ECOLOGICAL PARTITIONING BY DROSOPHILA POPULATIONS

A thesis presented for the degree of Doctor of Philosophy of the University of Stirling

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September, 1972.

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The work presented here is the result of my own investigations and has neither been accepted nor is being submitted for any other degree.

Candidate

Supervisor

ABSTRACT

Pure and mixed cultures of two populations of the fruit fly (*Drosophila melanogaster*) were grown together for up to 15 generations. Interbreeding and reproductively isolated mixtures of Bar and wild type genotypes were maintained in two experiments. Isolated mixtures only were maintained in a third, although the populations had been interbreeding previously for 30 generations.

Bar populations were as productive as wild type in pure cultures but less so in mixed cultures. Mixed cultures out-yielded pure cultures in the third experiment but not in the first two.

Flies from mixed cultures emerged earlier than those from pure cultures towards the end of Experiment 2.

The competitive ability of Bar flies from the reproductively isolated mixtures was inferior to that of Bar flies from pure cultures, while that of wild type flies from these cultures was only sometimes worse than wild type flies from pure cultures. However, competitive ability of Bar and wild type flies from interbreeding cultures was superior to that of flies from pure cultures.

This can be explained by the former suffering inbreeding depression, while the latter were becoming more outbred. It was also suggested that Bar, as it became a much worse competitor than wild type, was having strong selection pressures imposed upon it by the wild type population. By favouring only a part of the population, this would also reduce its genetic variability. This is a factor which has not been considered before in experiments of this type.

Replacement series tests, measuring performance of the two genotypes grown together at different frequencies, showed that frequency dependent selection was operating in the reproductively isolated mixtures, and in the pure cultures in the third experiment. Both Bar and wild type yielded proportionately higher when they were the minority component. This implied ecological differences, and probably competitive avoidance between the two populations. This is possibly supported by extinction rate tests, where some of the replicates of selected Bar (from reproductively isolated mixtures) appeared to reach stability. This was not true for unselected Bar (from pure cultures).

In the second part of this thesis, a single population was subjected to two unpleasant types of medium, one containing hydrochloric acid and the other sodium hydroxide.

Populations of flies grown on acid or base medium for 15 generations gave higher yields on their own type of medium than on the alternative medium and even higher than on normal medium. Flies grown on a choice of acid or base medium over this time also grew best on the medium from which they emerged in the final generation. Thus populations subjected to either directional or disruptive selection will produce flies adapted to either unpleasant types of medium. There was no indication of assortative mating having evolved between acid and base flies from the mixed cultures, so that although divergence had taken place, there was no incipient speciation.

ACKNOWLEDGMENTS

I should like to thank Professor Janis Antonovics, Dr. Elizabeth Berry and Dr. Michael Horne for supervising my research and for their useful advice and criticism, and Professor B. Clarke and John Endler for statistical advice. I am grateful to Professor Holliday and Professor Meidner for making me welcome in their department, and to all the staff and Post Graduates for taking an interest in my work and providing a friendly and encouraging atmosphere.
I should like to thank the technical staff for their assistance, particularly Mrs. Janice Maxwell for her advice on *Drosophila* and for maintaining my cultures while I was away.

I am grateful to Mrs. Abercorn for typing this thesis so well.

This work was supported by an S.R.C. Studentship.

CONTENTS

GENERAL INTRODUCTION

Ecology is concerned with the interactions between different kinds of plants and animals and their environment. Natural selection is the process by which those individuals interacting most favourably with their environment contribute more offspring to future generations.

Although natural selection operates on individuals, the unit in which changes in different characters can be seen is the population. Population genetics tends to deal with the changes in frequency of genes within a population due to the interactions of selection, mutation, migration and random processes. Ecological genetics deals with genetic changes of particular characters within a population and with concomitant quantitative changes of the population itself.

Interactions between populations and their non-living environment as well as between populations and their biotic environment are both the province of ecological genetics. These interactions may result in the elimination of unfavoured genotypes from a population or in polymorphisms where more than one genotype is maintained in a population.

The tremendous range of processes occurring in populations may appear at first sight to be only distantly related to the larger scale problems of speciation, but it has become clear in the past few decades that speciation is merely an extension of individual selection.

The alternative pathways of genetic divergence between isolated populations and divergence within a single population can, at least theoretically, both result in speciation.(See Mayr, 1942; Maynard Smith, 1966). The former has often been

considered the only possible mechanism or at least by far the most likely. As more is being understood about the maintenance and origin of polymorphism, the possibility of sympatric speciation (speciation with no previous geographical isolation) becomes more likely.

Theoretical models analysing the process of sympatric divergence predict that polymorphism could originate and be maintained within a single population if the different morphs are adapted to different niches in the environment and if selection pressures are high. (See Dickinson and Antonovics, 1972 for review and most extensive model). In addition, isolating mechanisms such as self fertility and assortative mating may evolve in situations where there are high selection pressures against each morph in the "wrong" niche. The maintenance of polymorphism in such multiple niche situations is generally attributed to disruptive selection, but such selection is also highly frequency dependent. Mather (1955) first mentioned the possibility of polymorphism and speciation being the result of disruptive selection, and indicated that under certain circumstances polymorphisms could be a prelude to sympatric speciation. Considerable evidence has come to light since then, indicating that polymorphism may be a frequent result of disruptive selection. The colour and banding morphs of the land snail, Cepaea nemoralis could be maintained by selection for one type in one niche and another type in another niche. For example, the brown unbanded snails are best hidden and protected in beech woods while yellow banded snails are best hidden in rough pasture (Cain and Sheppard, 1954). In addition, dark snails warm up more rapidly in misty, frosty

hollows while yellow snails are better protected from strong sunlight on hillocks (Jones, personal communication).

Perhaps the best example of a visible polymorphism maintained through disruptive selection is that shown by the Swallowtail Butterfly, Papilio dardanus. In many populations of this species, camouflaged, non-mimetic forms and mimetic forms (copying a sympatric distasteful species) occur together in the same area. Intermediates between these forms are neither well camouflaged nor good mimics and are rare in natural populations (Clarke and Sheppard, 1962).

When laboratory populations of the fruit fly, Drosophila melanogaster, are subjected to disruptive selection, increased variability and in some cases a bimodal distribution of a particular character may result. In two cases reproductive isolation has been obtained within a population (Thoday and Gibson, 1970; Cogne and Grant, 1972 for summary). The very high selection pressures may have favoured the chance of this happening, but as such small numbers of flies were used in initiating new generations, inbreeding would have been expected to reduce overall genetic variability and therefore counter such divergence (Robertson, 1970).

Another situation where selection for differing optima has taken place is that presented by grass populations living on ground contaminated by heavy metals. A very sharp change in level of heavy metal tolerance occurs over the edge of the contaminated area. There is considerable selection against non-tolerant plants on metal mines and some selection against tolerant plants on neighbouring pasture: this is enough to produce divergence between neighbouring populations despite the

high level of gene flow between them. Plants on the metal mine flower earlier than those off the mine and this difference is at least partially under genetic control. There is also an indication that some of the plants on the mine are slightly more self fertile than pasture plants, giving further reproductive isolation (Antonovics, 1968a, b).

Just as different genotypes may be maintained in a population by each occupying a different niche, so may different species coexist when they are not limited by identical resources and when they do not interfere with each other. As the degree of difference in ecological requirements increases, so does the opportunity for coexistence. It might be expected that species newly in competition would be forced to diverge further from each other (i.e. reduce their area of ecological overlap) in order to achieve coexistence. This may be reflected by an accentuation of the morphological differences between them where they occur together, a phenomenon that has been termed character displacement (see review by Brown and Wilson, 1956). An example of this is shown by the nuthatches, Sitta neumayer and S. tephronota, whose ranges overlap in parts of Asia. In the area of overlap S. neumayer becomes smaller, has a shorter beak and a smaller facial stripe, while S. tephronota is larger, has a longer beak and a larger facial stripe (Vaurie, 1951).

If character displacement could take place in populations which are polymorphic, then the increased differences between the forms might build up and this might be expected to aid the possibility of sympatric speciation under some circumstances. Sexual dimorphism may be the result of such forces (Selander,

1966).

Although many thorough studies of competing species have been carried out, fewer studies have been made on competing populations of the same species. Both theoretical models and intuitive concepts of the outcome of competition predict that for coexistence to take place the components must utilise, at least partially, different resources (Gause, 1934; Ludwig, 1950; de Wit, 1960; Schutz and Usanis, 1969; Ayala, 1969a).

The extent to which this kind of divergence takes place in a natural population is not known. It seems likely that populations living in a heterogeneous environment may be made up of a collection of phenotypes, each suited to a slightly different optimum, rather than a single optimal phenotype.

It was the purpose of this thesis to investigate two stages in the possible sequence from a single population through polymorphism to speciation. The aspects were
(i) the effect of competition between similar populations of the same species, particularly the extent to which ecological divergence could take place between them, and
(ii) the effect on a single population of being subjected to two opposing extreme environments.

COMPETITION BETWEEN RELATED POPULATIONS OF DROSOPHILA

Introduction

A considerable amount of work has been carried out on interspecific competition between *Drosophila melanogaster* and *D. simulans*. Moore (1952) found that populations of *D. simulans* which had previously experienced *D. melanogaster* will remain longer in a mixed culture than populations which had not experienced competition. This suggests that competitive ability may be inherited and can be selected. Narise (1965) showed that *D. simulans* is always a poor competitor over a wide range of frequencies but also claimed frequency dependent effects which were disputed by Putwain *et al.* (1967). Van Delden (1970) showed that the competitive performance of *D. simulans* (against *D. melanogaster*) improves through selection. However, the selected populations of *D. simulans* also perform better when in pure cultures than the original populations. Futuyma (1970), probably using inbred lines of the two species, showed that although the performance of the majority of the populations does not change, a few do improve, while a few become worse. Barker and Podger (1970) using the same two species at different densities as well as frequencies, showed that frequency dependent selection and increasing yield of mixed cultures are most evident at intermediate densities. Ayala (1969b) retained mixtures of *D. simulans* and *D. pseudoobscura* at apparent equilibrium. As the yield of this mixture was considerably below that predicted from the pure culture yields, he believed that this invalidated the competitive exclusion principle. Gilpin and Justice (1972),

Borowsky (1971) and Antonovics and Ford (1972) reinterpret these results and show how they are compatible with the principle of competitive exclusion.

Thus experiments using different species of Drosophila indicate that competitive ability is a phenomenon which can be selected and which in many cases can be shown to be frequency dependent. Whether co-existence can take place and whether the presence of two species leads to higher biomass or greater environmental exploitation seems to depend very much on the precise conditions of the experiment.

A considerable number of studies have also been carried out on competition between different mutants or naturally occurring polymorphisms. It is perhaps not surprising that most common mutants of Drosophila melanogaster perform rather poorly in competition with wild type populations. However there are examples of apparent equilibrium being reached with a mutant gene in a population. Jones and Barker (1966) found that the mutant ebony was maintained in a population for some 30 weeks at a frequency of about 2% but was eventually lost. While the ebony gene was still present, the overall population size was higher than after it was lost.

Several previous experiments, like the ones to be described here, have used the mutant Bar with wild type populations. Bakker (1961) showed that a wild type stock will outperform a Bar stock because wild type larvae eat more rapidly than Bar larvae. Weisbrot (1965) showed that when wild type or Bar populations are grown on medium which has previously been used by Bar or wild type, growth is sometimes enhanced and sometimes inhibited. This suggests that metabolites produced by either

populations could have an inhibitory or facilitory effect. Endler (personal communication) growing Bar and a wild type strain at different frequencies and allowing gene flow between populations of neighbouring frequencies (thus simulating a cline) showed that Bar is also less viable from egg to adult and in addition showed poorer mating ability. He also showed that Bar males emerged better at low frequencies, thus frequency dependent selection appeared to be operating.

It has been found that polymorphic populations are more fit, in terms of yield, than monomorphic populations (Beardmore et al., 1960; Dobzhansky and Pavlovsky, 1961; Beardmore, 1963). It seems likely that the environment is being more efficiently exploited by a polymorphic population than a monomorphic one suggesting that the two morphs are ecologically different. Lewontin (1955) found that larval viability was higher in mixtures of white eye and wild type cultures than in pure cultures suggesting some ecological differences at this stage of the life cycle between wild type and the mutant. Frequency dependent selection as a means of maintaining alternative alleles in a population has been abundantly discussed, and a few examples, where there is clear evidence of frequency dependent selection in populations of Drosophila, exist. The results of Kojima and Yarbrough (1967), Tobari and Kojima (1967), Beardmore (personal communication) and Briscoe (personal communication) all show that the fitness of enzyme alleles is dependent on gene frequency.

The experiments of Seaton and Antonovics (1967) involved two different populations of the same species and therefore fill the gap between those using two species of Drosophila

and those using polymorphic populations. They maintained two competing, yet reproductively isolated, populations of D. melanogaster (dumpy and wild type) for a number of generations. They showed that not only does competitive performance, in terms of proportion of the total, of the competing, yet isolated, lines improve but also that there is good evidence that frequency dependent selection is operating to a sufficient extent to retain both populations in the mixture. Biomass of the mixtures is higher than that of the pure cultures suggesting that the environment is being more efficiently exploited as a result of ecological divergence between the two initial populations.

Experimental plan and techniques

The two populations used in these experiments were a wild type population and a Bar population. The latter was considered a particularly suitable mutant as it is reasonably fit, performing nearly as well as wild type in pure cultures and also because it is sex linked and displays intermediate dominance. This made it possible to maintain interbreeding populations and yet prevent all heterozygotes (of the Bar gene) from contributing to the next generation. Heterozygote males do not exist and heterozygote females are easily identifiable as they have kidney shaped eyes. This allows a reduced level of gene flow (50% of that permitted by random mating and no selection), but also means that the X chromosome can be isolated in the two populations.

For Experiments 1 and 2, a wild type strain from the School of Plant Biology at Bangor (from a single mated female caught in 1966) and a Bar strain from the Institute of Animal

Genetics in Edinburgh were used. In Experiment 3, both wild type and Bar were from a Kaduna strain into which the Bar gene had been introduced by Endler in the Department of Zoology at Edinburgh.

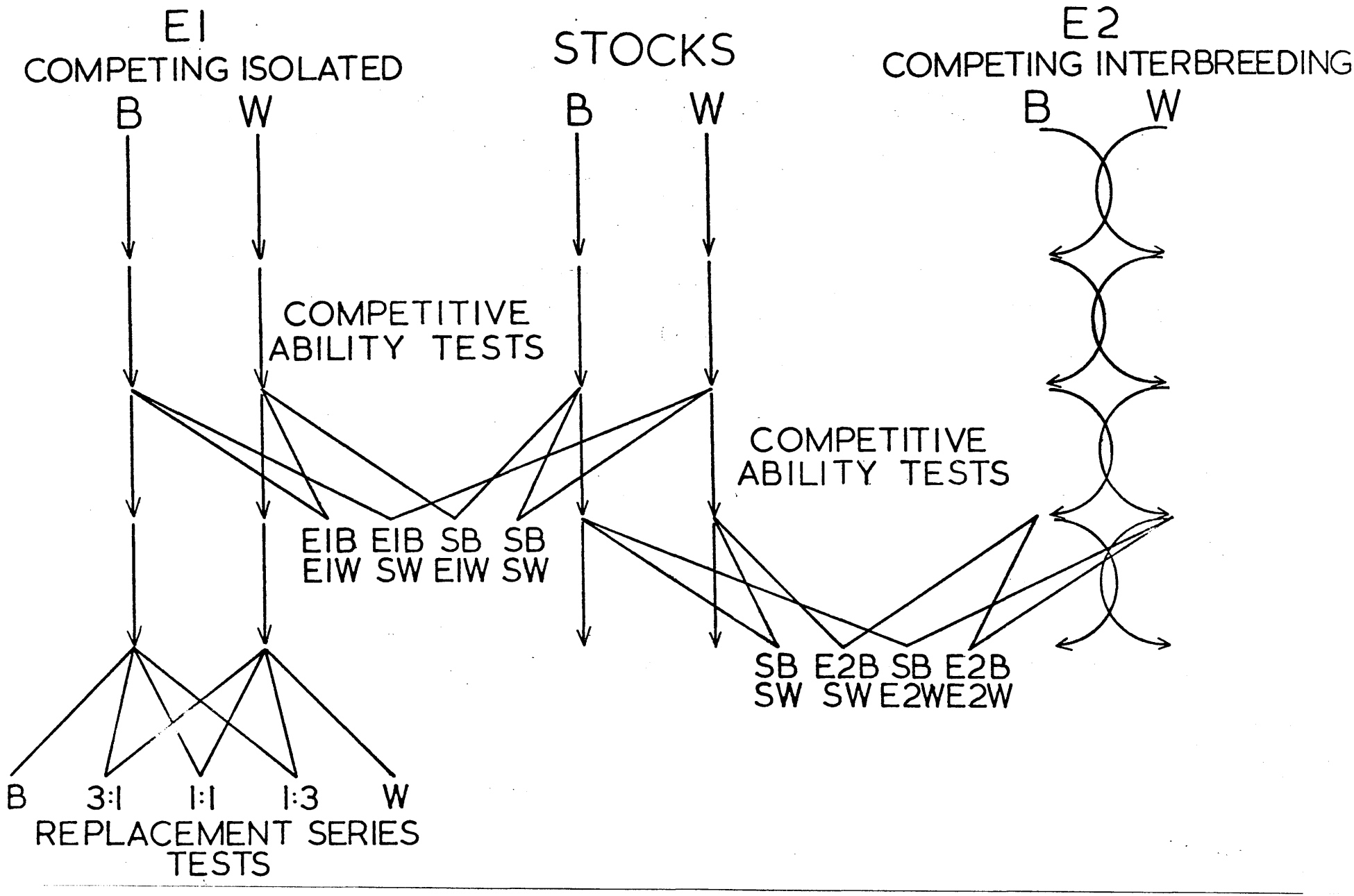
Figure 1 shows the basic plan used for all three experiments. Two types of pure culture, stock Bar (SB) and stock wild type (SW) were maintained as were two types of mixture. In the first type of mixture (E1) the two populations were kept reproductively isolated from each other. This was achieved by removing females before they had matured sufficiently to mate and putting them into vials to mate with males of their own genotype. Thus the two populations would be behaving as two very similar, yet reproductively isolated, species.

In the second type of mixture (E2) a limited level of gene flow was permitted. Virgins of each type were not collected, but mated females (equal numbers of each homozygote) were chosen randomly to initiate each generation, the heterozygotes being discarded. Individual replicates were numbered 1 to 4 and in Experiment 3, where there were 10 replicates, were numbered 1, 2, 3, 4, 5, 6, 7, 8, 9 and 0 (SB1, SB2, ... SB9, SB0; SW1, ... ; E11, ... ; E21, ...).

In Experiment 1, four replicate populations of each of SB, SW, E1 and E2 were maintained for 15 generations. As Seaton and Antonovics (1967) achieved positive results after only three generations, it was considered that 15 generations should be sufficient time for any changes in yields of mixtures or in competitive ability of selected and unselected populations to become evident. As the E1 lines performed very badly after

FIGURE 1

General plan for the competition experiments.


