorded every twelve hours (10AM and 10PM), giving two types of data: number of flies surviving on the medium and rate of development. The last set of data was collected in comparison with the control flies. Each experimental vial was paired with a vial containing the same type of medium and thirty control flies.

Food preference; In an attempt to discover whether any difference in food preferences existed among the selected and Control flies, virgin males and females were collected (as eggs and raised in shell vials) and offered a choice of the alternative food types. In order to control for uneven lighting, white, lidless cardboard boxes were made (30.5cm x 20cm x 47cm) over which long fluorescent lamps could be situated. With the room lights turned off, the lighting in the boxes seemed uniformly distributed. Plastic petri plates divided into four quadrants were used as test chambers. The opposing quadrants were filled with the alternative types of media, giving two distinct habitats. Ten lightly anesthetized virgin flies (all male or all female) were placed in one of the unfilled triangles and allowed to recover for thirty minutes. The flies could then be scored as to the type of food on which they were found each half-hour, for a period of eight hours. The plates were rotated 180° after each scoring.

Site of Oviposition; This test had the same design as the food preference test with several minor modifications. For the salt-pH comparisons, the media were dyed blue and smoothed to form a uniform surface. The flies used were all females and all had spent 24 hours with an equal number of males for mating. After having been lightly anesthetized, ten females were placed in the petri dish for eight hours (the dishes were turned each half-hour, for reasons des-

cribed above). At the end of this time, the number of eggs present on the different media were counted.

Mating tests; The mating tests between the flies from alternative food habitats were all performed during the fourteenth month. Twenty-four virgin flies (aged from two to seven days), equally divided as to sex and type were anesthetized, the males of one type and the females of one type were marked by subtly clipping one wing, and all twenty-four were placed in shell vials, once more according to sex and type. At dawn (when the lights came on) of the next day the flies were transferred to small, long-nosed plastic bottles from which they could be transferred to the mating chambers without anesthetization. The chambers were each constructed from a 90 x 15mm plastic petri plate with two openings (180° from one another) in the circumference. These openings, which were fitted with rubber gaskets, allowed both the introduction of the flies without anesthetization and the withdrawal of the mating pairs with an aspirator. Each test was run for two hours, and each mated pair of flies was removed with the aspirator to an empty shell vial for later scoring. Since the element of choice is reduced with each removal, only the first nine of the twelve (75%) possible matings were scored. Due to the fact that D. melanogaster make use of their wings in courtship behavior, it was necessary to preclude the possibility of mating discrimination on the basis of the wing

clipping. Grant and Mettler (1969) and Coyne and Grant (1972) tested this with control flies and found that there was no significant departure from random mating attributable to wing clipping. In order to insure that any effect of marking was balanced, the type of fly marked was alternated with each trial.

RESULTS

Migration. Table I presents the results of the migration test. A x^2 test comparing origins of flies within each extreme end of the cage shows that there is no departure from a one to one mixture.

Developmental rate of High pH and NaCl flies. The data presented in Table II are from a pilot study designed to determine whether an in depth study of developmental rate and survivorship were worthwhile. High pH flies develop faster than NaCl flies on both High pH and NaCl media but each type of fly develops faster on NaCl medium than on High pH medium. Although little could be concluded from these tests, they certainly suggested further study.

The results of the expanded developmental rate study are presented in Tables IIIa and IIIb and in Figure 2. Table IIIa presents the analysis of this data when grouped as presented (all three types of fly compared on one type of medium). After testing for homogeneity of variance (by Bartlett's test), an Analysis of Variance (ANOVA) was performed to determine whether or not there was any significant difference between the mean values. Where a significant F value was found, a Student-Newman-Keuls (SNK)

Table I. Migration data on 200 unselected flies. Flies with clipped wings (C) were placed in cage end A. Those with unclipped wings (U) were placed in cage end B. The x_1^2 is based on C vs. U in each extreme cage end.

Cage end	A		B	
	End	Middle	Middle	End
Flies	30C	49C	11C	9C
	31U	22U	31U	16U
	$x_1^2 = 0.018$		$x_1^2 = 1.960$	
	n. s.		n. s.	

Table II. Developmental rate and survivorship of NaCl and High pH flies on the various media. Pilot study.

Flies	Medium	Eclosion time in days ($\bar{x} \pm$ s. d.)	Number of flies surviving	% Survival
	NaCl	18.3 \pm 1.6	24	80
NaCl	High pH	23.1 \pm 2.1	10	33.3
	Control	13.1 \pm 0.2	16	53.3
	NaCl	15.8 \pm 0.9	12	40
High pH	High pH	18.1 \pm 1.6	18	60
	Control	14.3 \pm 1.4	22	73.3

Table IIIa. Developmental rate of High pH, NaCl and Control flies on the various media.

Included is an Analysis of Variance comparing the performance of all three types of fly on each type of medium. Student-Newman-Keuls test presented where appropriate. All groupings passed Bartlett's test of Homogeneity of Variance.

Flies	Medium	Mean days to eclosion ($\bar{x} \pm s. d.$)	$\frac{\text{Treatment DF}}{\text{Error DF}}$	ANOVA F	SNK
High pH		13.7 \pm 1.0			
NaCl	High pH	15.9 \pm 0.8	2/25	4.39*	n. s.
Control		15.0 \pm 1.7			n. s.
High pH		12.7 \pm 0.5			
NaCl	NaCl	12.5 \pm 0.3	2/29	2.44 n. s.	
Control		13.3 \pm 1.1			
High pH		10.1 \pm 0.3			
NaCl	Control	13.3 \pm 0.2	2/42	19.90**	
Control		11.1 \pm 1.2			

*significant at 0.05

**significant at 0.01

Table IIIb. Developmental rate of High pH, NaCl and Control flies on the various media.

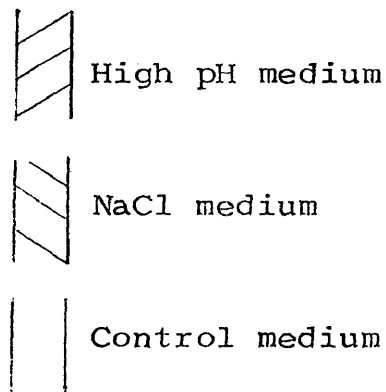
Included is an Analysis of Variance comparing the performance of each kind of fly on all three types of medium. Student-Newman-Keuls test presented where appropriate. All groupings passed Bartlett's test of Homogeneity of Variance.

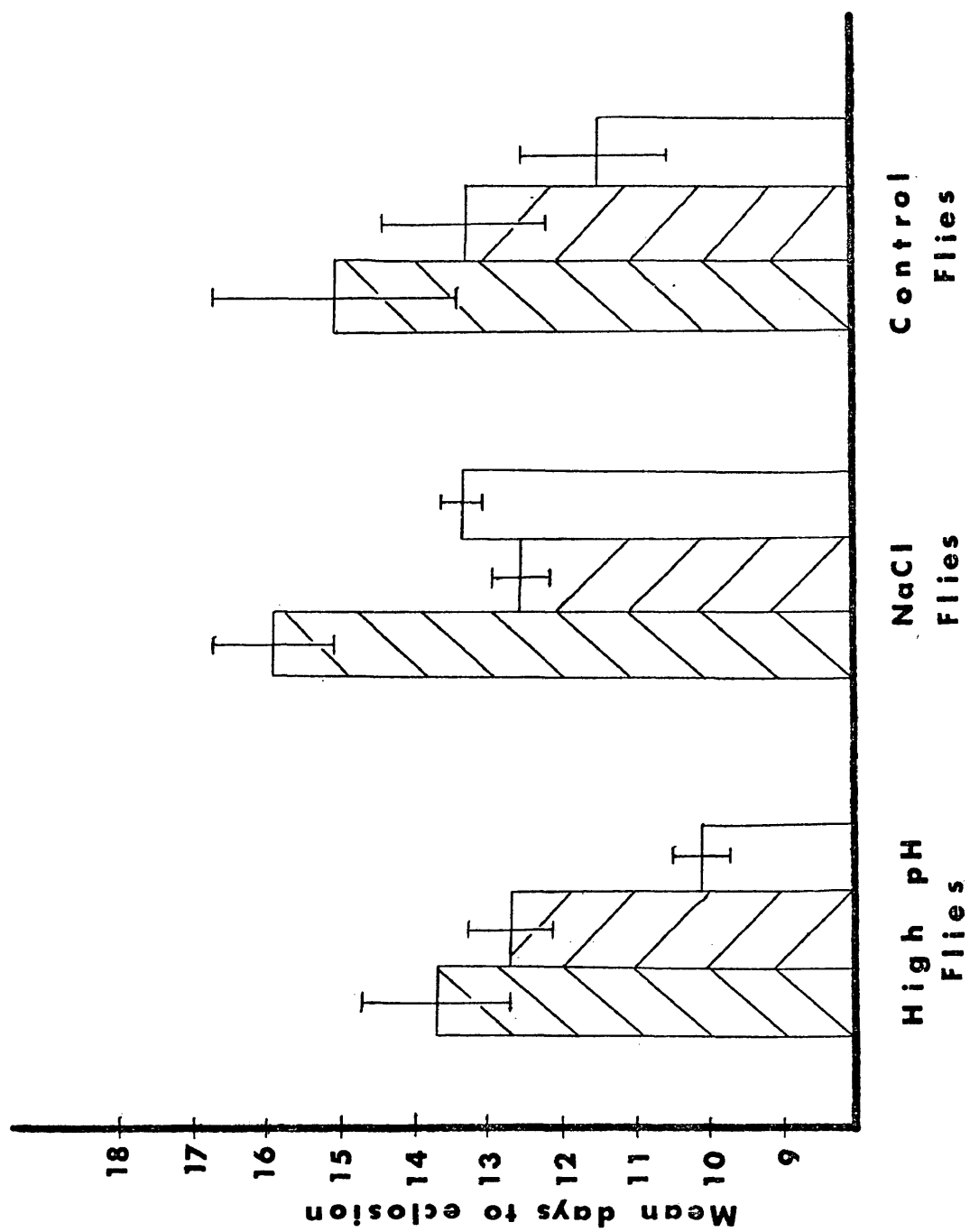
Flies	Medium	Mean days to eclosion ($\bar{x} \pm s. d.$)	$\frac{\text{Treatment DF}}{\text{Error DF}}$	ANOVA F	SNK
High pH	High pH	13.7 \pm 1.0			
	NaCl	12.7 \pm 0.5	2/21	58.95**	** n.s.
	Control	10.1 \pm 0.3			** **
NaCl	High pH	15.9 \pm 0.8			
	NaCl	12.5 \pm 0.3	2/18	77.18**	** **
	Control	13.3 \pm 0.2			** **
Control	High pH	15.0 \pm 1.7			
	NaCl	13.3 \pm 1.1	2/57	37.59**	** **
	Control	11.1 \pm 1.2			** **

*significant at 0.05

**significant at 0.01

Figure 2. Developmental rate of High pH flies, NaCl flies and Control flies. The values are the mean time (in days) taken until eclosion from the day the eggs were collected \pm the standard deviation. A media key is presented below.





test was run to determine to what values the differences should be attributed. On the High pH medium, High pH flies develop faster than either NaCl or Control flies and Control flies develop faster than the NaCl flies. The comparison between the performances of High pH and NaCl flies is highly significant. In the second row of comparisons (Table IIIa), on NaCl medium, the NaCl flies eclose first, High pH flies second and Control flies last. On the Control medium, High pH flies eclose first, Control flies next and NaCl flies last. Table IIIb presents the same data grouped to compare the performance of a given type of fly on the three types of media. High pH flies develop fastest on Control medium (highly significant when compared to both NaCl and High pH media), next on NaCl medium and slowest on High pH medium. The NaCl flies eclose first from NaCl medium, next from Control medium and last from High pH medium. The differences of all three of these values are highly significant. Control flies show their fastest development on Control medium, next on NaCl medium and slowest on High pH medium. All three comparisons reveal highly significant differences.