E1
COMPETING ISOLATED
B
W
STOCKS
B
W
E2
COMPETING INTERBREEDING
B
W
COMPETITIVE
ABILITY TESTS
COMPETITIVE
ABILITY TESTS
EIB EIB SB SB
EIW SW EIW SW
SB E2B SB E2B
SW SW E2WE2W
B
3:1
1:1
1:3
W
REPLACEMENT SERIES
TESTS

a few generations of this experiment, new lines had to be initiated at generation 8 from the SB and SW populations.

In Experiment 2, four replicates each of SB, SW, E1 and E2 were again maintained for 15 generations. After 8 generations, two further types of mixtures were initiated:- E5, where Bar and wild type flies were derived from the E1 mixtures and where interbreeding was permitted in the same way as in the E2 lines, and E6, where Bar and wild type flies were derived from the E2 mixtures and where the two genotypes were kept reproductively isolated yet still in competition. This was done in order to see whether changes which had taken place in the E1 and E2 lines would be maintained or reversed by changing the level of gene flow. Separate populations of E6B and E6W were maintained for 3 generations after generation 12; these were named "separated E6" (Sep.E6). As it was thought that the E1 lines in Experiment 1 had performed badly through inbreeding during the course of the experiment, a system of gene flow between the replicates was devised. In the E1 and E2 lines (and E5 and E6) 5 mated females of each genotype were kept to initiate the following generation in each replicate along with 5 from the previous replicate. For example, E12 was set up from 5 females of each genotype from E12 (previous generation) and 5 of each from E11 (previous generation), while 5 of each from E12 contributed to the next generation of E13. In the pure cultures, 10 mated females were retained and 10 were passed on to the following replicate.

In Experiment 3, ten replicates of SB, SW and E1 were maintained for 8 generations. The results of the tests on the E2 mixtures of the two previous experiments had shown very

consistent trends while those from the E1 mixtures had been less clear. Part of the reason for the latter was the large amount of variation between replicates. Thus the E2 mixtures were discontinued while the number of replicates of E1 was increased. The two genotypes for this experiment came from a polymorphic population in which Bar had been maintained for about 30 generations. It was therefore probably more genetically variable than the populations used in the previous experiments as well as there being a possibility that some niche divergence had already taken place between the Bar genotypes and the wild type genotypes.

The numbers of male and female of each genotype in each replicate of each type of culture were counted every generation. A comparison of the yields (total numbers of flies emerging) of each type of culture in generations 13, 14 and 15 in Experiments 1 and 2, and generations 6, 7 and 8 in Experiment 3, was made using a 't' test.

Tests

Two types of tests, similar to those carried out by Seaton and Antonovics (1967) were used.

(i) Competitive ability tests

To test the competitive ability of flies from pure and mixed populations, the numbers of flies emerging from the four combinations: SB x SW, E1B x SW, SB x E1W and E1B x E1W (and their E2, E5 and E6 equivalents) were counted. Equal numbers of each genotype were used to initiate each test. The proportions of Bar in the totals of each type of mixture were compared statistically with a modified 't' test:

were abbreviated to S followed by the replicate number (S1, S2, ... meaning SB1SW1, SB2SW2, ...).

Materials and procedure

All experimental lines and tests were carried out in half pint milk bottles (0.3 litres) stoppered with cotton wool. Medium was made up from 54 grams of sucrose, 32 grams of yeast (Allinson's Baking Yeast, Allinson, Ltd., London) and 19 grams of agar (Davis Standard Agar, Davis Gelatine Ltd., Warwick) in 1 litre of water. This was simmered for 20 minutes then allowed to cool to 60°C when 13 mls of a 1% solution of Nipagin in alcohol was added as a fungicide (following the recipe of Mittler and Bennett, 1962). About 70 mls of medium were used per bottle in Experiments 1 and 2 and exactly 40 mls of medium were dispensed in Experiment 3.

Flies which were to be used for initiating further generations or tests were anaesthetised with CO_2. Stock and selected cultures not used for future generations, and all tests, were anaesthetised with ether.

Six drops of live yeast suspension were added to the surface of the medium before 20 fertilised females were added to initiate each generation. Egg laying was allowed to continue for five days in the first two experiments and for 7 days in the third, after which the parents were removed. In the E1 and E6 lines, virgins were collected within ten hours of emergence between Days 10 and 14 until sufficient had been obtained for initiation of the following generation and for the tests. In the replacement series tests in Experiment 3, insufficient numbers of Bar were obtained in a few of the

replicates to make up a total of 20 flies at each ratio. In these cases, a total of 8, 12 or 16 flies were used per bottle, still retaining the correct ratios between Bar and wild type. In some cases, flies from more than one replicate were used in a single test (labelled e.g. E116 for E11 and E16, S90 for S9 and S0).

Peak emergence was usually between Days 11 and 14 and totals were counted up to Day 18 when the next generation was set up. In Experiment 2, totals were counted up to Day 21 at first, but this was discontinued as some second generation flies were beginning to emerge. Daily totals between Days 10 and 15 inclusive were also counted every third generation in Experiment 2 and these were used as a measure of the rate of emergence of each genotype in each culture.

All cultures were maintained in incubators at 25°C. However this temperature was certainly not maintained during Experiment 2 as all cultures were moved into a new building where electricity and heating were erratic and caused extreme difficulty in maintaining the incubators at the correct temperature. In April, 1971, the temperature in the laboratory rose to over 40°C and some of the cultures (SW) were rendered sterile. These were started again from the other lines.

RESULTS

i) Total numbers emerging each generation

Graphs of the mean numbers of flies in each type of culture in all three experiments give an indication of the variation in yield between different generations (Figures 2, 3, 4, 5 and 6). Differences between yields of pure cultures and mixed cultures can also be seen as can differences between yields of Bar and wild type in pure cultures. The yields of each pure culture and each kind of mixture were compared in the last three generations of each experiment (generations 13, 14 and 15 in Experiments 1 and 2 and generations 6, 7 and 8 in Experiment 3) using a 't' test (Tables 1, 2 and 3).

A large amount of variation in numbers from generation to generation is evident, and there appear to be indications of almost regular fluctuations with alternate peaks and troughs.

TABLE 1

Comparison of numbers of flies emerging from Stock Bar, Stock wild type, E1 and E2 in the last three generations of Experiment 1, using 't' values where $p < 0.05$, when $t > 1.96$ Red indicates $N_2 > N_1$, black $N_1 > N_2$. Generations as indicated below

Generations 13 14 15	N_1					
N_2	Stock wild type		E1		E2	
Stock Bar	2.41 0.62	1.00	0.33 1.03	0.76	1.88 0.87	2.61
Stock wild type	-		2.84 1.79	0.00	0.20 1.75	2.29
E1	-		-		2.21 0.31	1.88

FIGURE 2

Total numbers of flies emerging from E1, Stock Bar and Stock wild type each generation in Experiment 1.
(E1 was restarted at generation 7).

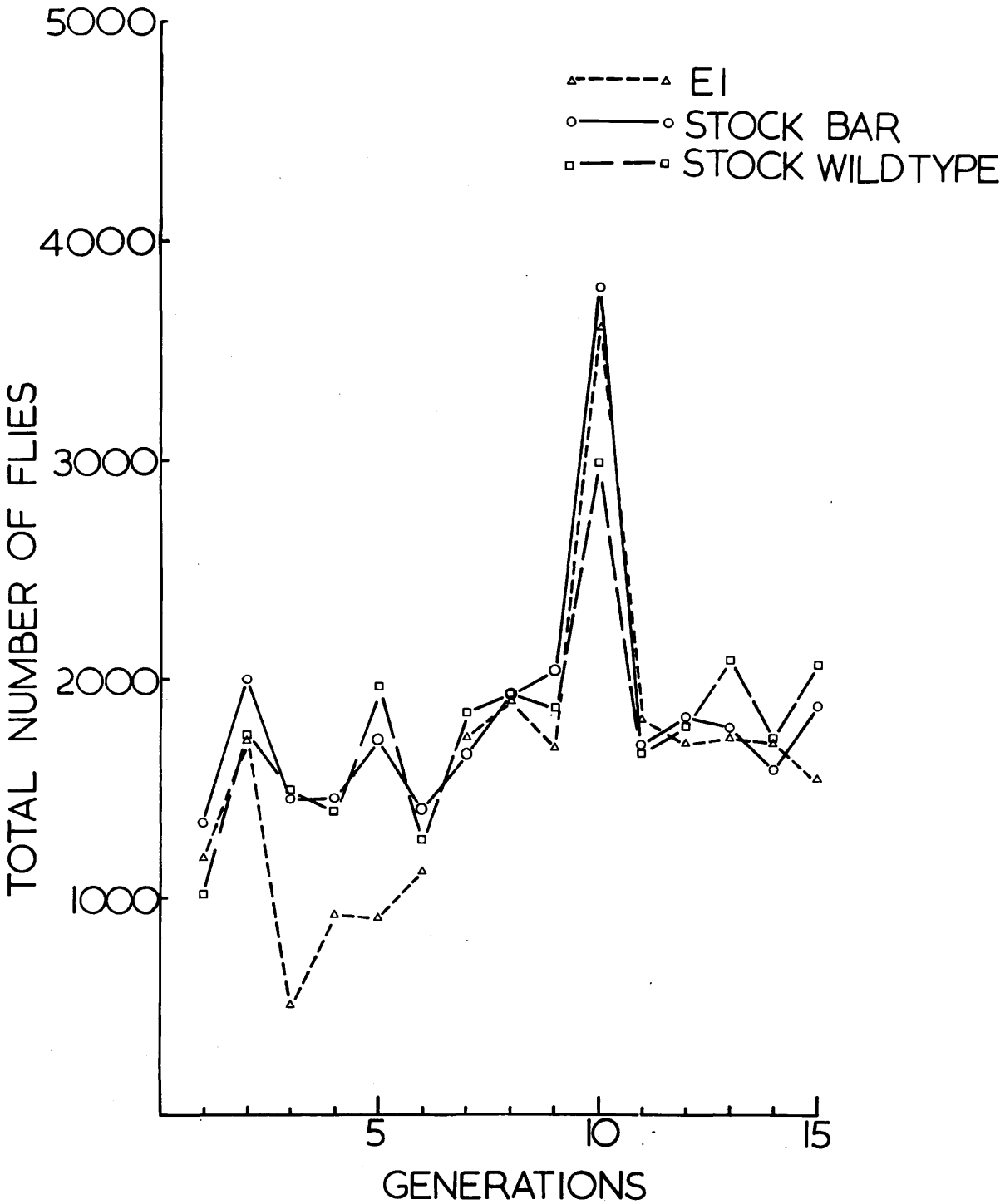
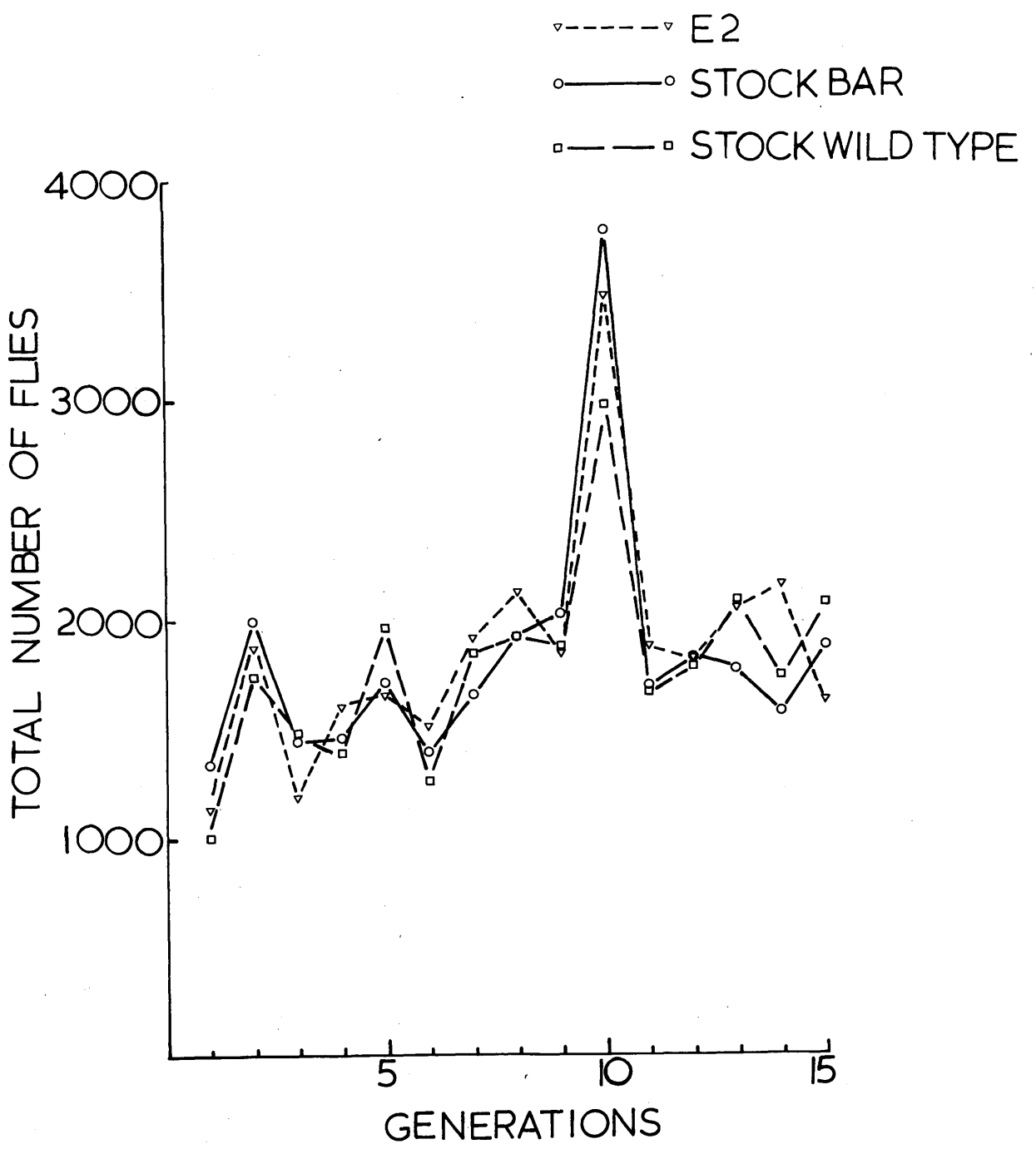

5000
4000
3000
2000
1000
TOTAL NUMBER OF FLIES
E1
STOCK BAR
STOCK WILDTYPE
5
10
15
GENERATIONS

FIGURE 3

Total numbers of flies emerging from E2, Stock Bar and Stock wild type each generation in Experiment 1.


E2
STOCK BAR
STOCK WILD TYPE
4000
3000
2000
1000
TOTAL NUMBER OF FLIES
5
10
15
GENERATIONS

In Experiment 1, after the E1 lines were restarted at generation 7, the two types of mixture and the two pure cultures gave very similar yields (Table 1). Stock wild type yielded higher than stock Bar and E1 once (generation 13) and E2 yielded higher than both stocks once (generation 15). There are, however, no other significant differences in yields between the four types of culture over the last three generations. All cultures gave very high yields in generation 10, probably because a different type of agar was used which gave a much more liquid medium.

In Experiment 2, the first 6 generations gave generally higher yields than generations 7 to 15. This was because counting was stopped on Day 17 in the later generations rather than on Day 21. Second generation flies had begun to emerge by Day 21 so that total numbers of flies were considerably higher. The very low total yield of stock wild type in generation 12 was because two replicates failed to produce any flies. The other two replicates produced a very small number of flies. This was most likely because of the high temperatures (over 40°C)experienced in the previous generation. The cultures were restarted from the other two replicates.

Table 2 shows that although there are many cases of a significant difference between yields of cultures in the last three generations of Experiment 2, there appear to be no consistent trends, mixtures are worse than pure cultures on eight occasions and better on four occasions: there is no indication that mixtures are consistently outyielding pure cultures.

TABLE 2

Comparison of numbers of flies emerging from Stock Bar, Stock wild type, E1, E2, E5 and E6 in the last three generations of Experiment 2, using 't' values where $p < 0.05$ when $t > 1.96$. Red indicates $N_2 > N_1$, black $N_1 \geq N_2$. Generations as indicated below.

Generations 13 14 15

N_2	N_1 Stock wild type	E1	E2	E5	E6
Stock Bar	2.29 0.35 5.47	0.08 1.20 2.61	2.65 3.55 0.67	1.30 0.61 1.21	0.15 2.61 10.47
Stock wild type	-	2.41 1.24 2.81	0.79 2.65 6.05	1.60 0.80 3.69	2.26 2.25 1.32
E1	-	-	3.01 2.08 3.18	1.55 0.91 1.05	0.09 1.43 2.79
E2	-	-	-	1.42 5.35 1.27	2.40 0.21 16.93
E5	-	-	-	-	1.24 2.77 4.10

FIGURE 4

Total numbers of flies emerging from E1, Stock Bar and Stock wild type each generation in Experiment 2.