Survival of High pH and NaCl flies. The indications from the survivorship pilot study (also Table II) seem much clearer than the considerations of developmental rate. The NaCl flies have a much higher survival on NaCl medium than on High pH medium while the High pH flies have a much higher survival rate on High pH medium than on NaCl medium.

Also, a comparison between the two types of fly reveals that the NaCl flies have a much higher survival rate on the NaCl medium than do the High pH flies and the High pH flies show a much higher survival rate on the High pH medium than the NaCl flies. This also suggested a more in depth study. The results of the expanded study are presented in Figure 3 and in Tables IVa and IVb. Table IVa treats the data grouped as all three types of fly on each of the three media. On the High pH medium, the High pH flies have a higher mean percent survival than either NaCl or Control flies (highly significant in both comparisons). The NaCl flies are next and the Control flies last. In Table IVb, the same data are arranged to facilitate comparisons of a given type of fly on all three types of media. High pH flies survive best on the NaCl medium while their performances on High pH and Control media are very similar. NaCl flies do best on NaCl medium, next on Control medium and poorest on High pH medium. The comparisons between High pH medium and both NaCl and Control media are highly significantly different. The Control flies have their greatest survival on NaCl medium also, followed by the Control medium and their lowest survival on High pH medium. The comparisons of Control flies on both the NaCl and Control media are highly significant when contrasted with the High pH medium.

Survival of Low pH and KCl flies. The data from the pilot study on developmental rate and survivorship of KCl and Low pH flies on the various media are presented in Table

Table IVa. Survival of High pH, NaCl and Control flies on the various media. Included is an Analysis of Variance comparing the performance of all three types of fly on each type of medium. Student-Newman-Keuls test presented where appropriate. All groupings passed Bartlett's test of Homogeneity of Variance.

Flies	Medium	Mean % survival ($\bar{x} \pm s. d.$)	$\frac{\text{Treatment DF}}{\text{Error DF}}$	ANOVA F	SNK
High pH		65.9 \pm 11.1			
NaCl	High pH	42.8 \pm 9.8	2/25	19.99**	**
Control		31.5 \pm 13.7			n. s.
High pH		80.9 \pm 13.8			
NaCl	NaCl	78.8 \pm 6.7	2/29	0.094 n. s.	
Control		81.8 \pm 13.8			
High pH		68.4 \pm 8.7			
NaCl	Control	74.3 \pm 11.0	2/42	0.87 n. s.	
Control		73.9 \pm 11.4			

*significant at 0.05

**significant at 0.01

Table IVb. Survival of High pH, NaCl and Control flies on the various media. Included is an Analysis of Variance comparing the performance of each kind of fly on all three types of medium. Student-Newman-Keuls test presented where appropriate.

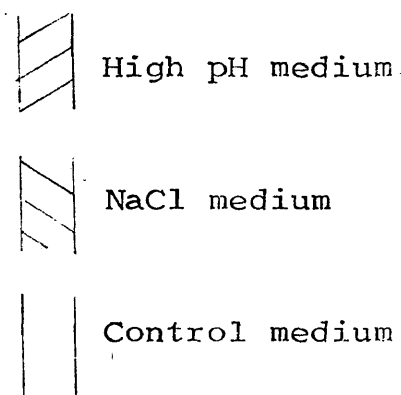
All groupings passed Bartlett's test of Homogeneity of Variance.

Flies	Medium	Mean % Survival ($\bar{x} \pm s. d.$)	ANOVA		SNK
			Treatment DF	Error DF	
High pH	High pH	65.9 \pm 11.1			
	NaCl	80.9 \pm 13.8	2/21	3.97*	n. s. n. s.
	Control	68.4 \pm 8.7			n. s.
NaCl	High pH	42.8 \pm 9.8			
	NaCl	78.8 \pm 6.7	2/18	29.7**	** **
	Control	74.3 \pm 11.0			n. s.
Control	High pH	31.5 \pm 13.7			
	NaCl	81.1 \pm 13.8	2/57	70.24**	** **
	Control	73.9 \pm 11.4			**

*significant at 0.05

**significant at 0.01

Figure 3. Survival of High pH flies, NaCl flies and Control flies on the different media. The values are presented as the mean percentage \pm the standard deviation. A media key is presented below.



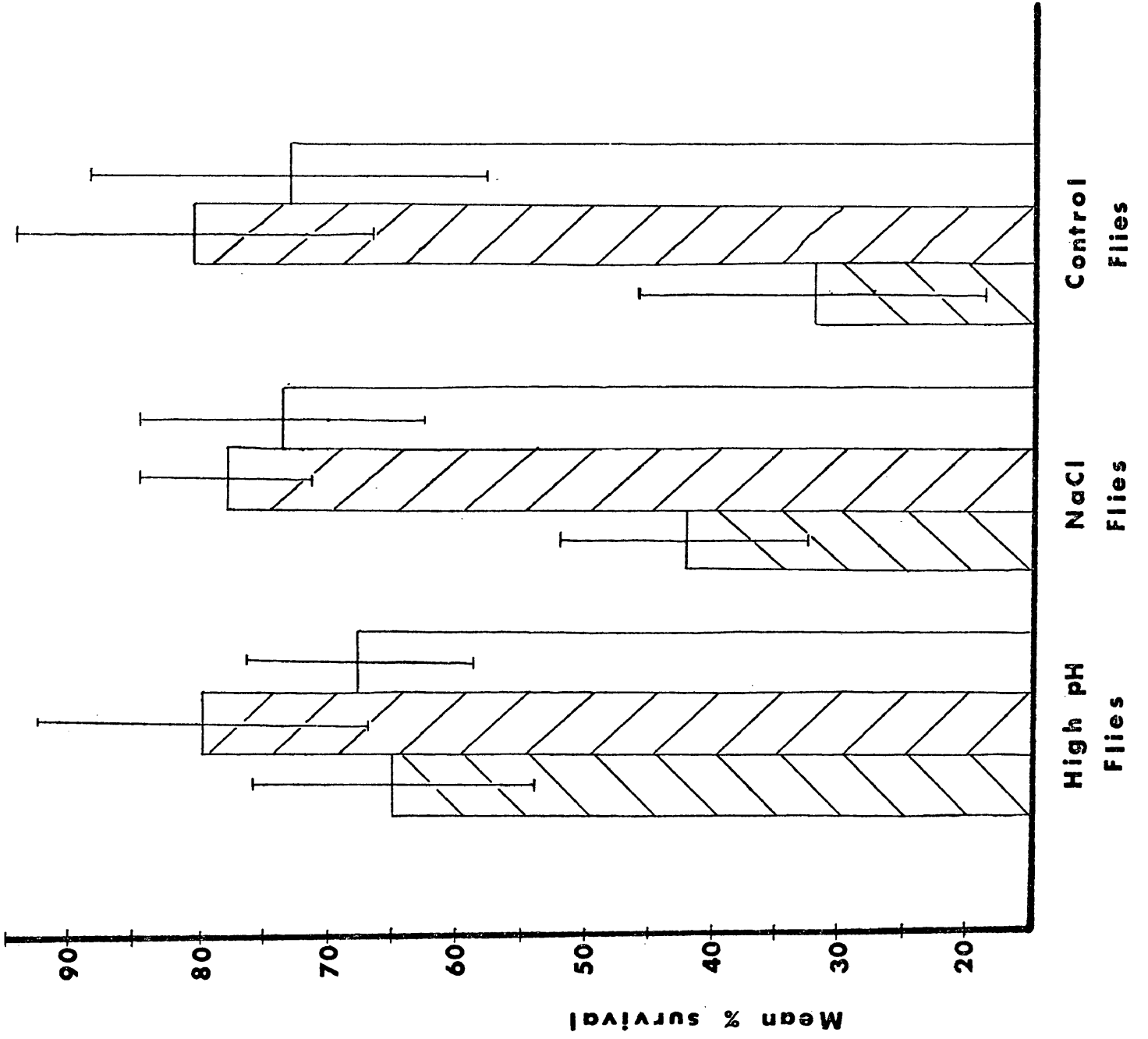


Table V. Developmental rate and survivorship of KCl and Low pH flies on the various media.
Pilot study.

Flies	Media	Ecdlosion time in days ($\bar{x} \pm$ s. d.)	Number of flies surviving	% Survival
KCl	KCl	16.2 \pm 1.2	20	66.7
	Low pH	25.3 \pm 1.6	20	66.7
	Control	14.5 \pm 1.1	19	63.3
Low pH	KCl	19.8 \pm 0.9	17	56.7
	Low pH	19.0 \pm 1.6	21	70.0
	Control	13.8 \pm 0.7	27	90.0

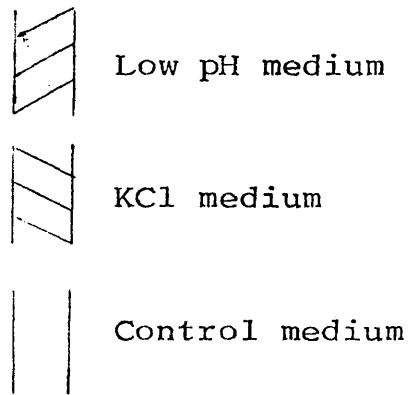
V. The survival data are in no way striking, but an indication is present that the Low pH flies do not survive as well on the KCl medium as do the KCl flies. There is also a rather large difference in survival of the two types of fly on the Control medium. A further study was undertaken to determine whether these differences were of any real significance. Figure 4 and Table VI present the results of this expanded test. The data in Table VI were grouped as were the data in Tables IIIa and IIIb and an ANOVA run on all six groupings. None of the F values were significant.

Developmental rate of Low pH and KCl flies. The data on developmental rate in Table V are much more striking than the survival data. The KCl flies developed more quickly than the Low pH flies on KCl medium, while the Low pH flies developed much more quickly on the Low pH medium than did the KCl flies. Tables VIIa, VIIb and Figure 5 present the data from the enlarged version of this test. A problem arose in the analysis of this data when Bartlett's test of Homogeneity of Variance produced four highly significant figures. Since ANOVA can be influenced by non-homogeneous variances, its non-parametric counterpart, the Kruskal-Wallis H test was used where indicated. Table VIIa presents the data grouped to compare the three types of fly on each type of medium. The KCl flies developed faster on Low pH medium than did the Control flies or the Low pH flies (H value is highly significant). The comparisons in Table

Table VI. Survival of Low pH flies, KCl flies and Control flies on the various media.