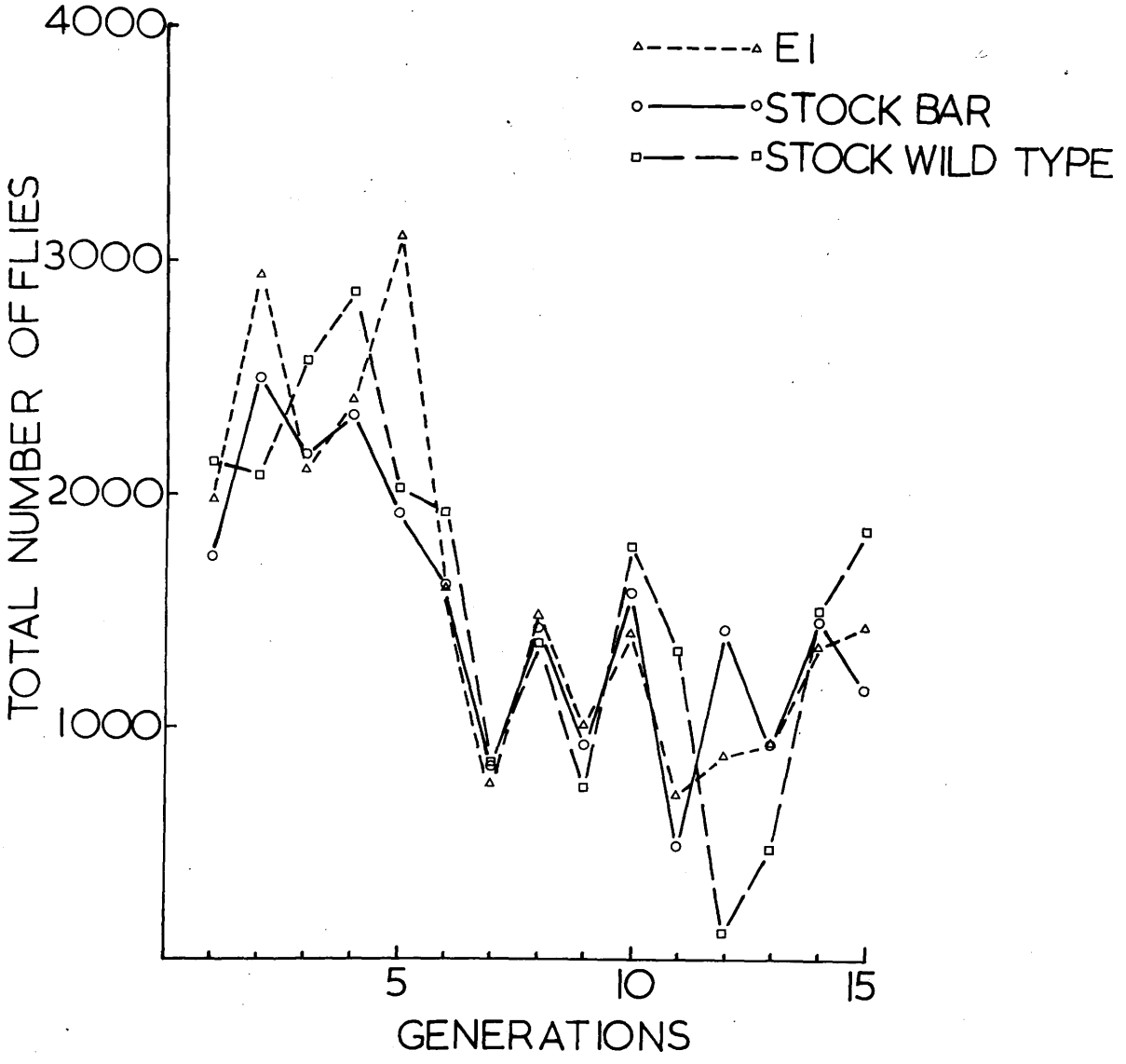

4000
3000
2000
1000
TOTAL NUMBER OF FLIES
5
10
15
GENERATIONS
E1
STOCK BAR
STOCK WILD TYPE

FIGURE 5

Total numbers of flies emerging from E2, E5 and E6 each generation in Experiment 2.
(E5 and E6 were initiated at generation 9).

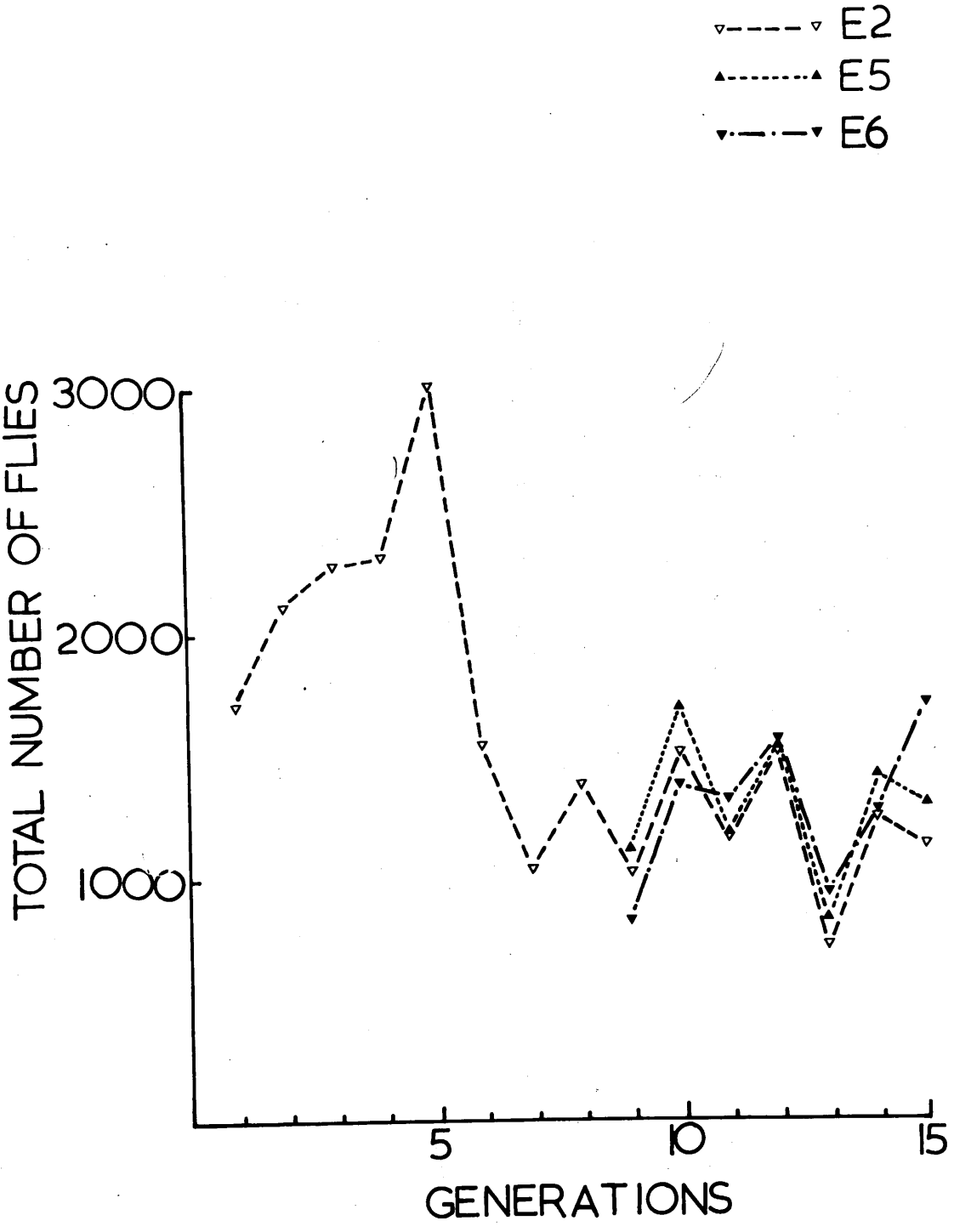

E2
E5
E6
TOTAL NUMBER OF FLIES
3000
2000
1000
5
10
15
GENERATIONS

TABLE 3

Comparison of numbers of flies emerging from Stock Bar, Stock wild type and E1 in the last three generations of Experiment 3, using 't' values where $p < 0.05$ when $t > 1.96$. Red indicates $N_2 > N_1$, black $N_1 > N_2$. Generations as indicated below.

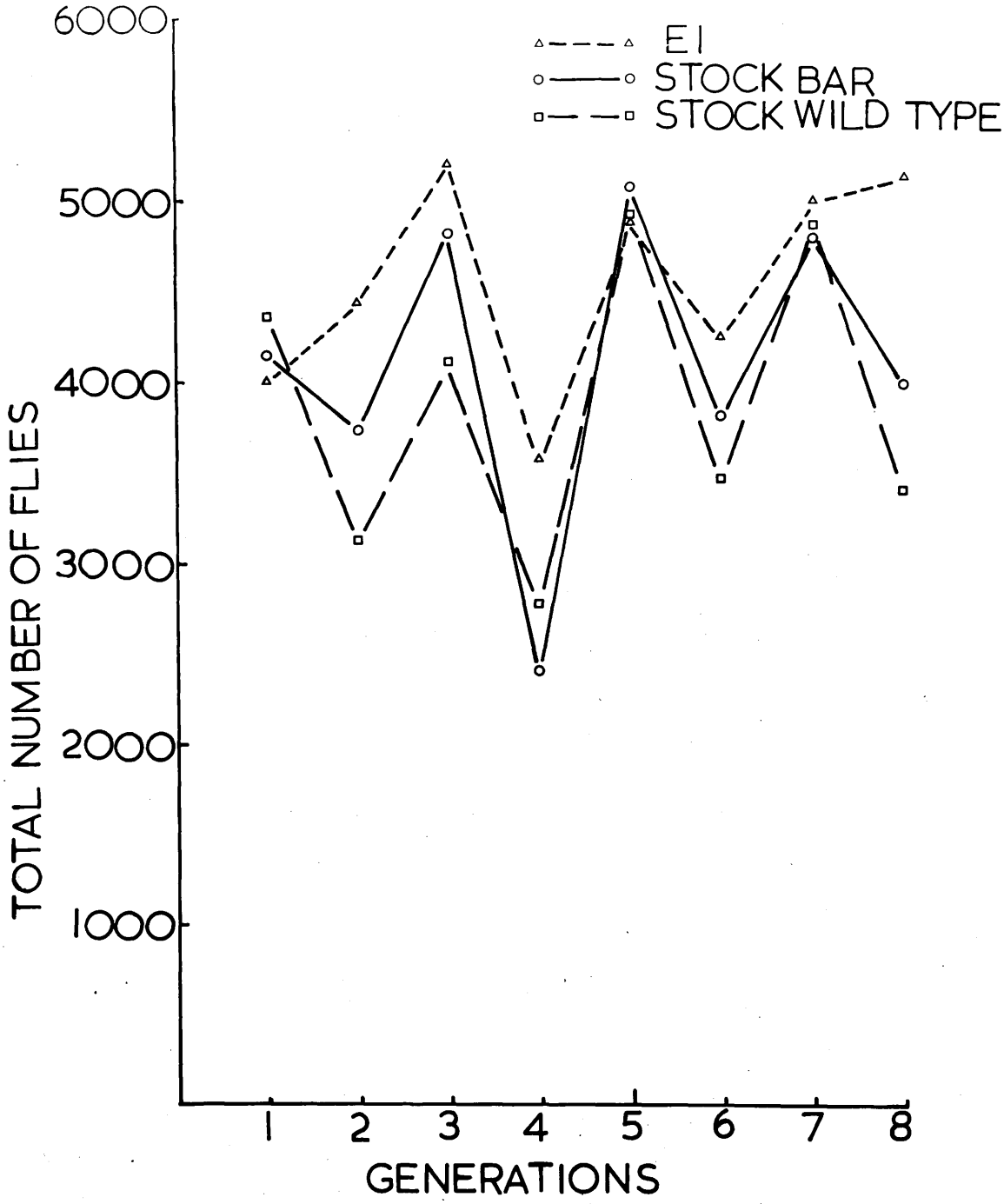
Generations 6 7 8

	N_1			
N_2	Stock wild type		E1	
Stock Bar	1.50	0.27	3.37	0.99
	1.53		3.40	
Stock wild type	-		1.40	0.47
			5.46	

Figure 6 shows that in Experiment 3, E1 usually gave higher yields than the pure cultures. Table 3 shows that in the last three generations E1 yielded significantly higher than stock Bar twice (generations 6 and 8) and stock wild type once (generation 8).

FIGURE 6

Total numbers of flies emerging from E1, Stock Bar and Stock wild type each generation in Experiment 3.


6000
5000
4000
3000
2000
1000
TOTAL NUMBER OF FLIES
E1
STOCK BAR
STOCK WILD TYPE
1
2
3
4
5
6
7
8
GENERATIONS

ii) Percentage of Bar in mixtures

Percentages of Bar emerging from isolated and interbreeding mixtures were calculated for each generation of each experiment. To make a fair comparison of Bar in the interbreeding mixtures, heterozygotes (kidney females) were excluded and only percentages of Bar and wild type were used.
A measure of the relative performances of stock Bar and stock wild type was also calculated for comparison with the percentage of Bar in the mixtures. This was calculated by dividing the total yield of stock Bar by the sum of the total yields of stock Bar and stock wild type, and multiplying by 100 to obtain a percentage.

These percentages were plotted for each generation in Experiments 1, 2 and 3 and are shown in Figures 7, 8 and 9.
A comparison of pure yields gave a percentage of Bar around 50% showing that Bar will perform as well by itself as will wild type. However, in both interbreeding and isolated mixtures it represented nearer 40% of the total of Bar and wild type. In both Experiments 1 and 2, there was wide fluctuation and Bar sometimes outyielded wild type in both types of mixture. In Experiment 3, with the larger number of replicates, Bar was consistently poorer than wild type in the E1 mixture (mean of 40.7%) while it is almost exactly equal to wild type in pure cultures (mean of 51.3%).

FIGURE 7

Percentage of Bar emerging each generation from Stocks, E1 and E2 in Experiment 1.

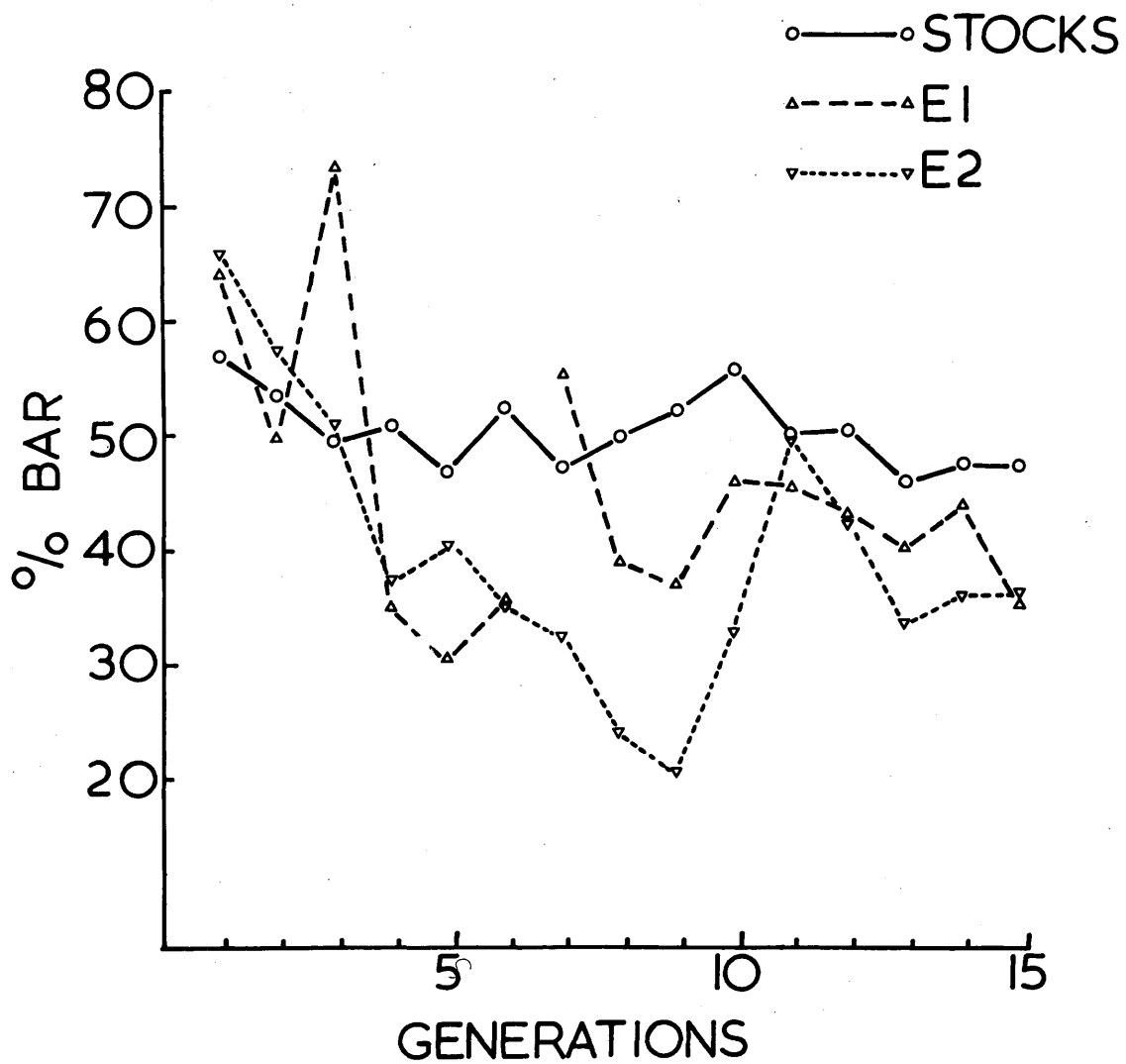

STOCKS
E1
E2
80
70
60
50
40
30
20
% BAR
5
10
15
GENERATIONS

FIGURE 8

Percentage of Bar emerging each generation from Stocks, E1, E2, E5 and E6 in Experiment 2.