Flies	Medium	Mean % survival ($\bar{x} \pm s. d.$)
Low pH		85.4 \pm 16.7
KCl	Low pH	82.1 \pm 15.1
Control		71.7 \pm 12.0
Low pH		72.5 \pm 16.1
KCl	KCl	72.1 \pm 13.8
Control		72.5 \pm 11.3
Low pH		84.6 \pm 9.8
KCl	Control	71.9 \pm 16.7
Control		73.9 \pm 11.4

Figure 4. Survival of Low pH flies, KCl flies and Control flies on the different media. The values are presented as the mean percentage \pm the standard deviation. A media key is presented below



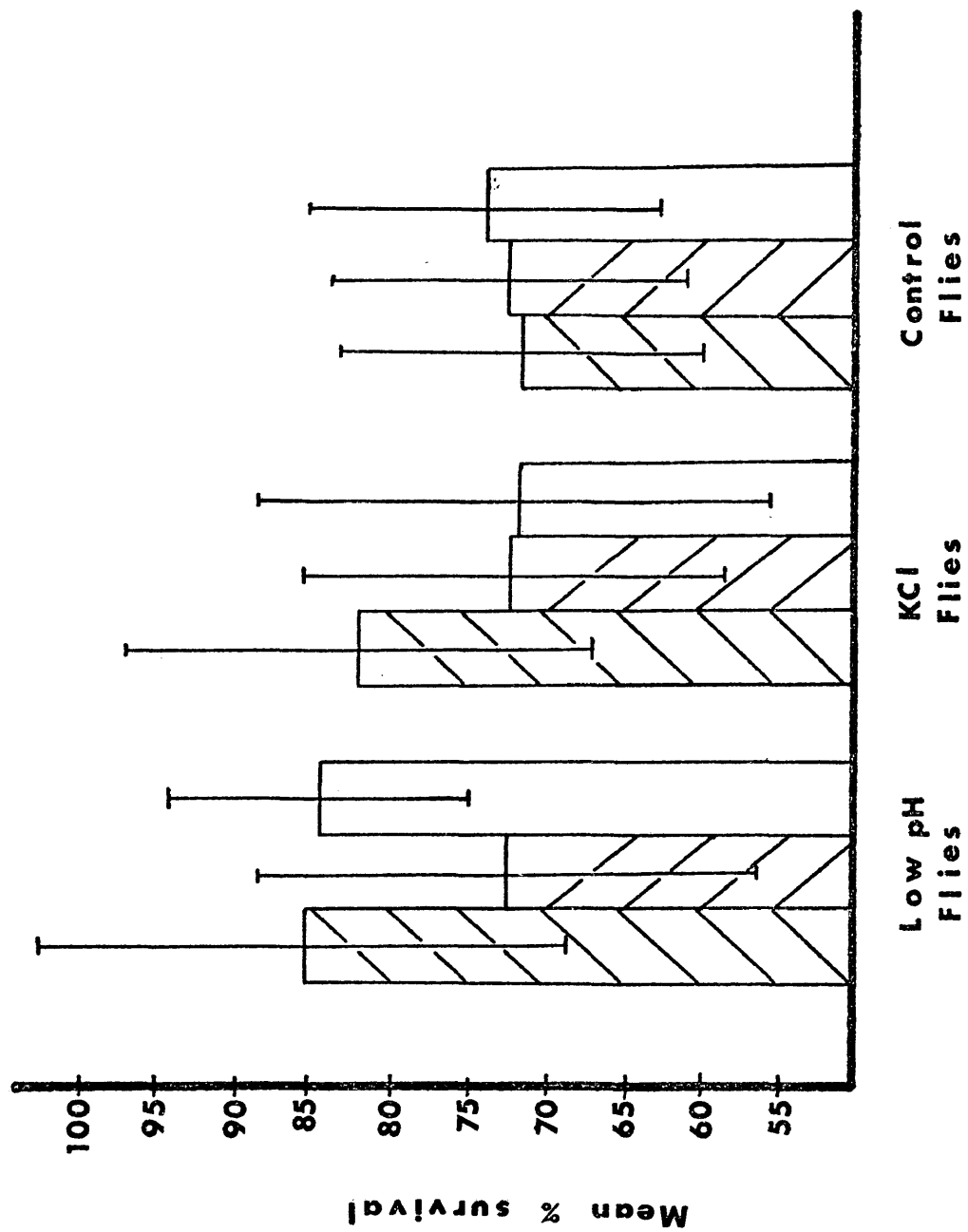


Table VIIa. Developmental rate of Low pH, KCl and Control flies on the various media. Where a grouping did not pass Bartlett's test of Homogeneity of Variance, the Kruskal-Wallis H test was used in place of ANOVA.

Flies	Medium	Mean days to eclosion ($\bar{x} \pm s. d.$)	ANOVA or	Kruskal-Wallis H test
Low pH		15.9 \pm 2.5		
KCl	Low pH	12.2 \pm 0.5		9.4**
Control		15.5 \pm 2.8		
Low pH		12.4 \pm 1.0		
KCl	KCl	11.3 \pm 0.5	3.04 n. s.	
Control		12.4 \pm 0.9		
Low pH		11.5 \pm 0.4		
KCl	Control	12.0 \pm 0.9		17.86**
Control		11.5 \pm 1.2		

*significant at 0.05

**significant at 0.01

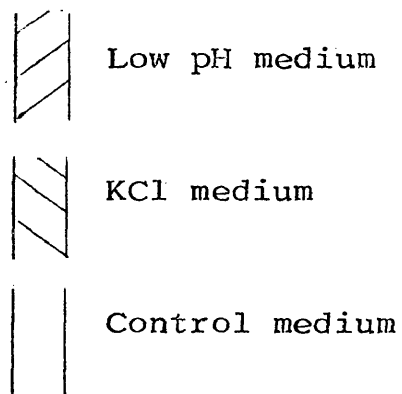
Table VIIb. Developmental rate of Low pH, KCl and Control flies on the various media, where a grouping did not pass Bartlett's test of Homogeneity of Variance, the Kruskal-Wallis H test was used in place of ANOVA.

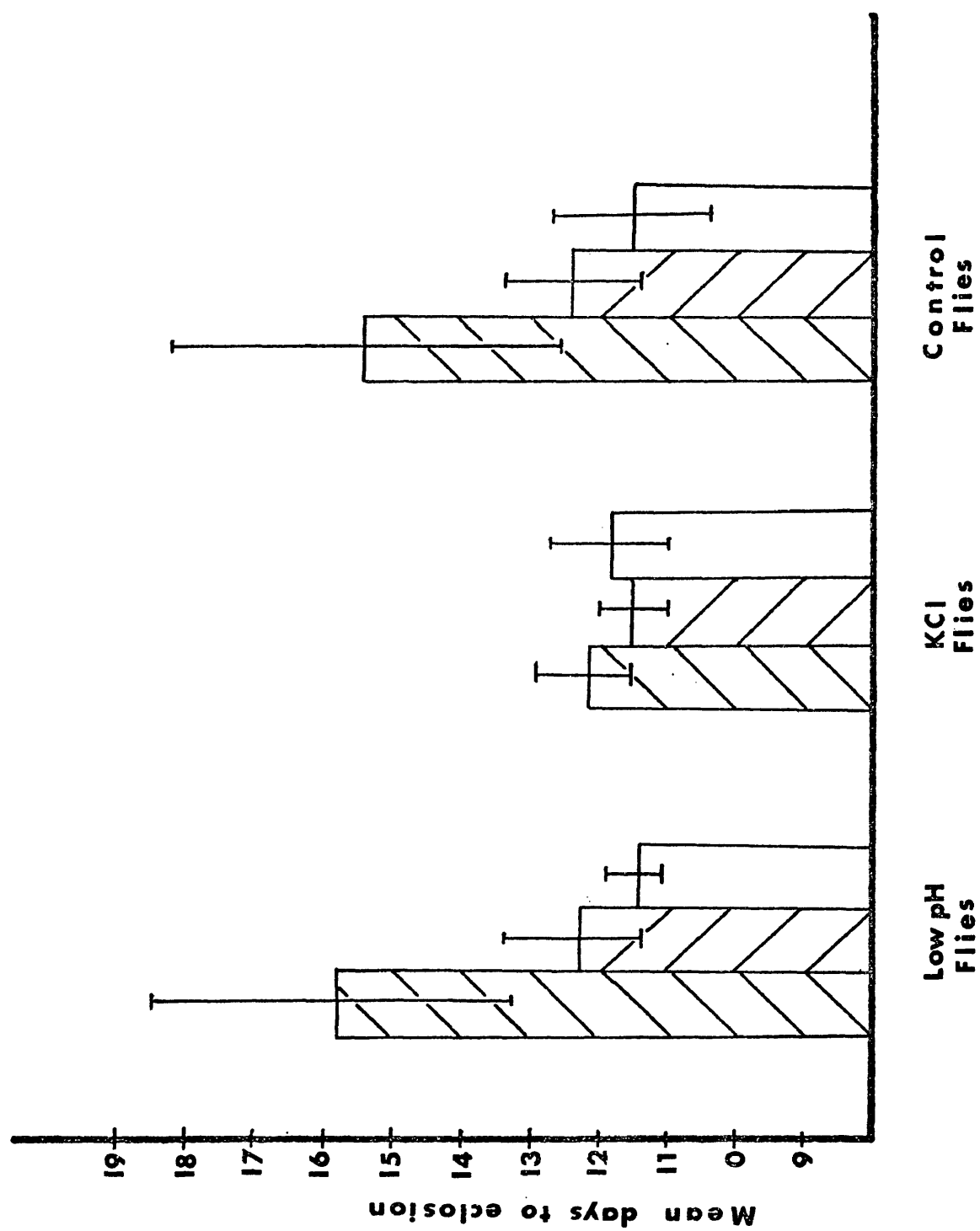
Flies	Medium	Mean days to eclosion ($\bar{x} \pm s. d.$)	ANOVA or Kruskal-Wallis H test
Low pH	Low pH	15.9 \pm 2.5	25.08**
	KCl	12.4 \pm 1.0	
	Control	11.5 \pm 0.4	
KCl	Low pH	12.2 \pm 0.5	1.9756 n. s.
	KCl	11.3 \pm 0.5	
	Control	12.0 \pm 0.9	
Control	Low pH	15.5 \pm 2.8	78.30**
	KCl	12.4 \pm 0.9	
	Control	11.5 \pm 1.2	

*significant at 0.05

**significant at 0.01

Figure 5. Developmental rate of Low pH flies, KCl flies and Control flies on the different media. The values are the mean time (in days) taken until eclosion from the day the eggs were collected \pm the standard deviation. A media key is presented below.





VIIb are of each type of fly on all three types of media. Low pH flies develop fastest on Control medium, next fastest on KCl medium and slowest on Low pH medium (highly significant H value). KCl flies eclose first from KCl medium. Control flies eclose first from Control medium, next from KCl medium and last from Low pH medium (H value is highly significant).

Food preference of High pH, NaCl and Control flies.

The data from these tests (all flies raised on Control media) are presented in Table VIIIa. The point of interest here is that in all significant trials, the flies, regardless of origin (salt, pH and Control), showed a preference for High pH medium (five of fifteen sets of results were significant).

Table VIIIb presents the food preference tests of the flies raised on their native media (*i. e.*, High pH flies reared on High pH medium). Once more, the three significant sets of values (two tests with Control and one with High pH flies) favor the High pH medium.

Food preference of Low pH, KCl and Control flies.

The data from the tests of these flies raised on Control medium are presented in Table IXa. All significant values are indications of a preference for the Low pH rather than the KCl medium.

In Table IXb, which reports the results of the food preference of these flies raised on their native medium, all significant preferences are for Low pH rather than KCl

Table VIIIa. Food preference of High pH, NaCl and Control flies raised on Control medium. The trials were run in sets of three as indicated by the horizontal groupings. H_0 (1:1)

Flies	High pH		NaCl		Control	
Medium NaCl	High pH	NaCl	High pH	NaCl	High pH	NaCl
28	40	22	25	12	23	
	$x_1^2=2.29$ n. s.	$x_1^2=0.19$ n. s.		$x_1^2=3.46$ n. s.		
25	75	9	23	21	37	
	$x_1^2=25.0^{**}$	$x_1^2=6.13^*$		$x_1^2=4.41^*$		
32	36	17	30	34	31	
	$x_1^2=0.24$ n. s.	$x_1^2=3.6$ n. s.		$x_1^2=0.14$ n. s.		
12	17	31	30	15	35	
	$x_1^2=0.86$ n. s.	$x_1^2=0.016$ n. s.		$x_1^2=8.0^{**}$		
21	19	16	42	20	26	
	$x_1^2=0.1$ n. s.	$x_1^2=11.66^{**}$		$x_1^2=0.78$ n. s.		