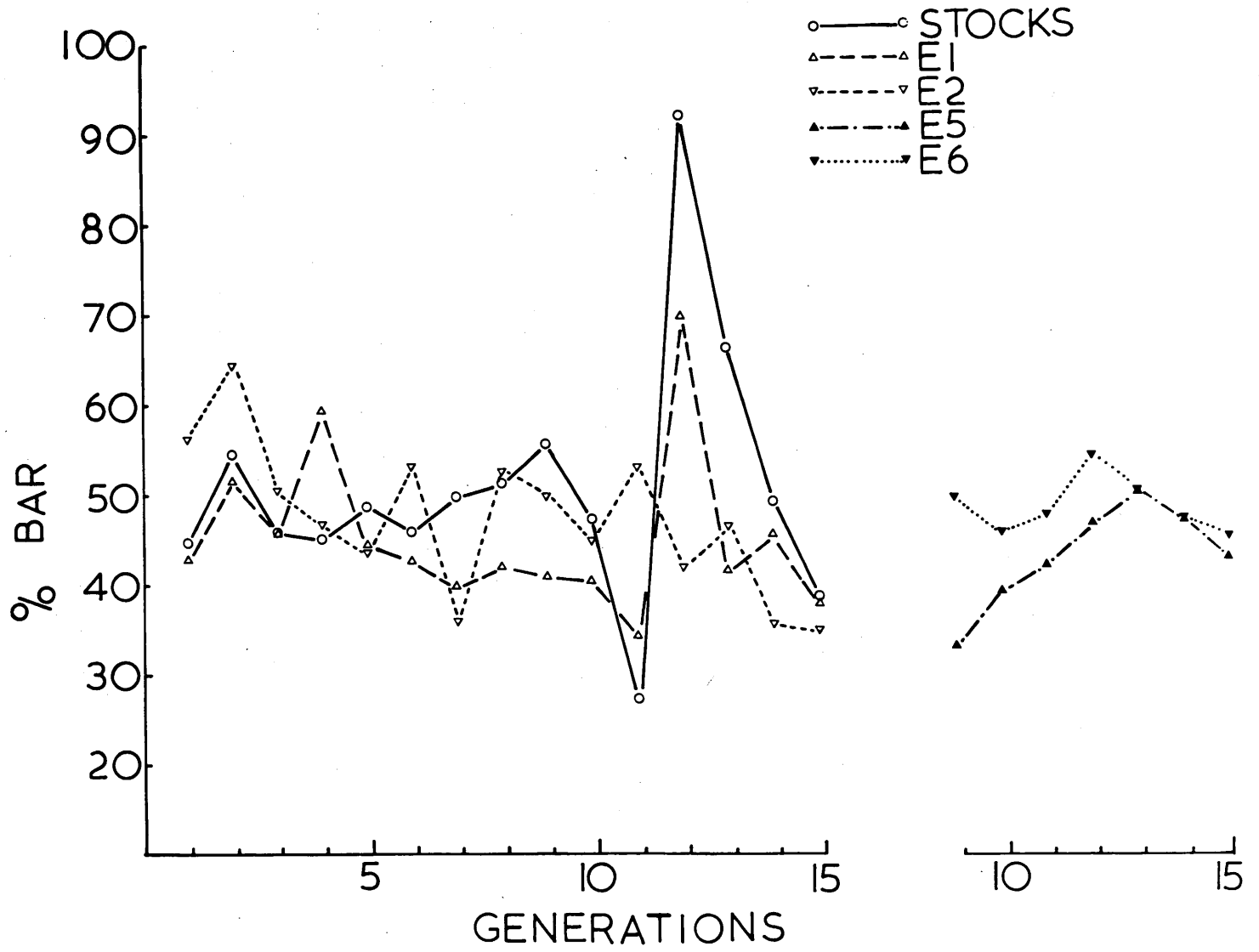
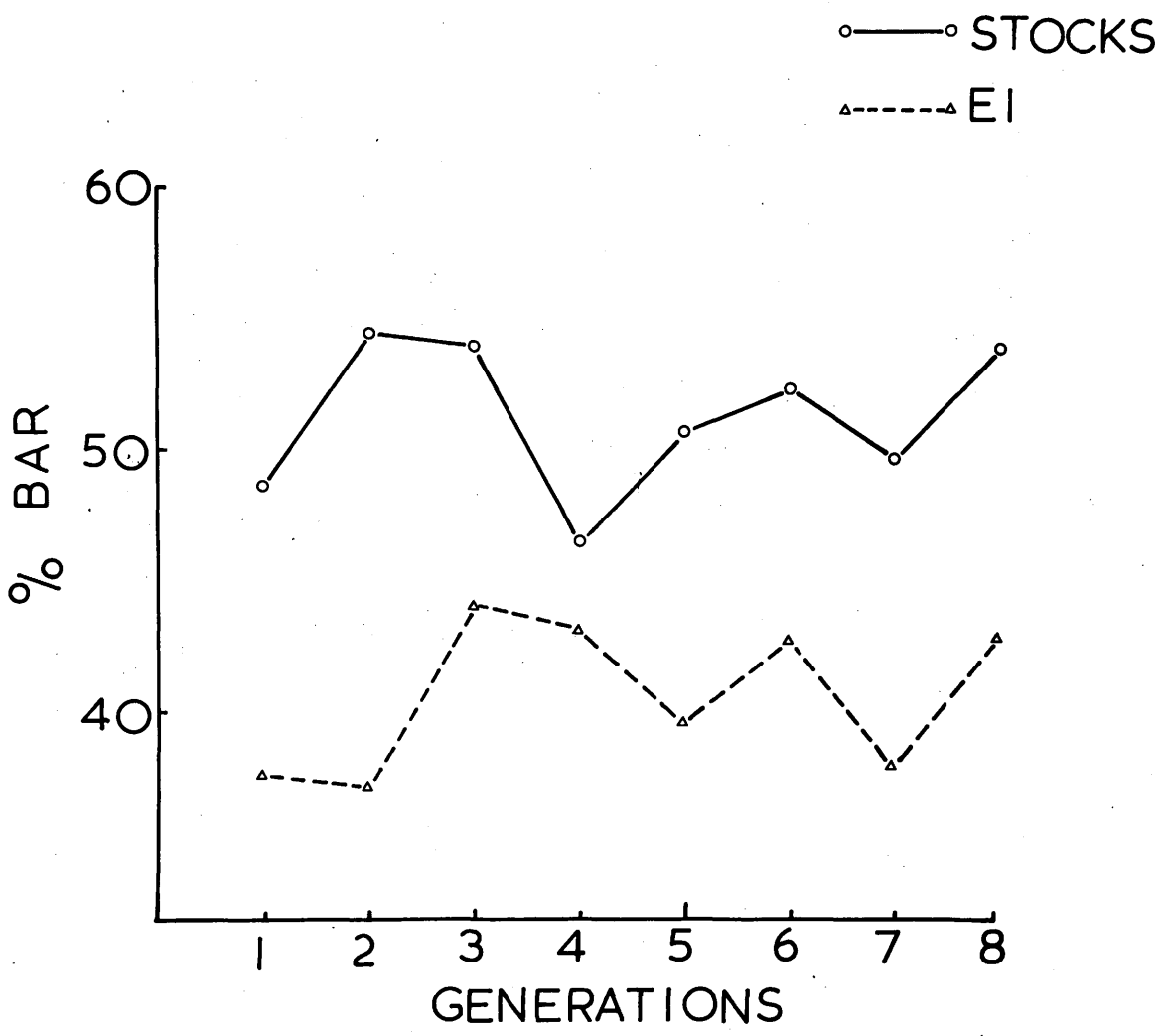

STOCKS
E1
E2
E5
E6
100
90
80
70
60
50
40
30
20
% BAR
5
10
15
10
15
GENERATIONS

FIGURE 9

Percentage of Bar emerging each generation from Stocks and E1 in Experiment 3.


STOCKS
EI
60
50
40
% BAR
1
2
3
4
5
6
7
8
GENERATIONS

iii) Rate of emergence

To obtain an idea of the effect of competition on the development rates of each genotype, the numbers of each genotype emerging each day from Day 10 to Day 15 inclusive were counted in generations 3, 6, 9, 12 and 15 of Experiment 2.

The mean day of emergence was calculated for each genotype in each culture and comparisons have been made on Figures 10, 11 and 12. This mean day of emergence is not a development time as it was calculated from the time each generation was initiated rather than from the time each egg was laid. A comparison between the cultures can indicate differences in development rate of a genotype when competing with itself or with the other genotype. A χ^2 analysis was carried out on emergence each day of different cultures, or, when there is heterogeneity, using Day 10-12 pooled and Day 13-15 pooled to see if there was any difference in rate of emergence. Levels of significance are given in appropriate generations on each graph.

Stock Bar was later than stock wild type, except in generation 3. E1 Bar was later than E1 wild type in generations 6 and 9, but it was significantly earlier than stock Bar in generations 12 and 15. E1 wild type was later than stock wild type in generations 6 and 9 yet earlier in generations 12 and 15. Thus, in the later generations, the E1 lines emerge relatively earlier than the stock lines.

E2 Bar is significantly earlier than stock Bar in generations 3, 9, 12 and 15 while E2 wild type is significantly later than stock wild type in generations 6, 9 and 12, yet earlier in generations 3 and 15.

FIGURE 10

Mean day of emergence in generations 3, 6, 9, 12 and 15 of Experiment 2.

i) Stock Bar and Stock wild type

ii) E1 Bar and E1 wild type

iii) Stock Bar and E1 Bar, and

iv) Stock wild type and E1 wild type

Levels of significance below each graph.

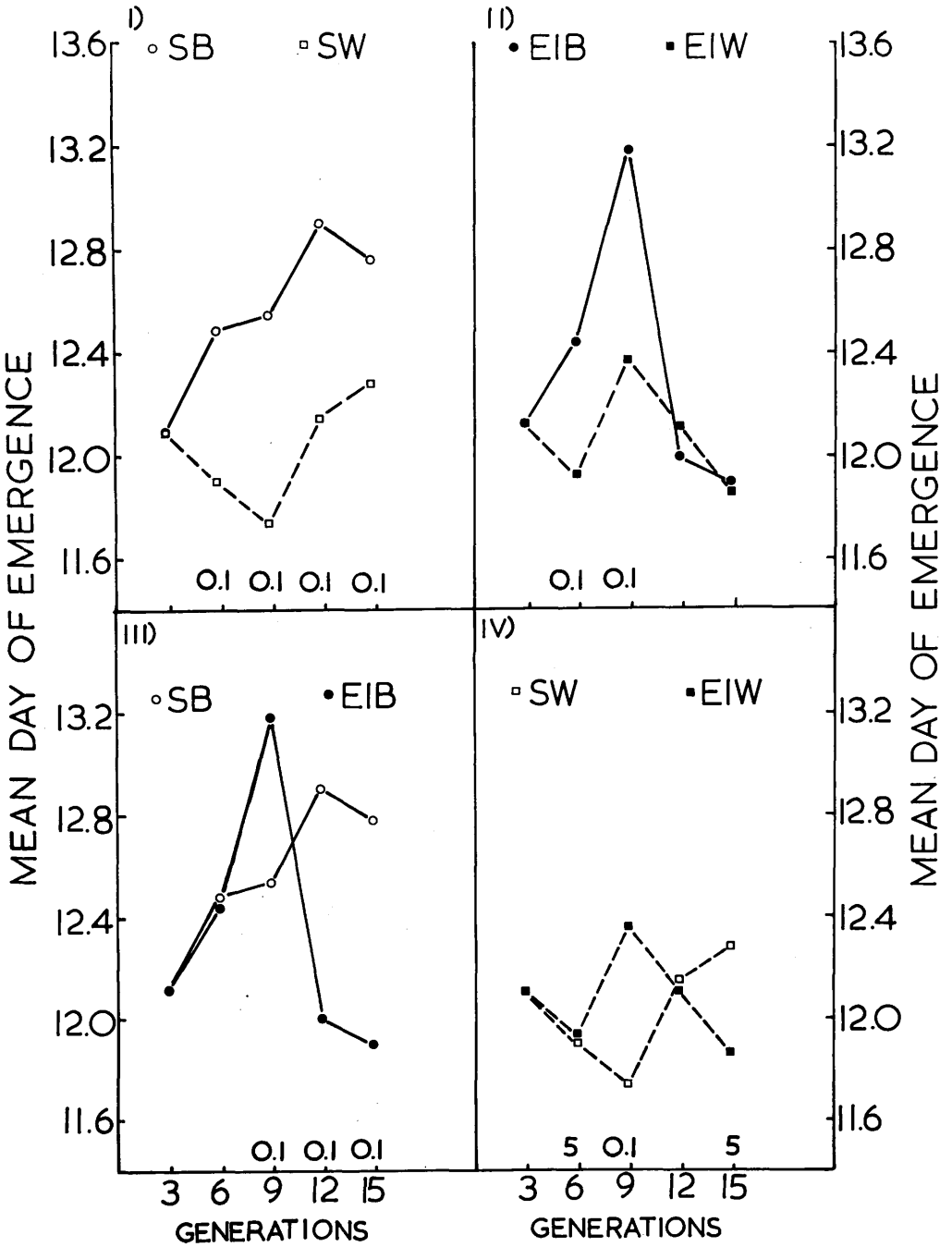

I)
SB
SW
II)
EIB
EIW
III)
SB
EIB
IV)
SW
EIW
13.6
13.2
12.8
12.4
12.0
11.6
MEAN DAY OF EMERGENCE
0.1 0.1 0.1 0.1
0.1 0.1
0.1 0.1 0.1
5 0.1 5
3 6 9 12 15
GENERATIONS

FIGURE 11

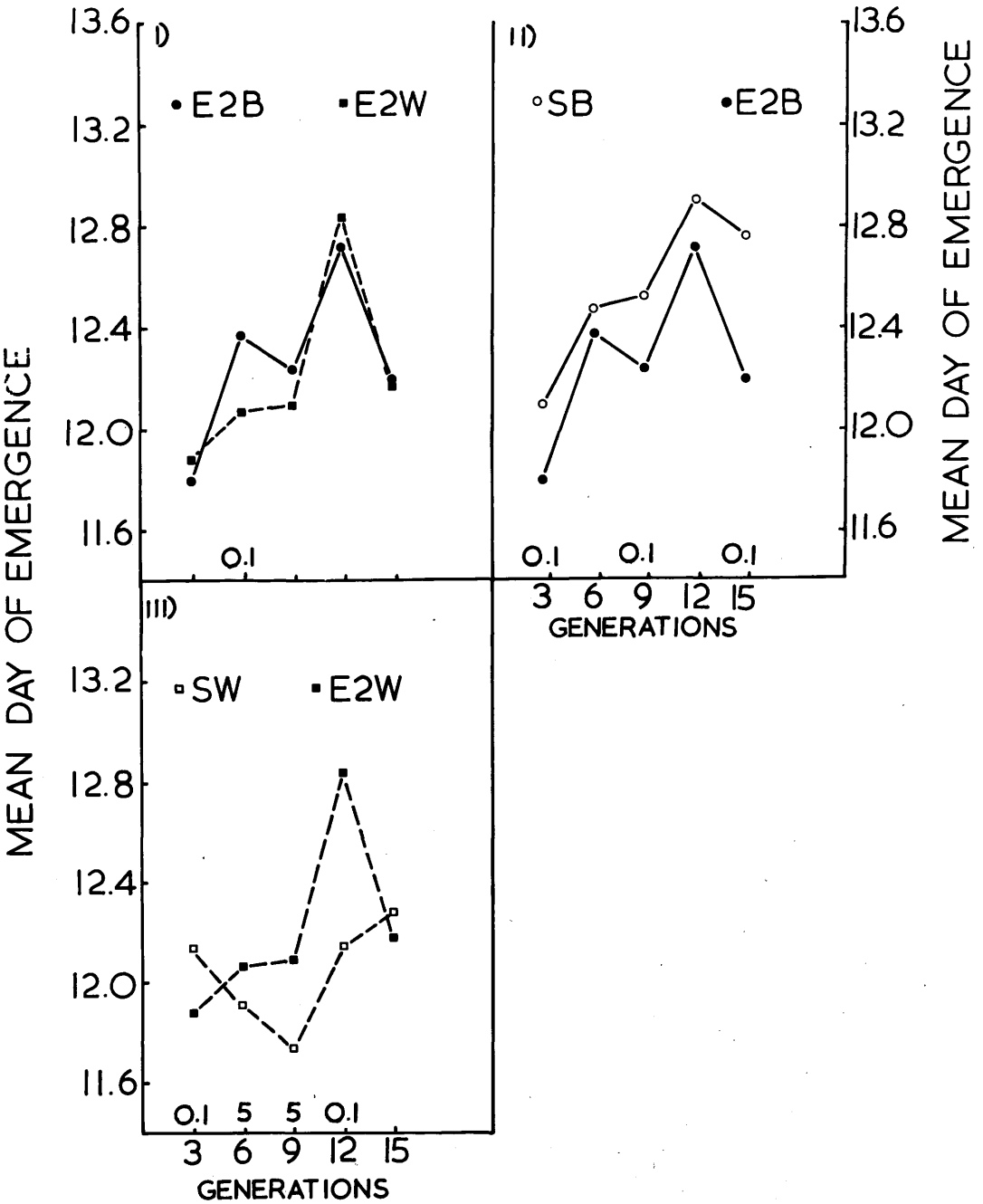
Mean day of emergence in generations 3, 6, 9, 12 and 15 of Experiment 2.

i) E2 Bar and E2 wild type

ii) Stock Bar and E2 Bar, and

iii) Stock wild type and E2 wild type.

Levels of significance below each graph.


I)
E2B
E2W
II)
SB
E2B
III)
SW
E2W
MEAN DAY OF EMERGENCE
13.6
13.2
12.8
12.4
12.0
11.6
0.1
0.1 0.1 0.1
5 5
3 6 9 12 15
GENERATIONS

The numbers of each type emerging each day in the E5 and E6 lines were counted in generations 9, 12 and 15, or 1, 4 and 7 generations after they were initiated. E5 Bar was later than stock Bar in generation 9 but earlier in generations 12 and 15, just like E1 Bar. E5 wild type was later than stock wild type in generations 9 and 12 but earlier in generation 15; it was also later than E1 wild type in all generations. E6 Bar was earlier than stock Bar in generations 12 and 15, while E6 wild type was later than stock wild type in generations 9 and 12, but earlier in generation 15.

FIGURE 12

Mean day of emergence in generations 9, 12 and 15 of Experiment 2.

i) E5 Bar and E5 wild type

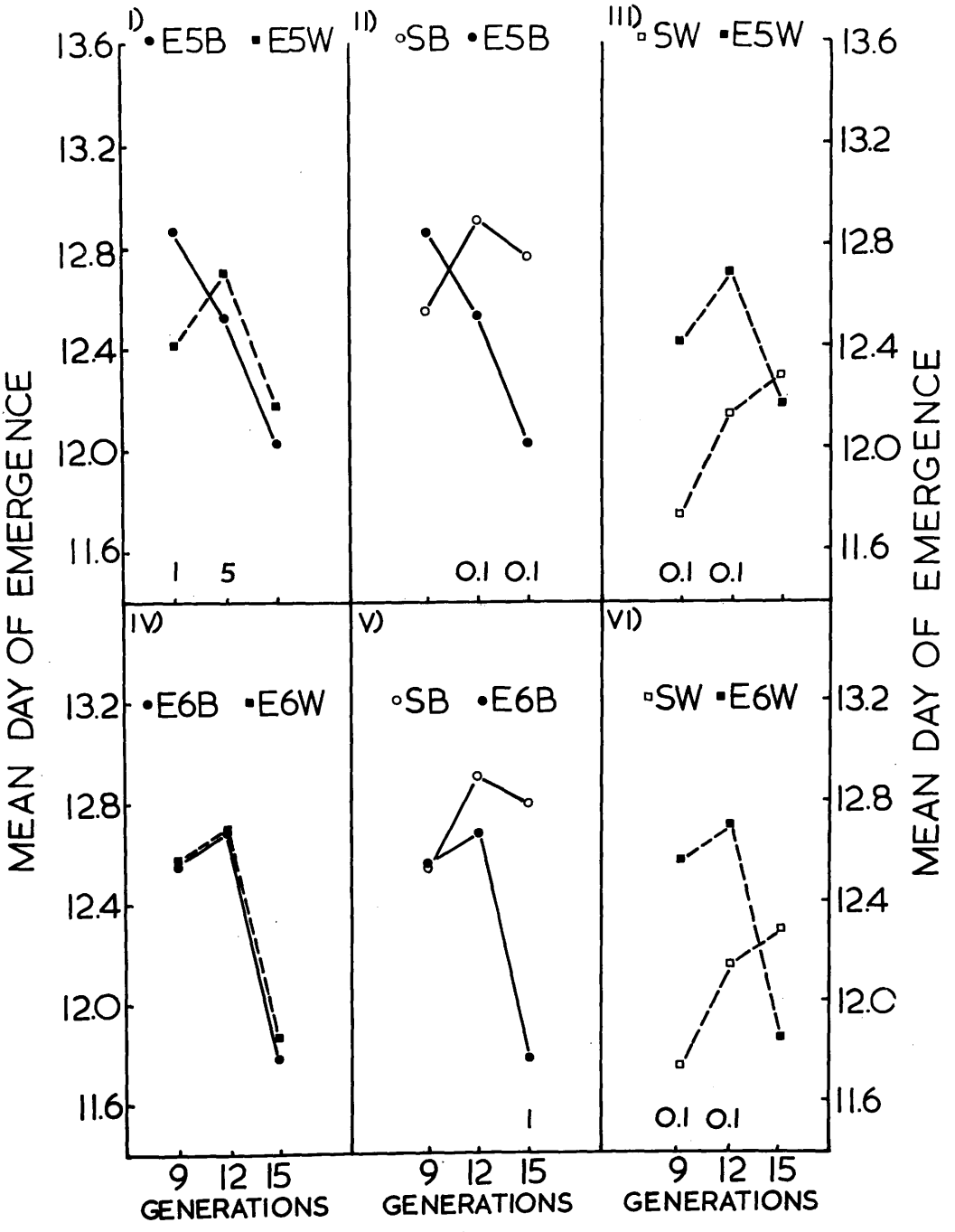
ii) Stock Bar and E5 Bar

iii) Stock wild type and E5 wild type

iv) E6 Bar and E6 wild type

v) Stock Bar and E6 Bar and

vi) Stock wild type and E6 wild type


I)
•E5B ▪E5W
II)
○SB •E5B
III)
□SW ▪E5W
13.6
13.2
12.8
12.4
12.0
11.6
1 5
0.1 0.1
0.1 0.1
IV)
•E6B ▪E6W
V)
○SB •E6B
VI)
□SW ▪E6W
13.2
12.8
12.4
12.0
11.6
1
0.1 0.1
9 12 15
GENERATIONS
9 12 15
GENERATIONS
9 12 15
GENERATIONS
MEAN DAY OF EMERGENCE
MEAN DAY OF EMERGENCE

iv) Competitive ability tests

These tests compare the performance of stock and selected populations subjected to different competitors and investigate any changes in competitive ability which have taken place due to selection or other factors.