*significant at 0.05

**significant at 0.01

Table VIIIb. Food preference of High pH, NaCl and Control flies raised on their native medium. The trials were run in sets of three as indicated by the horizontal groupings. H_0 (1:1)

Flies	High pH		NaCl		Control	
Medium	NaCl	High pH	NaCl	High pH	NaCl	High pH
	36	40	77	100	16	54
	$x_1^2=0.21$ n. s.		$x_1^2=2.99$ n. s.		$x_1^2=20.63^{**}$	
	30	43	36	53	28	18
	$x_1^2=2.32$ n. s.		$x_1^2=3.25$ n. s.		$x_1^2=2.17$ n. s.	
	66	94	39	44	41	43
	$x_1^2=4.90^*$		$x_1^2=0.30$ n. s.		$x_1^2=0.04$ n. s.	
	33	49	36	53	23	39
	$x_1^2=3.12$ n. s.		$x_1^2=3.25$ n. s.		$x_1^2=4.13^*$	
	13	16	37	32	34	44
	$x_1^2=0.31$ n. s.		$x_1^2=0.36$ n. s.		$x_1^2=1.28$ n.s.	

*significant at 0.05

**significant at 0.01

Table IXa. Food preference of Low pH, KCl and Control flies raised on Control medium. The trials were run in sets of three as indicated by the horizontal groupings. H_0 (1:1)

Flies Medium	Low pH		KCl		Control	
	KCl	Low pH	KCl	Low pH	KCl	Low pH
	11	72	40	48	6	19
	$x_1^2=44.84^{**}$		$x_1^2=0.72$ n. s.		$x_1^2=6.92^{**}$	
	18	50	27	82	18	30
	$x_1^2=15.06^{**}$		$x_1^2=27.76^{**}$		$x_1^2=3.0$ n. s.	
	14	32	30	64	5	17
	$x_1^2=7.04^{**}$		$x_1^2=12.3^{**}$		$x_1^2=6.54^*$	
	16	27	37	68	7	20
	$x_1^2=2.82$ n. s.		$x_1^2=9.16^{**}$		$x_1^2=6.26^*$	
	11	23	23	71	21	37
	$x_1^2=4.24^*$		$x_1^2=24.52^{**}$		$x_1^2=4.42^*$	

*significant at 0.05

**significant at 0.01

Table IXb. Food preference of Low pH, KCl and Control flies raised on their native medium. The trials were run in sets of three as indicated by the horizontal groupings. H_0 (1:1)

Flies	Low pH		KCl		Control	
Medium	KCl	Low pH	KCl	Low pH	KCl	Low pH
	12	56	22	47	4	17
	$x_1^2=28.48^{**}$		$x_1^2=9.06^{**}$		$x_1^2=8.04^{**}$	
	31	46	15	31	14	29
	$x_1^2=2.92$ n. s.		$x_1^2=5.56^*$		$x_1^2=5.24^*$	
	14	61	36	54	17	31
	$x_1^2=29.46^{**}$		$x_1^2=3.6$ n. s.		$x_1^2=4.08^*$	
	21	39	17	28	19	27
	$x_1^2=5.4^*$		$x_1^2=2.68$ n. s.		$x_1^2=1.4$ n. s.	
	22	27	25	31	14	20
	$x_1^2=0.52$ n.s.		$x_1^2=0.64$ n. s.		$x_1^2=1.06$ n. s.	

*significant at 0.05

**significant at 0.01

medium.

Oviposition preference tests. The results of the oviposition tests of High pH, NaCl and Control females are presented in Table X. Two of these trials show a significant preference for the High pH medium while the rest show a highly significant preference for the High pH medium.

Table XI presents the results of the oviposition preference tests of Low pH, KCl and Control females. All nine tests show highly significant preferences for the Low pH medium.

Mating tests. Table XII presents a summary of the results of the mating tests. Also present is an adjusted heterogeneity chi-square value and Schaffer's measure of mating discrimination (Schaffer, 1968), which ranges from -100% to +100% for completely negative to completely positive assortative mating, respectively. There is no significant departure from random mating.

Table X. Oviposition preference of High pH, NaCl and Control females raised on their native medium. The trials were run in sets of three as indicated by the horizontal groupings. H_0 (1:1)

Flies	NaCl		High pH		Control	
	NaCl	High pH	NaCl	High pH	NaCl	High pH
	28	56	17	34	33	67
	$\chi^2_1=9.33^{**}$		$\chi^2_1=5.67^{**}$		$\chi^2_1=11.56^{**}$	
	20	67	26	49	15	47
	$\chi^2_1=25.39^{**}$		$\chi^2_1=7.05^{**}$		$\chi^2_1=16.51^{**}$	
	35	60	25	59	12	36
	$\chi^2_1=6.579^*$		$\chi^2_1=13.76^{**}$		$\chi^2_1=12.0^{**}$	

*significant at 0.05

**significant at 0.01

Table XI. Oviposition preference of Low pH, KCl and Control females raised on their native medium. The trials were run in sets of three as indicated by the horizontal groupings. H_0 (1:1)

Flies	KCl		Low pH		Control	
Medium	KCl	Low pH	KCl	Low pH	KCl	Low pH
	25	62	31	57	20	63
	$\chi^2_1=15.74^{**}$		$\chi^2_1=7.68^{**}$		$\chi^2_1=22.28^{**}$	
	15	138	27	77	36	79
	$\chi^2_1=98.88^{**}$		$\chi^2_1=24.04^{**}$		$\chi^2_1=16.08^{**}$	
	42	75	15	68	17	43
	$\chi^2_1=9.31^{**}$		$\chi^2_1=33.84^{**}$		$\chi^2_1=11.27^{**}$	

*significant at 0.05

**significant at 0.01

Table XII. Tests for sexual isolation. S is Schaffer's measure of mating discrimination.