The results are summarised in Tables 4, 6, 8,10 and 12 and are shown graphically on histograms (Figures 13, 14, 15, 16 and 17). A comparison of the performance of the two components was made with a modified 't' test (see Experimental plan and techniques section). The 't' values are presented for each test on Tables 5, 7, 9, 11 and 13.

TABLE 4

Summary of competitive ability tests of (a) E1 and (b) E2 in Experiment 1, showing the mean yields of each genotype and the proportion (p) of Bar (no. Bar/total).

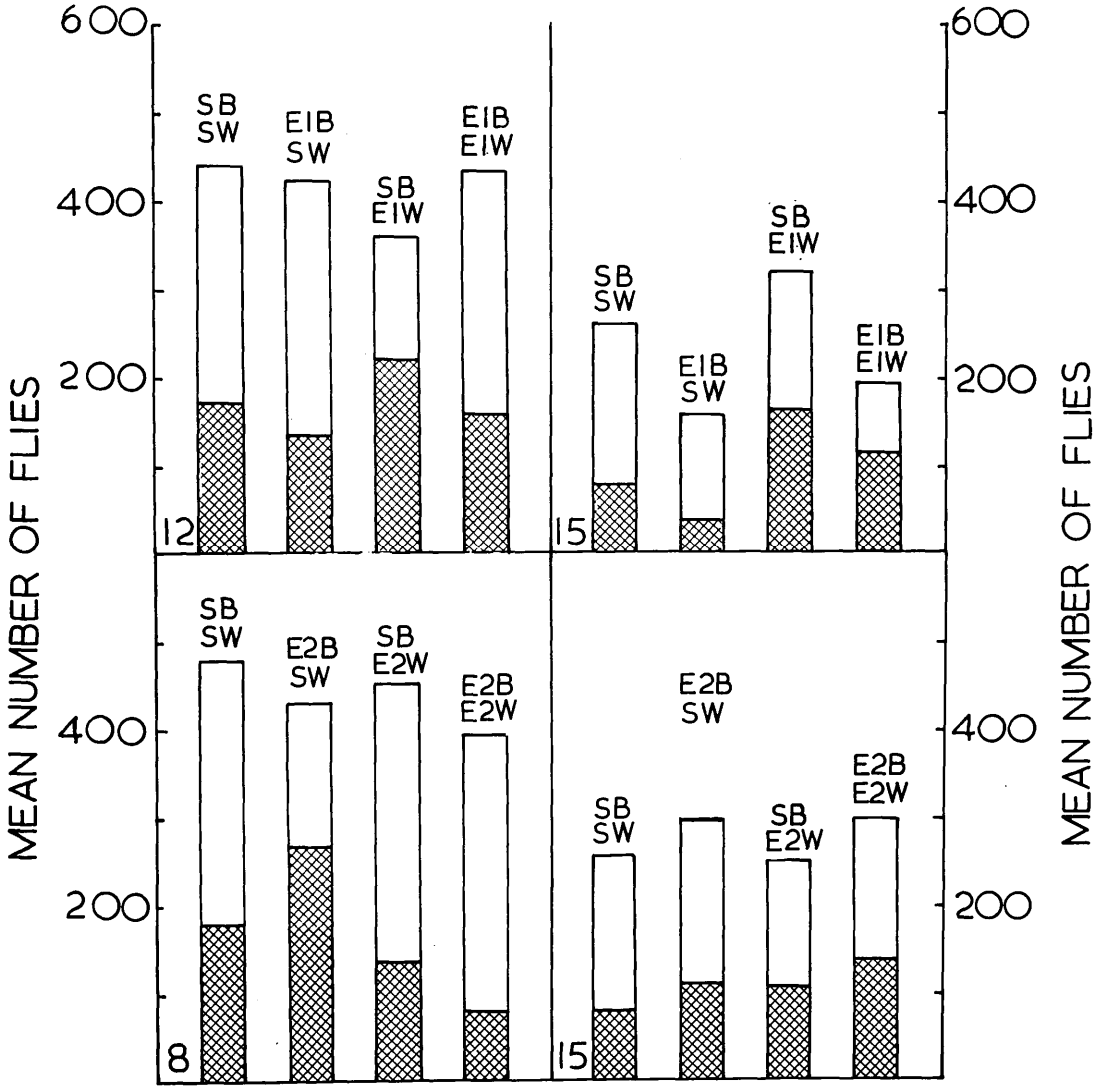
(a)	SBSW		p	E1BSW		p	SBE1W		p	E1BE1W		p
Gen.12	182	257	.41	136	289	.32	222	140	.61	160	227	.41
Gen.15	80	177	.31	41	121	.25	166	156	.52	117	79	.60
Total	262	434	.38	177	410	.30	388	296	.57	277	306	.48

(b)	SBSW		p	E2BSW		p	SBE2W		p	E2BE2W		p
Gen.8	180	298	.38	243	188	.56	137	315	.30	102	323	.24
Gen.15	80	177	.31	112	181	.38	107	143	.43	138	161	.46
Total	260	475	.35	355	369	.49	244	458	.35	240	484	.33

In the E1 lines of Experiment 1 (Tables 4, 5 and Figure 13) after both 12 and 15 generations (only 6 and 9 generations after E1 was restarted) there is evidence that the selected populations did less well than stock populations when they competed with them. Statistical analysis (Table 5) shows that selected Bar did worse against stock wild type than did stock

FIGURE 13

Mean numbers of flies emerging from competitive ability tests on E1 in generations 12 and 15, and E2 in generations 8 and 15 of Experiment 1.
Shaded area Bar, unshaded area wild type.


MEAN NUMBER OF FLIES
600
400
200
SB SW
E1B SW
SB E1W
E1B E1W
12
SB SW
E1B SW
SB E1W
E1B E1W
15
SB SW
E2B SW
SB E2W
E2B E2W
8
SB SW
E2B SW
SB E2W
E2B E2W
15
MEAN NUMBER OF FLIES

Bar, and worse once and better once against selected wild type. Selected wild type performed worse than stock wild type against stock and selected Bar.

TABLE 5

Comparison of proportions of different populations of Bar in competitive ability tests in Experiment 1, using 't' values, where $p < 0.05$ when $t > 1.96$.

(a) E1 tested in generations 12 and 15.

(b) E2 tested in generations 8 and 15.

Red indicates $\theta_1 \leq \theta_2$, black $\theta_1 \geq \theta_2$.

(a)	θ_1					
θ_2	E1BSW		SBE1W		E1BE1W	
SBSW	5.37	2.31	10.50	8.41	2.68	10.68
E1BSW	-		16.70	11.17	2.92	13.25
SBE1W	-		-		14.98	3.07

(b)	θ_1					
θ_2	E2BSW		SBE2W		E2BE2W	
SBSW	13.69	3.16	4.01	4.74	10.58	6.59
E2BSW	-		18.59	1.85	24.26	3.81
SB E2W	-		-		6.58	1.79

In the E2 tests (also Tables 4 and 5 and Figure 13) the opposite was true. After 8 generations of selection, selected Bar was better than stock Bar against stock wild type though worse than stock Bar against selected wild type. Selected wild type was better than stock wild type against both stock and selected Bar. After 15 generations, selected Bar was better than stock Bar against stock wild type and no worse against selected wild type. Selected wild type was worse than

stock wild type against both stock and selected Bar.

These tests were carried out more extensively in Experiment 2; the results for the E1 tests are shown in Figure 14, those for E2 in Figure 15, and those for E5 and E6 in Figure 16. The results are summarised in Tables 6, 8 and 10 and the results of the 't' tests are shown in Tables 7, 9 and 11.

TABLE 6

Summary of competitive ability tests of E1 in Experiment 2 showing mean numbers of each genotype and the proportion (p) of Bar

	SBSW		p	E1BSW		p	SBE1W		p	E1BE1W		p
Gen. 2	283	225	.56	223	275	.45	273	235	.54	241	285	.46
Gen. 4	266	161	.62	263	159	.62	254	133	.66	256	236	.52
Gen. 6	132	137	.49	73	120	.38	117	133	.49	74	115	.39
Gen. 8	114	172	.40	74	185	.29	160	186	.46	103	244	.30
Gen.10	129	147	.47	39	99	.29	95	135	.41	61	118	.34
Gen.13	245	281	.47	214	243	.47	222	217	.51	175	275	.39
Total	1169	1123	.51	886	1081	.45	1121	1039	.52	910	1273	.42

TABLE 7

Comparison of proportions of different populations of Bar in competitive ability tests on E1 in Experiment 2, using 't' values where $p < 0.05$ when $t > 1.96$. Red indicates $\theta_1 \leq \theta_2$, black $\theta_1 > \theta_2$. Generations as indicated below.

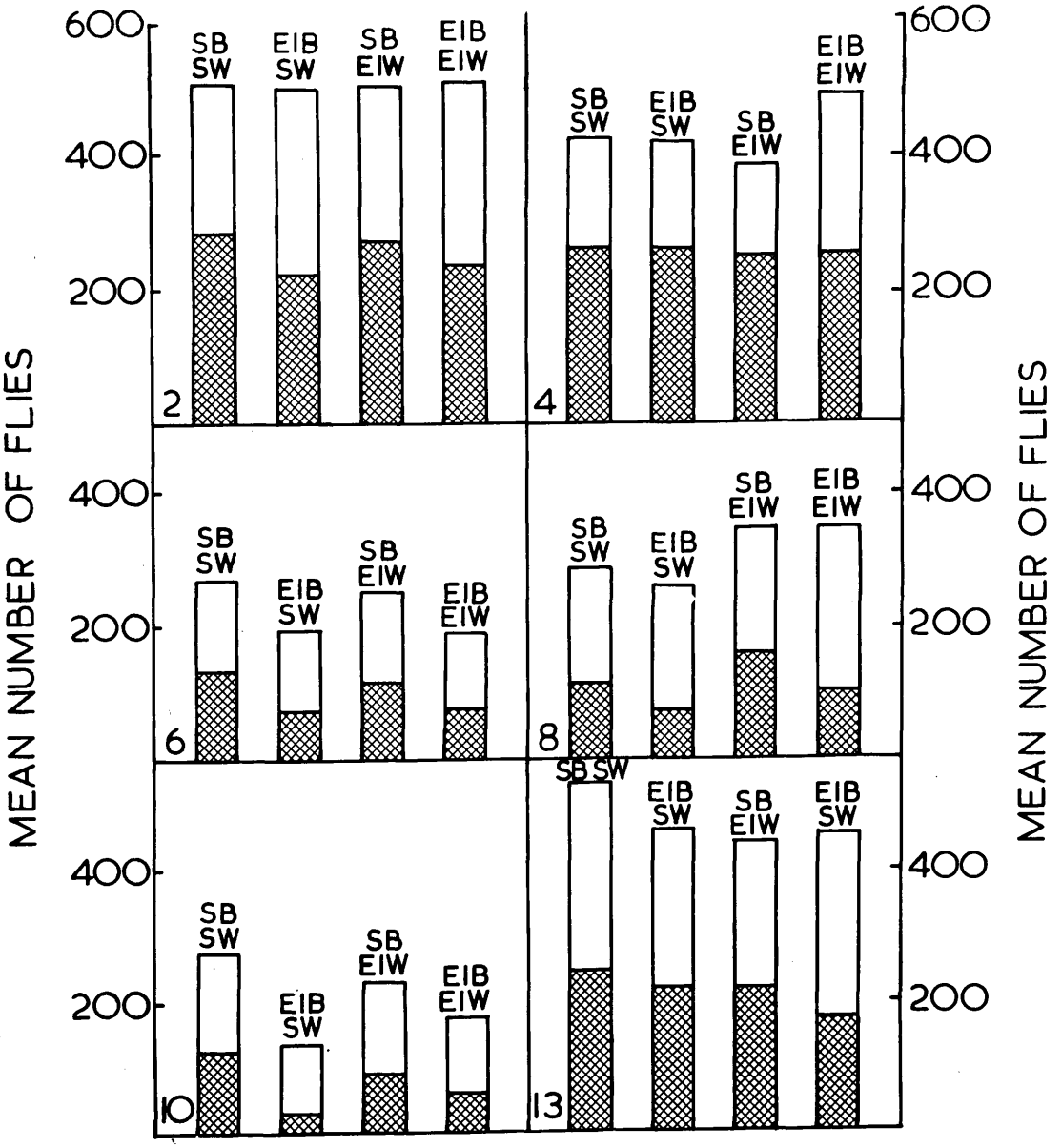
Generations 2 4
6 8
10 13

θ_2	θ_1 E1BSW		SBE1W		E1BE1W	
SBSW	6.99 4.66 7.03	0.00 5.60 0.11	1.22 1.11 2.27	1.96 3.06 2.59	6.40 4.30 5.10	6.44 5.61 4.67
E1BSW	-		5.77 3.57 4.93	1.98 8.75 2.51	0.67 0.75 2.16	6.42 0.34 4.52
SBE1W	-		-		5.17 2.76 2.87	8.33 9.10 6.97

FIGURE 14

Mean numbers of flies emerging from competitive ability tests on E1 in generations 2, 4, 6, 8, 10 and 13 of Experiment 2.

Shaded area Bar, unshaded wild type.


MEAN NUMBER OF FLIES
MEAN NUMBER OF FLIES
600
400
200
SB SW
EIB SW
SB EIW
EIB EIW
2
4
6
8
10
13

The results of the E1 tests are similar to those of Experiment 1. Selected Bar did poorly against stock wild type and selected wild type. Selected wild type was no worse against stock Bar than was stock wild type but was better against selected Bar than was stock wild type on two out of the six tests. Thus Bar has become a much poorer competitor while wild type had perhaps slightly improved.

TABLE 8

Summary of competitive ability tests of E2 in Experiment 2, showing mean numbers of each genotype and the proportion (p) of Bar

	SBSW		p	E2BSW		p	SBE2W		p	E2BE2W		p
Gen. 3	277	193	.59	442	129	.77	204	388	.34	241	360	.40
Gen. 5	285	140	.67	296	94	.76	88	338	.21	202	189	.52
Gen. 7	140	235	.37	175	158	.53	108	252	.30	185	165	.53
Gen. 9	229	225	.50	270	129	.68	176	255	.41	197	207	.49
Gen.14	220	225	.49	252	126	.67	97	298	.25	120	190	.39
Gen.15	180	136	.57	216	159	.58	122	233	.34	108	181	.37
Total	1331	1154	.54	1651	795	.68	795	1764	.31	1053	1292	.45

TABLE 9

Comparison of proportions of different populations of Bar in competitive ability tests on E2 in Experiment 2, using 't' values where $p < 0.05$ when $t > 1.96$. Red indicates $\theta_1 < \theta_2$ black $\theta_1 > \theta_2$. Generations as indicated below

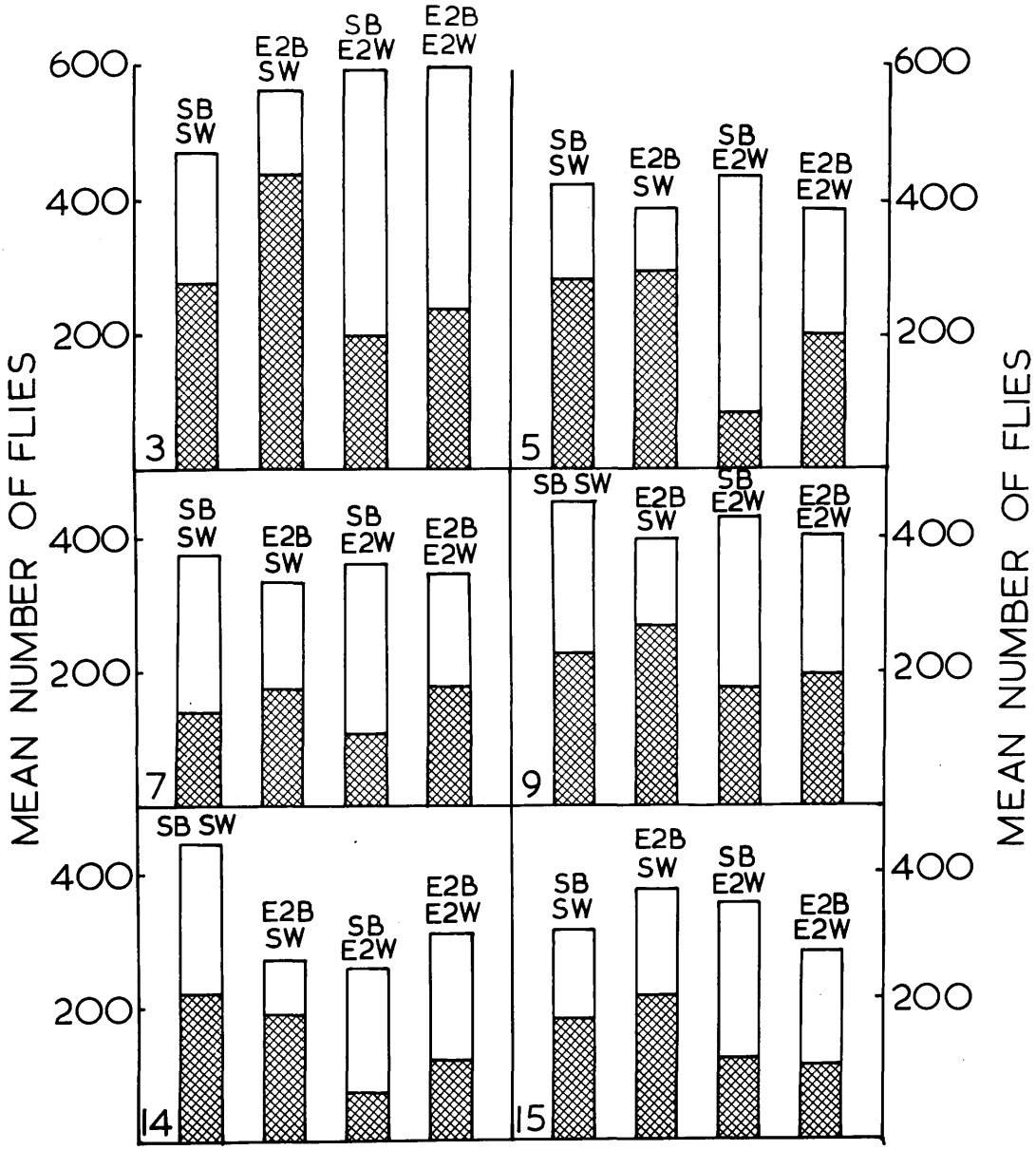
Generations	3	5
	7	9
	14	15

θ_2	θ_1 E2BSW		SBE2W		E2BE2W	
SBSW	12.33	52.80	16.09	27.80	7.58	8.96
	8.06	10.18	4.07	5.82	7.30	3.13
	9.63	0.33	13.30	9.60	6.72	9.15
E2BSW	-		30.00	32.47	20.04	13.70
			11.93	15.68	0.25	12.01
			12.44	9.51	14.17	9.02
SBE2W	-		-		7.78	16.79
					10.58	2.00
					4.66	0.22

FIGURE 15

Mean numbers of flies emerging from competitive ability tests on E2 in generations 3, 5, 7, 9, 14 and 15 of Experiment 2.

Shaded area Bar, unshaded wild type.


MEAN NUMBER OF FLIES
600
400
200
SB SW
E2B SW
SB E2W
E2B E2W
3
5
7
9
14
15

As in Experiment 1, the E2 lines gave results contrary to the E1 lines. Selected Bar was superior to stock Bar against both stock and selected wild type, in all but generation 15 in the former and generations 14 and 15 in the latter. Selected wild type was superior to stock wild type in all generations against stock Bar and in all but generation 7 against selected Bar.

TABLE 10

Summary of competitive ability tests of (a) E6 and (b) E5 in Experiment 2, showing mean number of each genotype and the proportion (p) of Bar

(a)	SBSW		p	E6BSW		p	SBE6W		p	E6BE6W		p
Gen.10	129	147	.47	255	84	.75	43	162	.21	153	175	.47
Gen.13	245	281	.47	255	172	.60	200	321	.38	245	250	.49
Total	374	428	.47	510	256	.67	243	483	.33	398	425	.48

(b)	SBSW		p	E5BSW		p	SBE5W		p	E5BE5W		p
Gen.11	254	65	.80	331	34	.91	178	260	.41	176	206	.46
Gen.14	220	225	.49	244	152	.62	138	295	.32	170	190	.47
Gen.15	180	136	.57	259	113	.70	131	232	.36	201	231	.47
Total	654	426	.61	834	299	.74	447	787	.36	547	627	.47

In the E6 tests (E6 initiated from E2 by preventing interbreeding but continuing competition) selected Bar retained its superiority over stock Bar when competing with stock and selected wild type, while selected wild type also retained its superiority against stock and selected Bar.

In the E5 tests (E5 initiated from E1 by allowing interbreeding) there was a rapid change in the performance of selected lines after interbreeding was started. After only

TABLE 11

Comparison of proportions of different populations of Bar in competitive ability tests on (a) E6 and (b) E5 in Experiment 2, using 't' values where $p < 0.05$ when $t > 1.96$. Red indicates $\theta_1 < \theta_2$, black $\theta_1 > \theta_2$

	E6		E5	
Generations	10	13	11	14
			15	

(a)

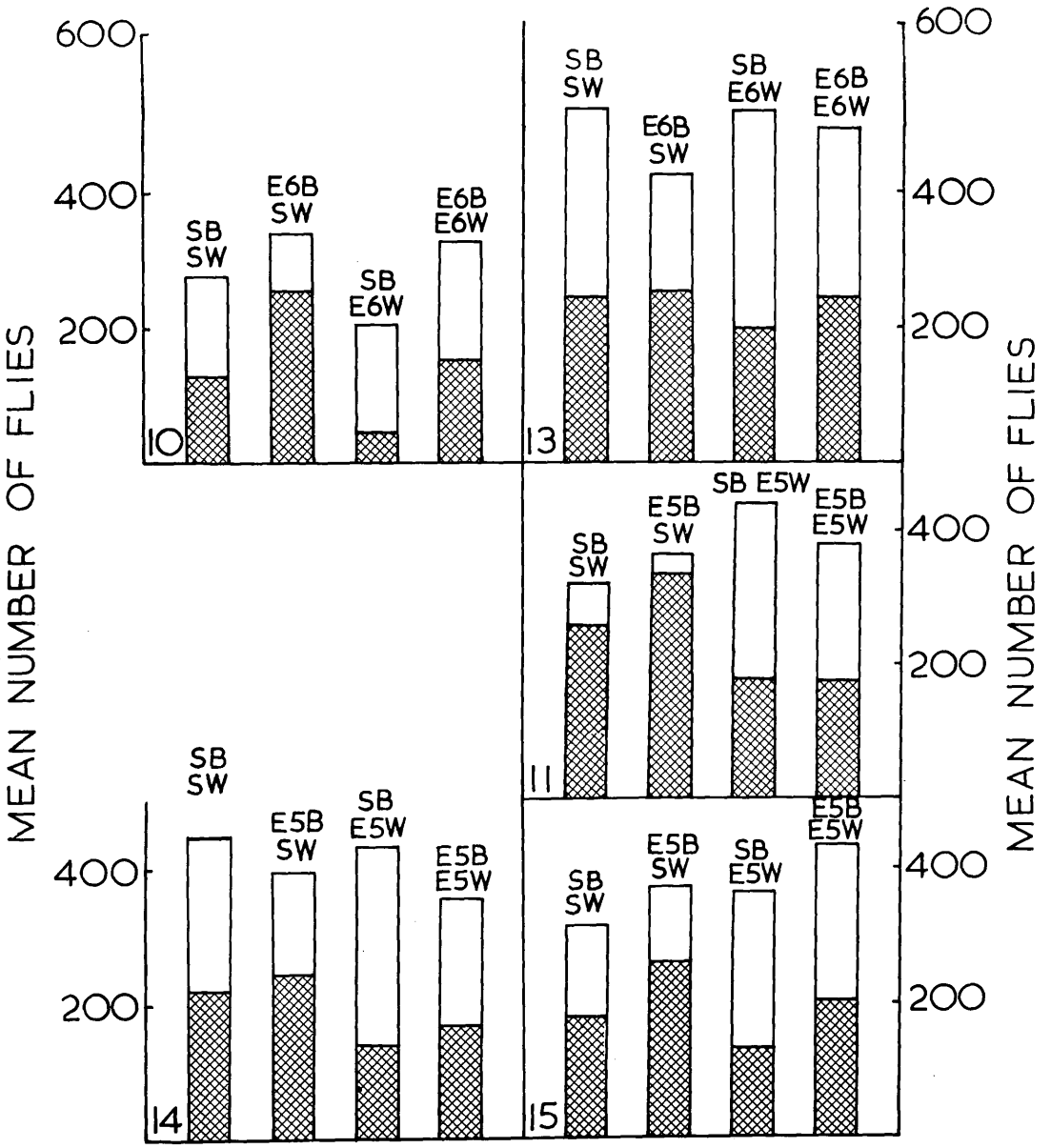
	θ_1					
θ_2	E6B	SW	SB	E6W	E6B	E6W
SB SW	14.72	8.14	11.88	5.42	0.00	1.67
E6B SW			25.87	13.26	15.43	6.45
SB E6W					12.78	7.01

(b)

	θ_1					
θ_2	E5B	SW	SB	E5W	E5B	E5W
SB SW	6.42 6.94	10.56	14.61 11.16	7.75	13.83 5.94	1.61
E5B SW			22.09 18.83	17.57	22.19 13.72	8.22
SB E5W					2.55 5.88	8.68

FIGURE 16

Mean numbers of flies emerging from competitive ability tests on E6 in generations 10 and 13, and E5 in generations 11, 14 and 15 of Experiment 2.
Shaded area Bar, unshaded wild type.


MEAN NUMBER OF FLIES
600
400
200
SB SW
E6B SW
SB E6W
E6B E6W
10
SB SW
E6B SW
SB E6W
E6B E6W
13
SB SW
E5B SW
SB E5W
E5B E5W
11
SB SW
E5B SW
SB E5W
E5B E5W
14
SB SW
E5B SW
SB E5W
E5B E5W
15
MEAN NUMBER OF FLIES

three generations of interbreeding, selected Bar, a poor competitor in the E1 lines, was superior to stock Bar against stock wild type and no worse against selected wild type. It was superior against both after 6 and 7 generations of interbreeding. Selected wild type was also superior to stock wild type against both stock and selected Bar, in all three tests.

TABLE 12

Summary of competitive ability tests of E1 in Experiment 3, showing mean numbers of each genotype and the proportion of Bar

	SBSW		p	E1BSW		p	SBE1W		p	E1BE1W		p
Gen.2	163	275	.37	153	348	.30	190	324	.37	150	292	.34
Gen.4	212	268	.44	233	275	.46	193	254	.43	205	278	.43
Gen.6	216	282	.44	167	359	.32	212	305	.41	199	343	.37
Gen.8	151	237	.39	125	310	.29	158	215	.42	133	230	.37
Total	742	1062	.41	678	1292	.34	753	1098	.41	687	1143	.38

TABLE 13

Comparison of proportions of different populations of Bar in competitive ability tests on E1 in Experiment 3, using 't' values where $p < 0.05$ when $t > 1.96$. Red indicates $\theta_1 < \theta_2$ black $\theta_1 > \theta_2$. Generations as indicated below

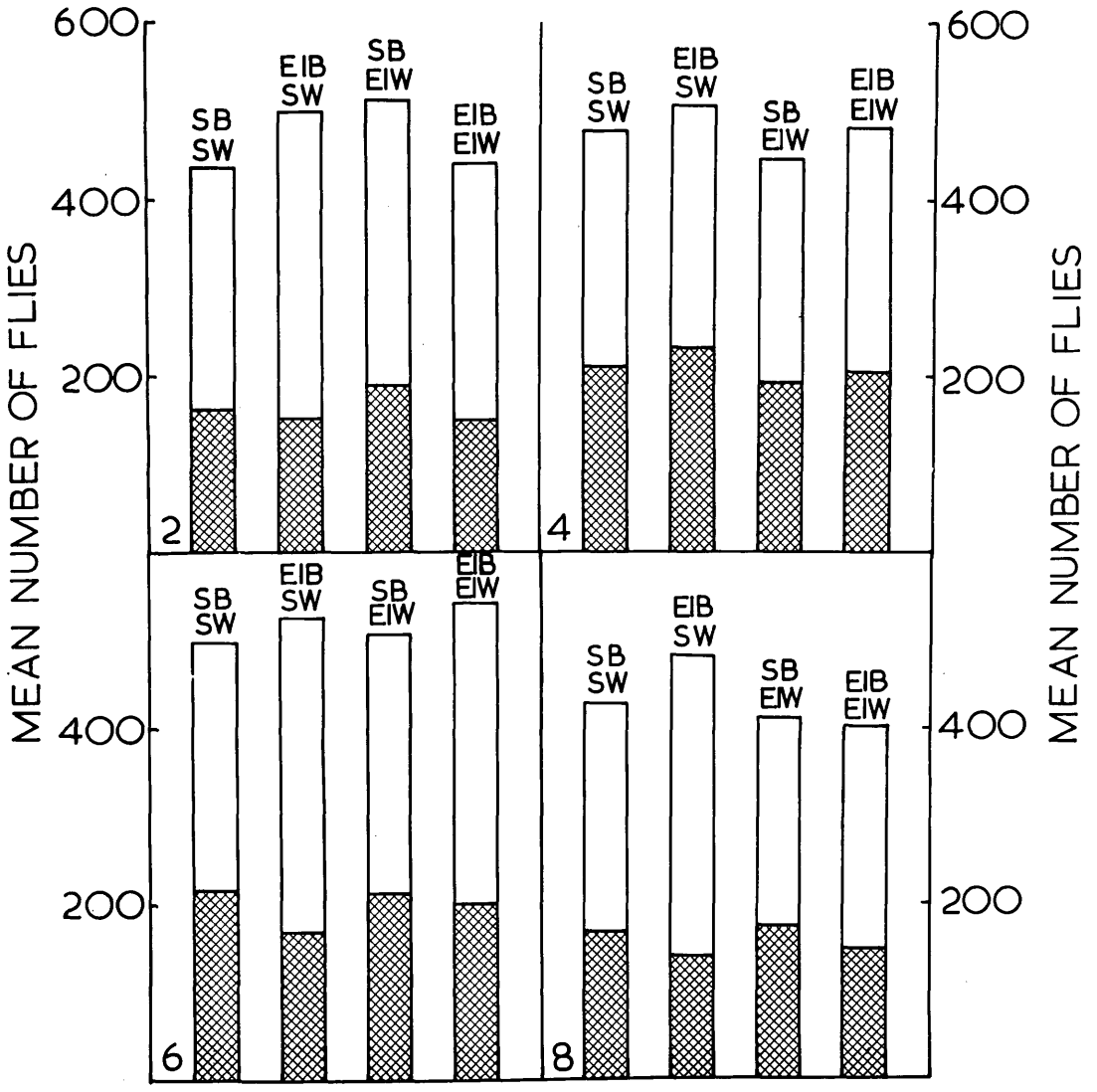
Generations 2 4
6 8

θ_2	θ_1 E1BSW		SBE1W		E1BE1W	
SBSW	6.93	1.73	0.34	0.84	3.28	1.54
	13.05	9.76	3.16	3.11	7.83	1.89
E1BSW			6.84	2.54	3.55	3.32
			9.98	12.83	5.42	7.61
SBE1W					3.05	0.67
					4.67	4.95

Experiment 3 differed from the two previous experiments in having 10 replicates and using very outbred lines. The results are presented on Figure 17 with a summary on Table 12 and 't' test analysis on Table 13. The tests after 2, 6 and 8 generations indicate that E1 Bar was a poor competitor against both stock and selected wild type, compared with stock Bar. Selected wild type was worse than stock wild type both against selected Bar in generations 2, 6 and 8, and stock Bar in generation 8, though better against selected Bar in generation 4 and stock Bar in generation 6.

FIGURE 17

Mean numbers of flies emerging from competitive ability tests on E1 in generations 2, 4, 6 and 8 of Experiment 3. Shaded area Bar, unshaded wild type.


MEAN NUMBER OF FLIES
600
400
200
SB SW
EIB SW
SB EIW
EIB EIW
2
4
6
8

(v) Extinction rate tests

The first of these tests was initiated from Bar and wild type flies from the last (generation 8) of the competitive ability tests on E1 in Experiment 3. The ratios of Bar and wild type from each individual competitive ability test were used as input ratios. This was repeated over 4 subsequent tests, so that an effective extinction rate of the Bar genotype was measured over 5 generations. The frequency of Bar in each generation for the individual replicates of each of the four mixtures was plotted, using a log scale.

The frequency of Bar in the last competitive ability test of stock Bar/stock wild type was about 0.25 - 0.40 (Figure 18). After 5 generations, the frequency of Bar was below 0.10 in most of the replicates though around 0.20 in two replicates which had started near to 0.50 in the first test.

In the selected Bar/stock wild type tests, the frequency of Bar was already low, between 0.20 and 0.40, after the competitive ability test (Figure 19). Although in subsequent generations Bar was reduced to extinction in some of the replicates, it was still retained at a much higher frequency in four of the replicates, two at around 0.20 and two at around 0.10.

In the stock Bar/selected wild type tests, the frequency of Bar was quite high; most replicates were around 0.40 after the competitive ability test (Figure 20). This was unlike the previous test and in common with the competitive ability tests carried out after 2, 4 and 6 generations of selection. However, the frequency fell very rapidly until after 5 generations of selection the frequency of Bar in all except one

replicate was below 0.10. Although there is considerable variation between replicates, the overall pattern contrasts with that of the selected Bar/stock wild type tests where the extinction rate was much less steep in some of the replicates. In the single replicate where the frequency of Bar does not drop rapidly, it is maintained throughout at a frequency of about 0.50.

In the selected Bar/selected wild type tests, the frequency of Bar was between 0.30 and 0.40 in the competitive ability test (Figure 21). In most of the replicates, the frequency of Bar fell to below 0.10 after 5 generations although in two it was retained at around 0.20.

FIGURE 18

Extinction rate curves for Bar in individual replicates of Stock Bar/Stock wild type mixtures in Experiment 3. (Log scale).

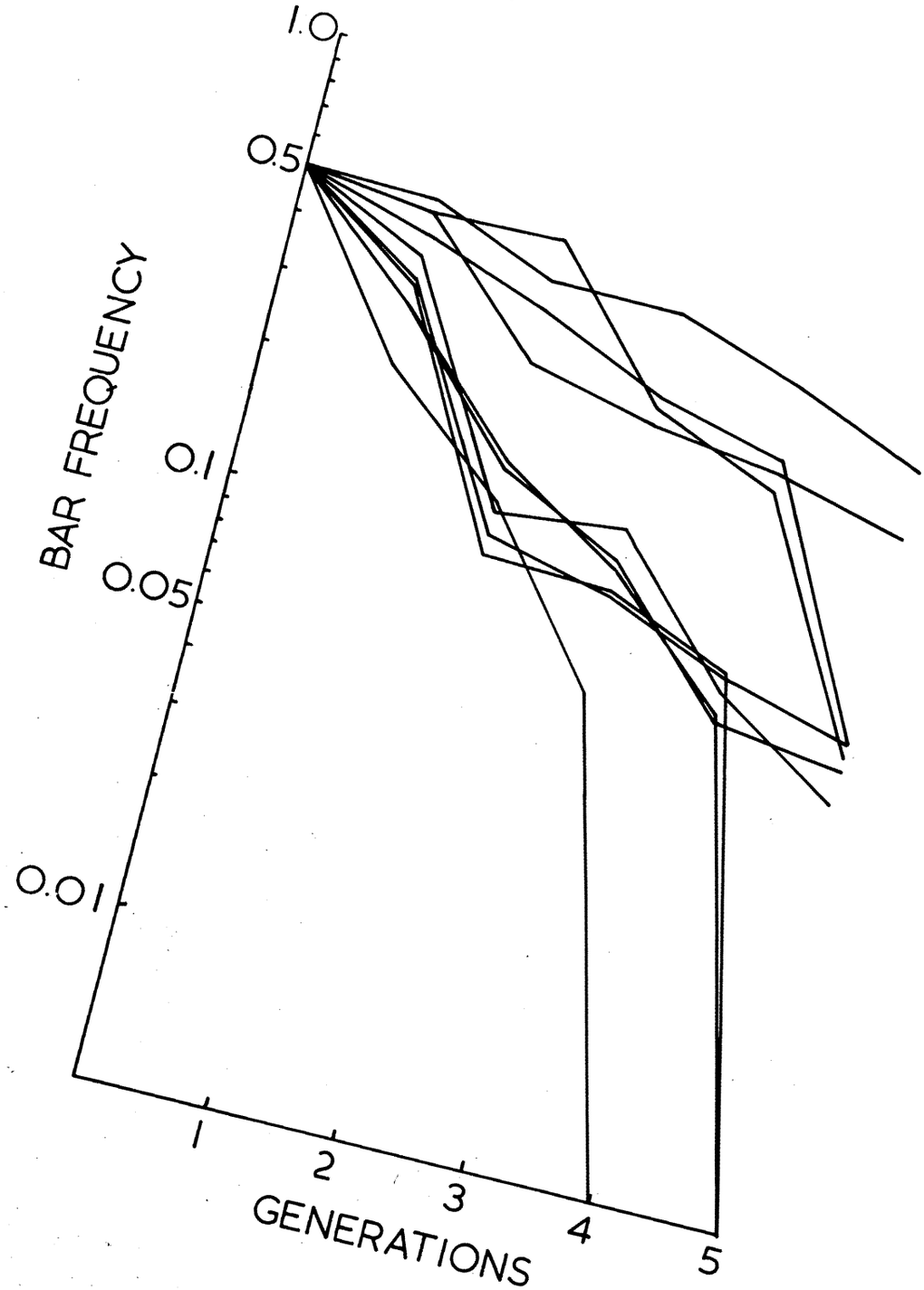

1.0
0.5
0.1
0.05
0.01
BAR FREQUENCY
1
2
3
4
5
GENERATIONS

FIGURE 19

Extinction rate curves for Bar in individual replicates of E1 Bar/Stock wild type mixtures in Experiment 3. (Log. scale).