	KCl	Low pH
KCl	24	23
Low pH	28	20
	Adjusted heterogeneity $x_1^2 = 0.2556$ n. s.	
	S = -7.235	

	NaCl	High pH
NaCl	31	25
High pH	25	29
	Adjusted heterogeneity $x_1^2 = 0.5769$ n. s.	
	S = +9.055	

DISCUSSION

Environmental heterogeneity has been proposed by a number of authors (e. g., Levins, 1968; Grant and Mettler, 1969; Antonovics and Bradshaw, 1970; Powell, 1971) as a mechanism which maintains diversity within a population. If different habitats are utilized differentially, and sufficient selective pressures are imposed by these habitats, the outcome is a form of disruptive or diversifying selection. There are three other possible outcomes to diversifying selection besides the maintenance of genetic variation. Sabath (1974) has looked at sympatric populations of eleven species of drosophilid flies and found that there was no correlation between niche breadth and genetic variability, i. e., outcome nothing. Polymorphism as a third possibility was suggested by Mather (1955) and has been reported by Clarke and Sheppard (1960, 1962), Thoday (1960) and Giesel (1970). The fourth and most controversial possibility is reproductive isolation which has been reported from laboratory experiments by Thoday and Gibson (1962), Coyne and Grant (1972) and Beardmore and Baldawi (cited by Thoday, 1972).

The results of the experiments presented here sup-

port the contention that diversity can be maintained by exposure to a heterogeneous environment. The NaCl-High pH cage gave rise to some very pertinent results, especially when one considers the developmental rate data. There are several ways in which a shortened developmental rate may be considered advantageous. First, Wallace (1948), working with Drosophila pseudoobscura noted that only five percent of the larvae in a crowded population cage food cup survive the very intense competition, and that those requiring the least time to develop have a tremendous advantage. His argument, although valid, is not particularly relevant to the experiments under consideration here; the food cups in our cages were certainly not overcrowded. Nagle (1964) discussed the mold (fungus) which sometimes overruns a dirty cage and offered some proof that it allowed Drosophila arizonensis to co-exist with the normally superior competitor D. mojavensis baja because baja was slower to develop and many larvae were lost when mold totally overran the cup. This is not particularly relevant either for there was never a mold blanket covering the cups in our experiments. A third possibility is that the sooner a fly has eclosed, the sooner it is able to reproduce, which results in a decreased generation time. Last and perhaps most important to this study is that if the medium is sufficiently deleterious to larval survival, there will be a premium on rapid development, i. e., the shorter the exposure to severe en-

vironmental vicissitudes, the greater the probability of survival. High pH medium slowed the development of all three types of fly (Tables IIIa and IIIb). The High pH flies appear to have adapted to High pH media for they developed faster on it than did NaCl or Control flies. This rapid development was carried over to the Control medium, where High pH flies were also the fastest developers. The performance of NaCl flies suggests that they were better adapted to NaCl medium, for they were able to develop faster on it than on either the High pH medium or the Control medium. From the survival data also (Tables IVa and IVb), it appears that High pH flies have adapted to their own medium. All three types of fly did poorly on the High pH medium but the High pH flies did less poorly. Although it is possible that the decreased developmental time of the High pH flies was one of the adaptations allowing greater overall survival on this medium than the other flies, such a conclusion is premature based on the evidence of a simple correlation.

The food preference and oviposition preference (Tables VIIIa, VIIIb and X) was rather unexpected in light of the survival and rate of development data but does explain events which were at first rather puzzling. The surprising fact is that the flies, when offered a choice of food, generally chose the medium which was most stringent in terms of survival and developmental rate; all tests sug-

gest that ovipositing females preferred the medium upon which their progeny did poorest. This, perhaps, was the reason for the early paucity of flies found in the NaCl end of the cage, which led the scrutator to reduce the concentration of salt in the medium.

The results from the KCl-Low pH cage are much less clear but still support the basic thesis. The KCl flies developed more rapidly than did the Low pH or Control flies on all media excluding the Control medium. They did not develop more rapidly on any particular medium, but did exhibit a relatively constant rate of development regardless of the medium; whereas, the Low pH and Control flies varied greatly with the different media. Once more the food and oviposition preferences were always toward the pH medium but this seems to be less paradoxical in a population where none of the media caused a marked reduction in survivorship.

One last point which must be brought out is that each of the two cages contained a single interbreeding population (Table XII), *i. e.*, the differences reported between flies from the alternate habitats reflect diversity within a population.

The results of the experiments described herein lend experimental support to the supposition that increased environmental heterogeneity leads to greater phenotypic diversity. The two populations were subjected to diversifying selection and each responded with an increased ecological amplitude.

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Epilogue

- Spoken by Prospero

Now my charms are all o'erthrown,
And what strength I have 's mine own,—
Which is most faint: now 'tis true,
I must be here confined by you,
Or sent to Naples. Let me not,
Since I have my dukedom got,
And pardon'd the deceiver, dwell
In this bare island by your spell;
But release me from my bands
With the help of your good hands.
Gentle breath of yours my sails
Must fill, or else my project fails,
Which was to please. Now I want
Spirits to enforce, art to enchant;
And my ending is despair
Unless I be relieved by prayer;
Which pierces so, that it assaults
Mercy itself, and frees all faults.
As you from crimes would pardon'd be,
Let your indulgence set me free.

The Tempest

William Shakespeare

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