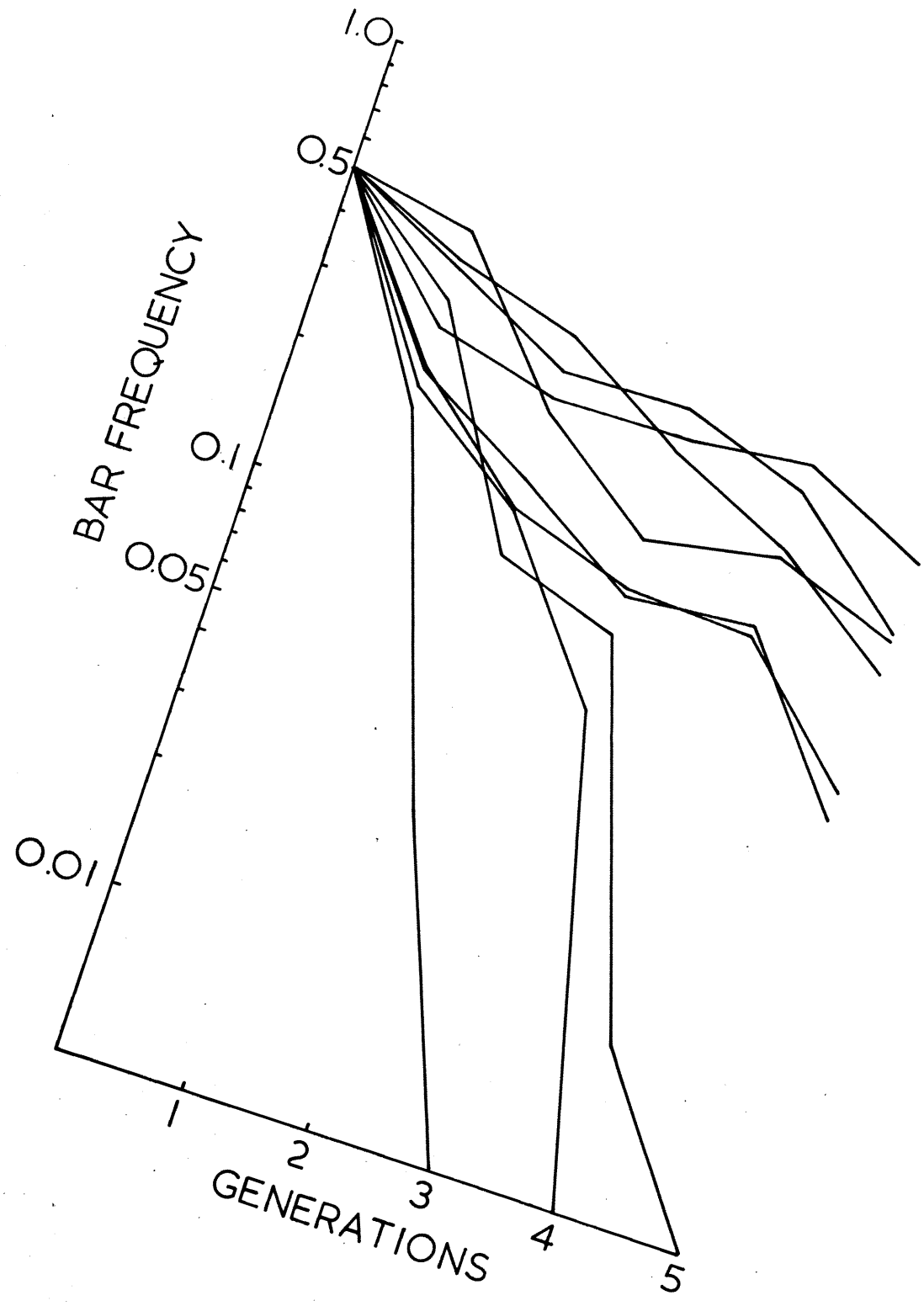

1.0
0.5
0.1
0.05
0.01
BAR FREQUENCY
1
2
3
4
5
GENERATIONS

FIGURE 20

Extinction rate curves for Bar in individual replicates of Stock Bar/E1 wild type mixtures in Experiment 3. (Log. scale).

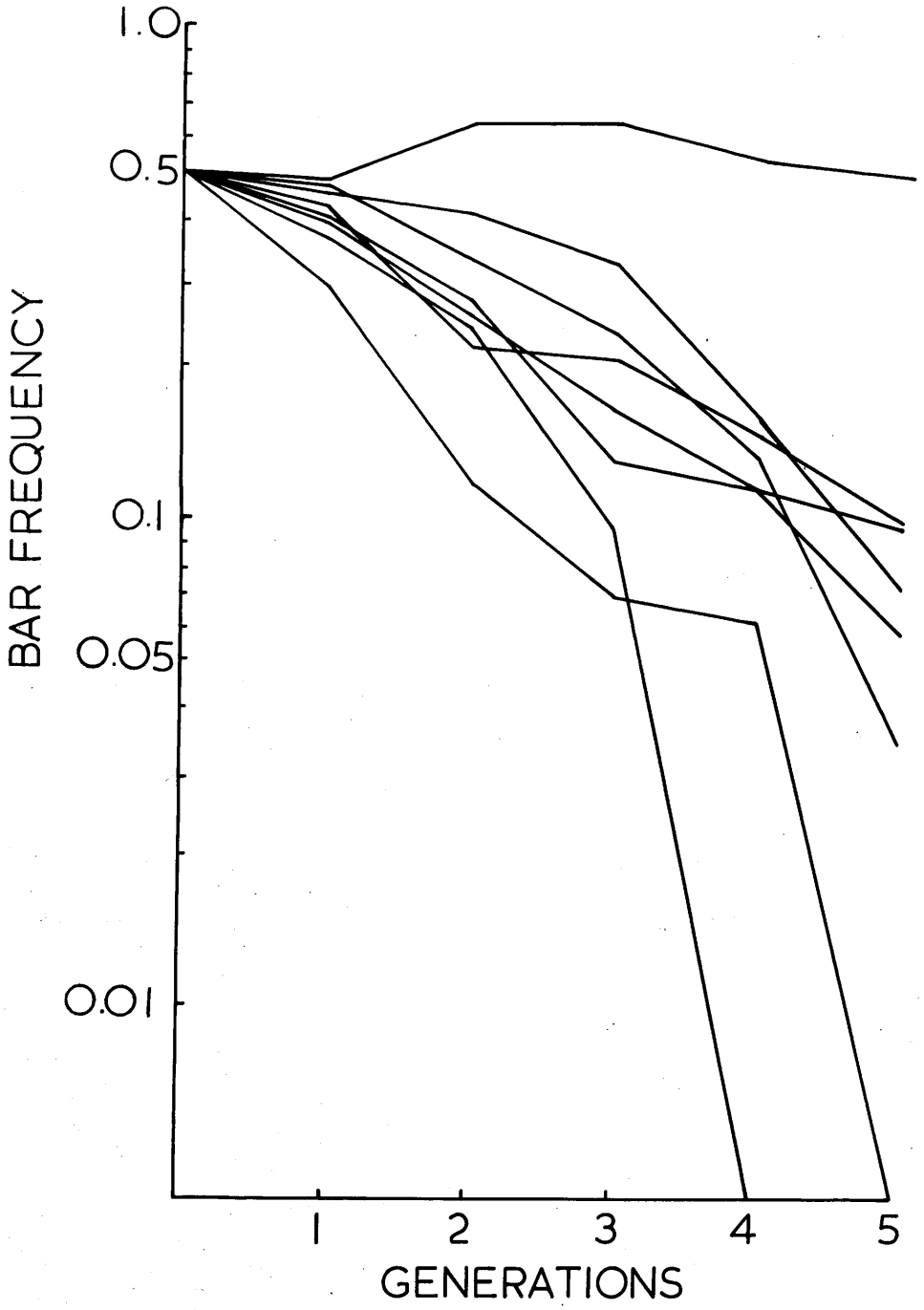
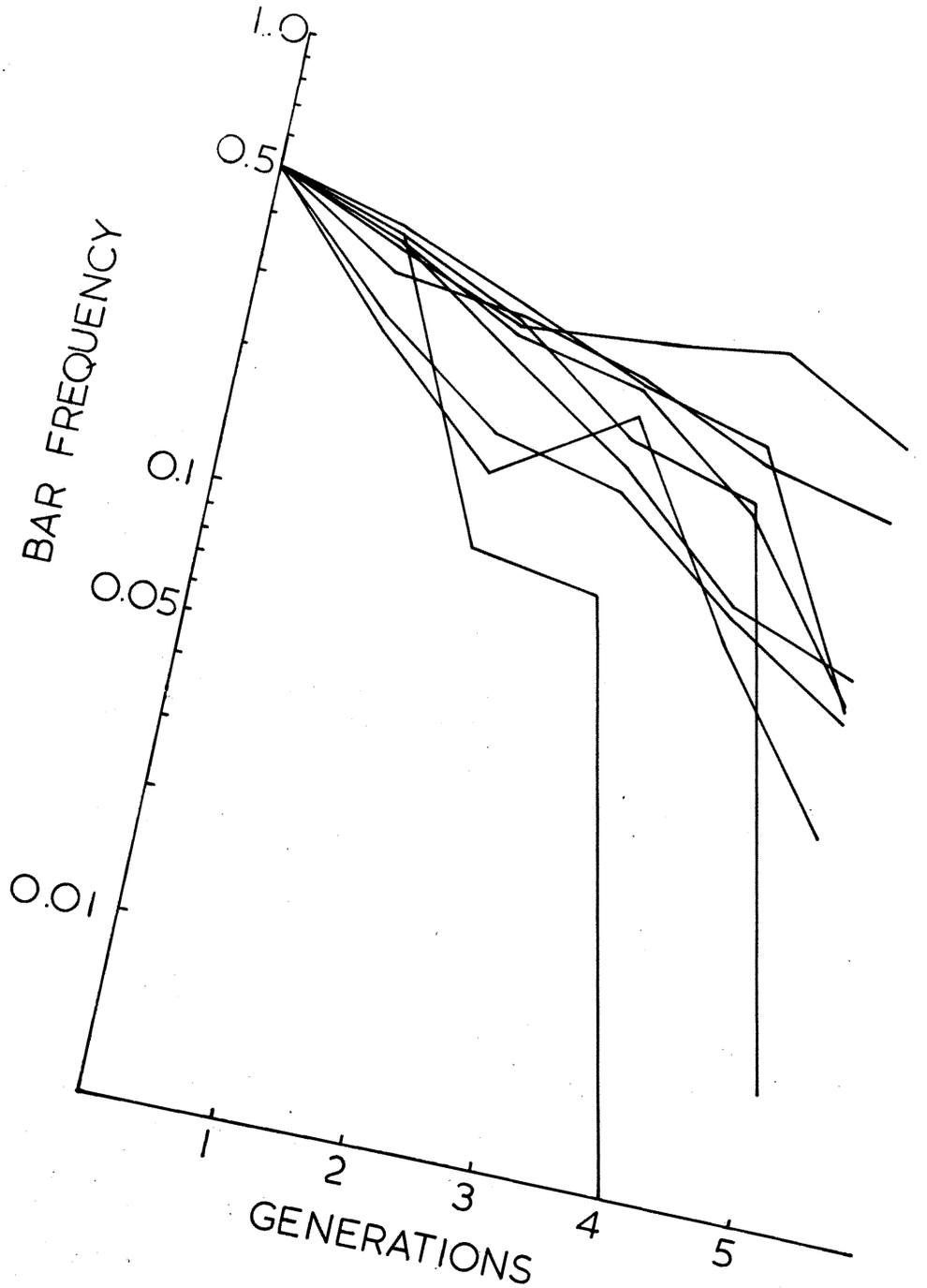

1.0
0.5
0.1
0.05
0.01
BAR FREQUENCY
1
2
3
4
5
GENERATIONS

FIGURE 21

Extinction rate curves for Bar in individual replicates of E1 Bar/E1 wild type mixtures in Experiment 3. (Log scale).


1.0
0.5
0.1
0.05
0.01
BAR FREQUENCY
1
2
3
4
5
GENERATIONS

(vi) Replacement series tests.

Analysis and presentation.

In the replacement series tests, the graphs show the yields of both components, the total yields at each frequency of mixture and the yields of the two pure cultures. The total yields of the mixed cultures give an idea of the total productivity, itself a parameter of resource utilisation, compared with the pure cultures. The resource utilisation of pure and mixed cultures can be compared statistically. Expected total yields can be calculated on the assumption that each parent produces, on average, as many offspring in mixed as in pure cultures. A χ^2 analysis can then be applied to these predicted and observed totals. Individual replicates within each test can be pooled by summing individual χ values, taking into account the direction of these values. A value of $\frac{(\Sigma\chi)^2}{n}$ can then be calculated, whose level of significance can be found in χ^2 tables using one degree of freedom (n being the number of values summed). These $\frac{(\Sigma\chi)^2}{n}$ values and their level and direction of significance can then be presented in tables. In a similar way, results from individual tests for each replicate can be pooled.

The relative yields of the two components in the three ratios of mixtures are also of importance, as these may give an indication of frequency dependent selection resulting possibly from ecological differences between the components. Expected values can also be calculated here, using yields at all three frequencies of mixture to determine the Cross Product Ratio, as used by Allen (1972).

$$CPR = \frac{(\text{Bar at } 3:1) + (2 \times \text{Bar at } 1:1) + (3 \times \text{Bar at } 1:3)}{(3 \times \text{wild at } 3:1) + (2 \times \text{wild at } 1:1) + (\text{wild at } 1:3)}$$

(Note that all ratios refer to Bar first).

These Cross Product Ratios can then be used to determine the proportional yield of each component at each frequency. Unlike the analysis used by de Wit (1960), pure culture yields are not used since these do not take into account any of the facets of competition and are therefore not considered relevant to the prediction of the yield of each genotype in mixed cultures. They could be used only in the simplest hypothesis, that relative yield in pure cultures is the same as relative yield in equal mixtures. This is not true in these experiments, as can be seen later. χ^2 values can again be determined for each replicate in each test and, by pooling the individual χ values from individual replicates or tests, a value for $\frac{(\Sigma\chi)^2}{n}$ can be calculated. These values are presented on tables along with the numbers of times (the number of replicates or number of tests) where greater or lesser yields of Bar are obtained than expected.

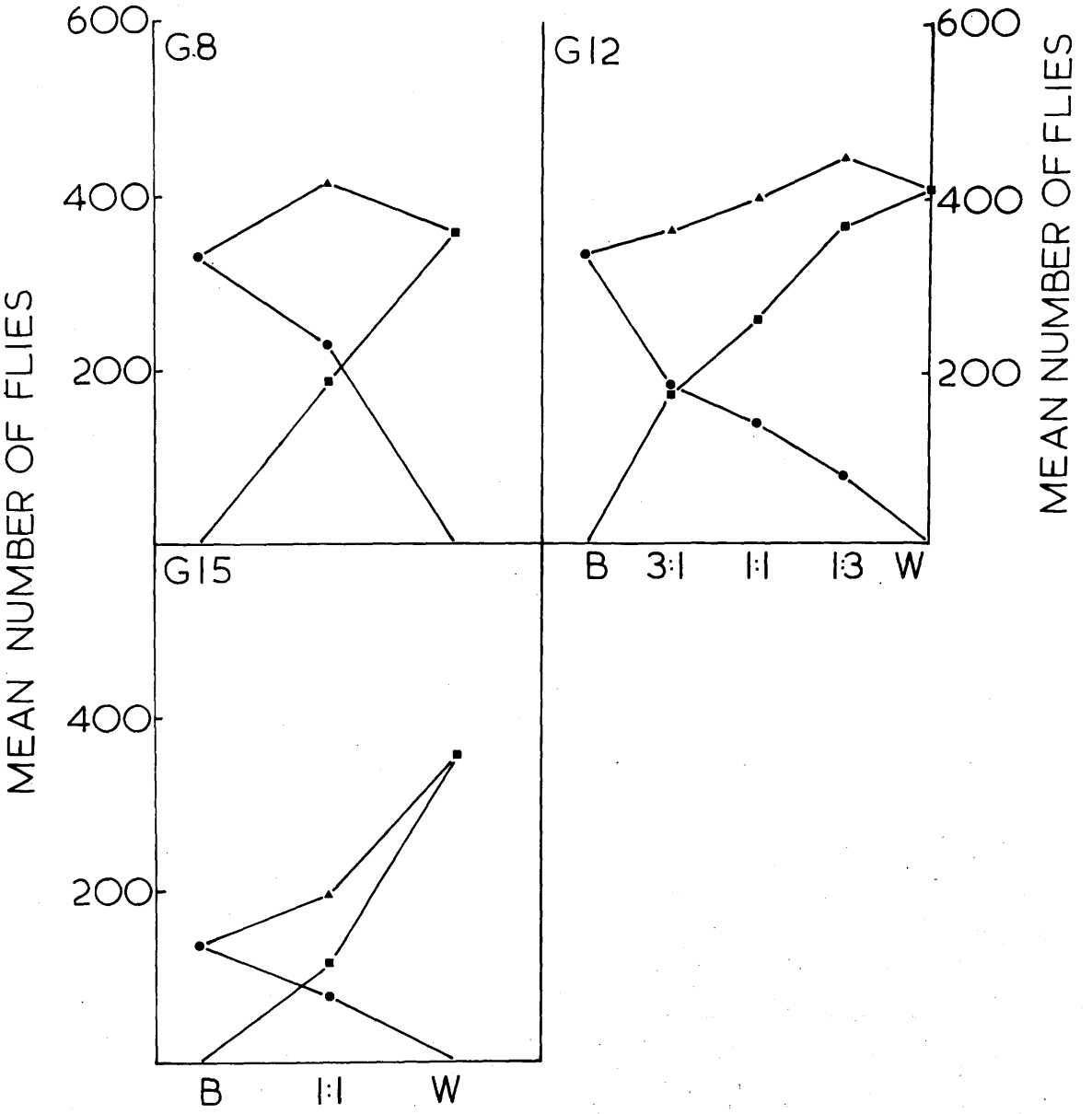
The relative performance of the two genotypes at different frequencies can also be displayed by means of ratio diagrams and the slope of the regression line was calculated. This slope was compared with unity, at which value no frequency dependent effects are present.

Results

The replacement series graphs of the test carried out on E1 in generations 8, 12 and 15 (after 2, 6 and 9 generations of selection as E1 was restarted after 6 generations) of Experiment 1 are shown in Figure 22. Only a 1:1 mixture was

FIGURE 22

Replacement series graphs of competition tests carried out on E1 in generations 8, 12 and 15 of Experiment 1.


MEAN NUMBER OF FLIES
600
400
200
G8
G12
G15
B
3:1
1:1
1:3
W
MEAN NUMBER OF FLIES

used in the first and the last of these tests as it was thought at the time that the total yields obtained from mixtures would be more valuable than the relative yields of the two components at the intermediate frequencies.

TABLE 14

(a) Values of $\frac{(\Sigma X)^2}{n}$, direction (d) and levels of significance (p), comparing observed and expected total yields (calculated from the pure culture yields) at 1:1 ratio of replacement series tests in generations 8 and 15 of Experiment 1, and at 3:1, 1:1 and 1:3 ratios of tests in generation 12. All replicates are pooled at each generation.

(b) Values of $\frac{(\Sigma X)^2}{n}$, direction and levels of significance for individual replicates, pooling the three tests at the 1:1 ratio.

(a)

	Input Ratio								
	3:1			1:1			1:3		
	$\frac{(\Sigma X)^2}{n}$	d	p	$\frac{(\Sigma X)^2}{n}$	d	p	$\frac{(\Sigma X)^2}{n}$	d	p
Gen. 8				64.24	+	.1%			
Gen.12	5.55	+	5%	12.22	+	.1%	36.18	+	.1%
Gen.15				49.28	-	.1%			

(b)

E11			E12			E13			E14		
$\frac{(\Sigma X)^2}{n}$	d	p	$\frac{(\Sigma X)^2}{n}$	d	p	$\frac{(\Sigma X)^2}{n}$	d	p	$\frac{(\Sigma X)^2}{n}$	d	p
20.49	-	.1%	16.33	+	.1%	7.05	+	1%	9.05	+	1%

Table 14 shows that total yields of the mixtures at 1:1 in the test in generation 8 and total yields at all frequencies in generation 12 were significantly higher than expected from the pure culture yields. The 1:1 mixture in generation 15 gave

significantly lower than expected yields. When the three tests were pooled (1:1 ratio only) (Table 14b) and individual replicates were tested, E11 gave a significantly lower total yield than expected while the other three replicates gave significantly higher yields than expected.

TABLE 15

Values of $\frac{(\Sigma X)^2}{n}$, direction (d), and levels of significance (p), comparing observed and expected numbers of Bar and wild type from the replacement series test carried out after 12 generations of Experiment 1. (+ signifies more Bar, - less Bar than expected). Also the numbers of individual replicates showing significantly less than (sig<E), more than (sig>E) or not different from (NSD) expected values of Bar.

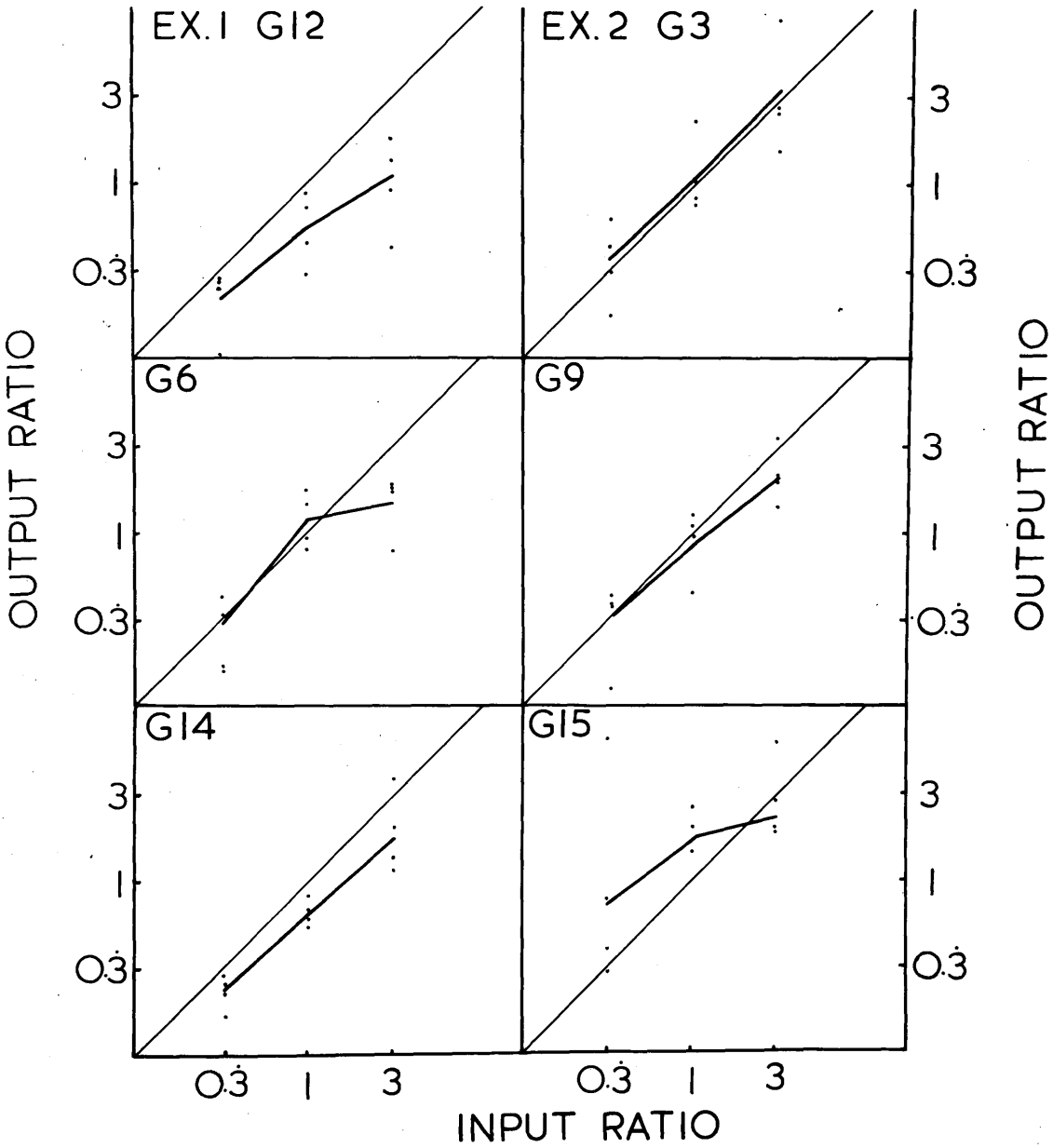
Input Ratio	$\frac{(\Sigma X)^2}{n}$	d	p	Number of replicates		
				Sig <E	Sig >E	NSD
3:1	48.44	-	.1%	2	0	2
1:1	3.05	+	NS	1	2	1
1:3	14.44	+	.1%	0	2	2

Table 15 shows how at generation 12, when Bar is the majority component (i.e. 3:1 ratio) it gave significantly lower yields than expected, yet where it was the minority component (i.e. 1:3 ratio) it gave higher yields than expected. Two replicates were significant in each case. The ratio diagram (Figure 23 (i)) shows a slope of greater than one, and this too is significant.

The replacement series graphs obtained for the tests carried out on E1 (in generations 3, 6, 9, 14 and 15) in

FIGURE 23

Ratio diagrams of competition tests carried out on E1 in generation 12 of Experiment 1, and generations 3, 6, 9, 14 and 15 of Experiment 2. Thin line represents the equilibrium line.


EX.1 G12
EX.2 G3
G6
G9
G14
G15
3
1
0.3
OUTPUT RATIO
INPUT RATIO

Experiment 2 are shown in Figure 24. Table 16 shows that total yields in the 3:1 and 1:3 mixtures were generally lower than those predicted from pure culture yields although this is only significantly so in generation 15 of the 1:3 mixture. The 1:1 mixtures gave higher than expected yields in generations 3 and 9 but lower yields in generation 15.

It can be seen from Table 17 that after 3 generations of selection, the numbers of Bar and wild type did not differ significantly from expected at any of the ratios. Individual replicates, however, were significantly different from expected. In generation 6, when Bar was the majority component in the test ratio, it yielded lower than expected, and when at 1:1 it yielded higher than expected while as the minority component it yielded no different from expected: two replicates were higher and two were lower. Generations 9 and 15 showed that Bar as the majority component yielded lower than expected though it was better than expected as the minority component. In the tests after 14 generations none of the frequencies showed Bar giving different yields from expected.

The overall result, summing χ values from all replicates in all tests, shows that Bar was a poor performer when it was more common and a better performer when it was rare. This is supported by the summary of the individual results, where Bar was poorer on 10 occasions and better on 4 occasions at 3:1, poorer 3 times and better 7 times at 1:1, and poorer 4 times and better 10 times at 1:3.

The summary of the analysis for each individual replicate (pooling tests) is shown on Table 18. Replicates E12, E13 and E14 showed strong frequency dependent selection while E11 did not.

FIGURE 24

Replacement series graphs of competition tests on E1 in generations 3, 6, 9, 14 and 15 of Experiment 2.

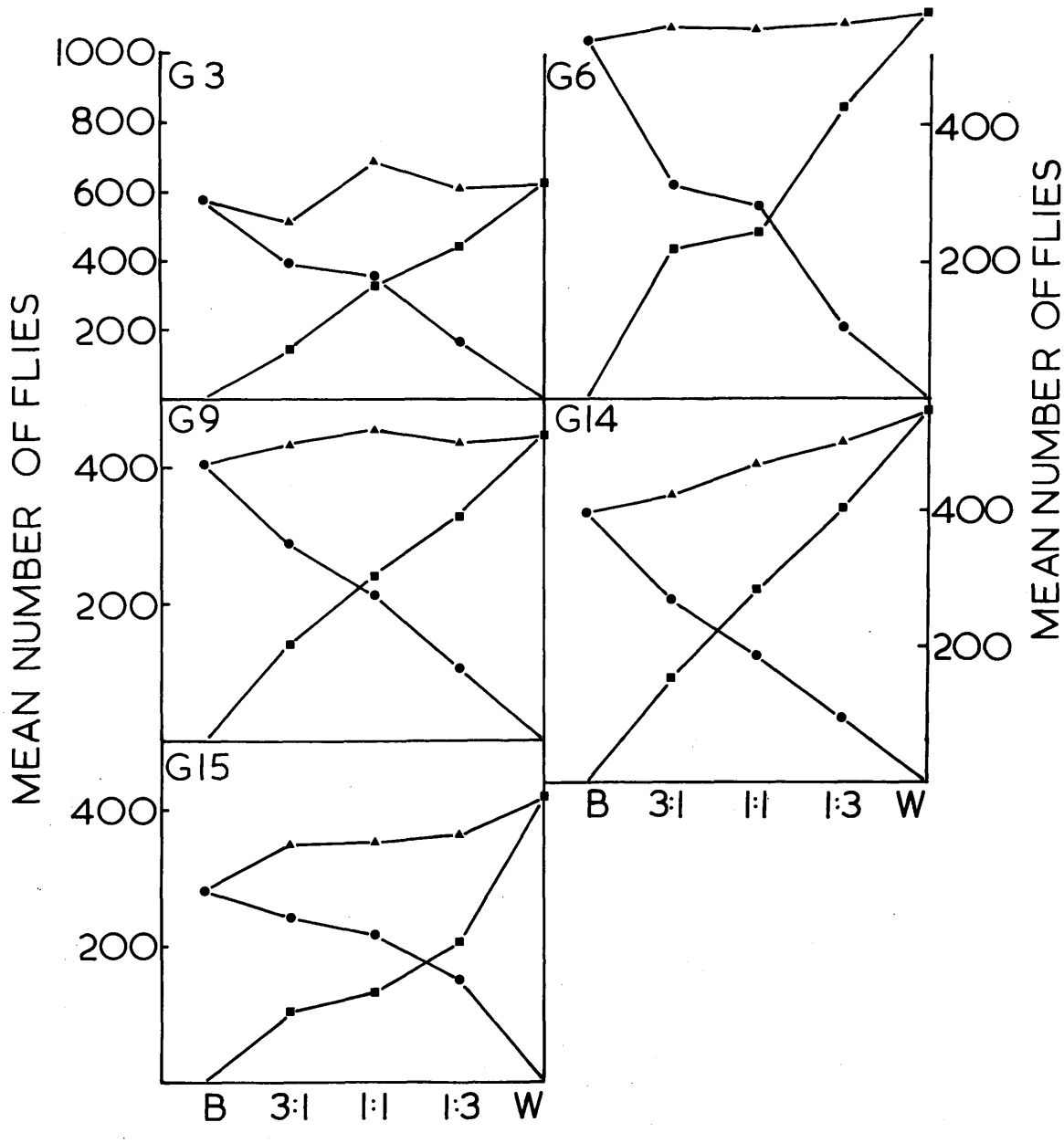

MEAN NUMBER OF FLIES
1000
800
600
400
200
G 3
G6
G9
G14
G15
400
200
B
3:1
1:1
1:3
W

TABLE 16

(a) Values of $\frac{(\Sigma X)^2}{n}$, direction (d) and levels of significance (p), comparing observed and expected total yields at the three ratios in tests on E1 during Experiment 2.

(b) Similar values for individual replicates for each ratio over all five tests.

	Input Ratio								
	3:1			1:1			1:3		
	$\frac{(\Sigma X)^2}{n}$	d	p	$\frac{(\Sigma X)^2}{n}$	d	p	$\frac{(\Sigma X)^2}{n}$	d	p
Gen. 3	30.30	-	.1%	71.53	+	.1%	0.54	-	NS
Gen. 6	0.88	-	NS	0.42	-	NS	0.26	+	NS
Gen. 9	0.00	-	NS	7.31	+	1%	2.42	+	NS
Gen. 14	1.54	-	NS	0.03	-	NS	0.99	-	NS
Gen. 15	0.19	-	NS	97.78	-	.1%	62.81	-	.1%
All Gens.	13.31	-	.1%	0.04	+	NS	11.51	-	.1%

	Input Ratio								
	3:1			1:1			1:3		
Replicate	$\frac{(\Sigma X)^2}{n}$	d	p	$\frac{(\Sigma X)^2}{n}$	d	p	$\frac{(\Sigma X)^2}{n}$	d	p
E11	0.76	+	NS	16.70	-	.1%	39.08	-	.1%
E12	3.44	+	NS	2.38	-	NS	11.28	-	.1%
E13	10.16	-	.1%	16.34	+	.1%	0.28	-	NS
E14	15.52	-	.1%	4.00	+	5%	11.23	+	.1%

TABLE 17

Values of $\frac{(\Sigma X)^2}{n}$, direction (d), and levels of significance (p), comparing observed and expected numbers of Bar and wild type from the tests on E1 in Experiment 2. (+ signifies more Bar, - less Bar than expected). Also the numbers of individual replicates showing significantly less than (sig<E), more than (sig>E) or not different from (NSD) expected values of Bar.

	Input Ratio	$\frac{(\Sigma X)^2}{n}$	d	p	Number of replicates sig <E	sig >E	NSD
	3:1	2.31	-	NS	1	2	1
Gen. 3	1:1	0.26	+	NS	1	2	1
	1:3	0.48	+	NS	2	2	0
	3:1	113.53	-	.1%	3	0	1
Gen. 6	1:1	90.82	+	.1%	0	3	1
	1:3	0.00	+	NS	2	2	0
	3:1	12.04	-	.1%	2	1	1
Gen. 9	1:1	1.90	+	NS	0	1	3
	1:3	4.20	+	5%	0	2	2
	3:1	2.96	-	NS	2	1	1
Gen. 14	1:1	0.03	+	NS	0	0	4
	1:3	2.05	+	NS	0	2	2
	3:1	53.29	-	.1%	2	0	2
Gen. 15	1:1	7.51	-	5%	2	1	1
	1:3	78.94	+	.1%	0	2	2
	3:1	121.67	-	.1%	10	4	6
All Gens.	1:1	15.70	+	.1%	3	7	10
	1:3	34.45	+	.1%	4	10	6

TABLE 18

Values of $\frac{(\Sigma X)^2}{n}$, direction (d) and levels of significance (p) of observed and expected numbers of Bar and wild type, for individual replicates, of E1 in Experiment 2 for each ratio over all five tests. (+ signifies more Bar, - less than expected). Also the numbers of tests where significantly less than (sig<E), more than (sig>E) or not different (NSD) from expected values of Bar.

					Number of generations		
	Input Ratio	$\frac{(\Sigma X)^2}{n}$	d	p	Sig <E	Sig >E	NSD
E11	3:1	3.73	-	NS	2	2	1
	1:1	4.01	+	5%	0	2	3
	1:3	0.22	-	NS	1	2	2
E12	3:1	62.16	-	.1%	4	0	1
	1:1	2.87	+	NS	1	2	2
	1:3	19.52	+	.1%	1	4	0
E13	3:1	13.25	-	.1%	2	1	2
	1:1	0.47	+	NS	1	2	2
	1:3	7.27	+	1%	1	2	2
E14	3:1	74.03	-	.1%	2	1	2
	1:1	12.51	+	.1%	1	1	3
	1:3	25.90	+	.1%	1	2	2

Thus, a degree of frequency dependent selection was operating, at least after 6 generations of selection, in the E1 lines. This does not necessarily mean that the weaker competitor, Bar, would have been maintained in the mixtures at the lower ratio. It merely implies that if this differential selection was acting, there should theoretically have been a frequency at which Bar could reach stability. This can be seen better from the ratio diagrams, where frequency dependent selection could be inferred if the regression line had a slope of greater than one, but where stability is reached only when the equilibrium line is crossed. The ratio diagrams for the E1 tests in Experiment 2 are shown in Figure 23. The slopes of the regression lines are all greater than one, although none significantly so. In generations 6, 9 and 15, the equilibrium line is crossed.

Replacement series tests were carried out on the E6 lines after 12 and 15 generations (4 and 7 generations after the E6 lines were initiated from the E2 lines). The replacement series graphs are shown on Figure 25 i) and ii). Analysis of the observed and expected total yields (Table 19) shows that both unequal mixtures yielded higher than expected in generation 12, but that the 3:1 mixtures yielded lower than expected in generation 15. The 1:1 mixture did not yield significantly different from expected.

A summary of the χ^2 analysis on the frequencies of the two components is shown on Table 20. Both tests showed a frequency dependent effect with Bar performing worse than expected when it was common and better than expected when it was rare. Considering the two tests together (Table 20), the

FIGURE 25

Replacement series graphs of competition tests on

i) E6 in generation 12 and

ii) E6 in generation 15

iii) separated E6 in generation 15 and

iv) Stocks in generation 15

All in Experiment 2.

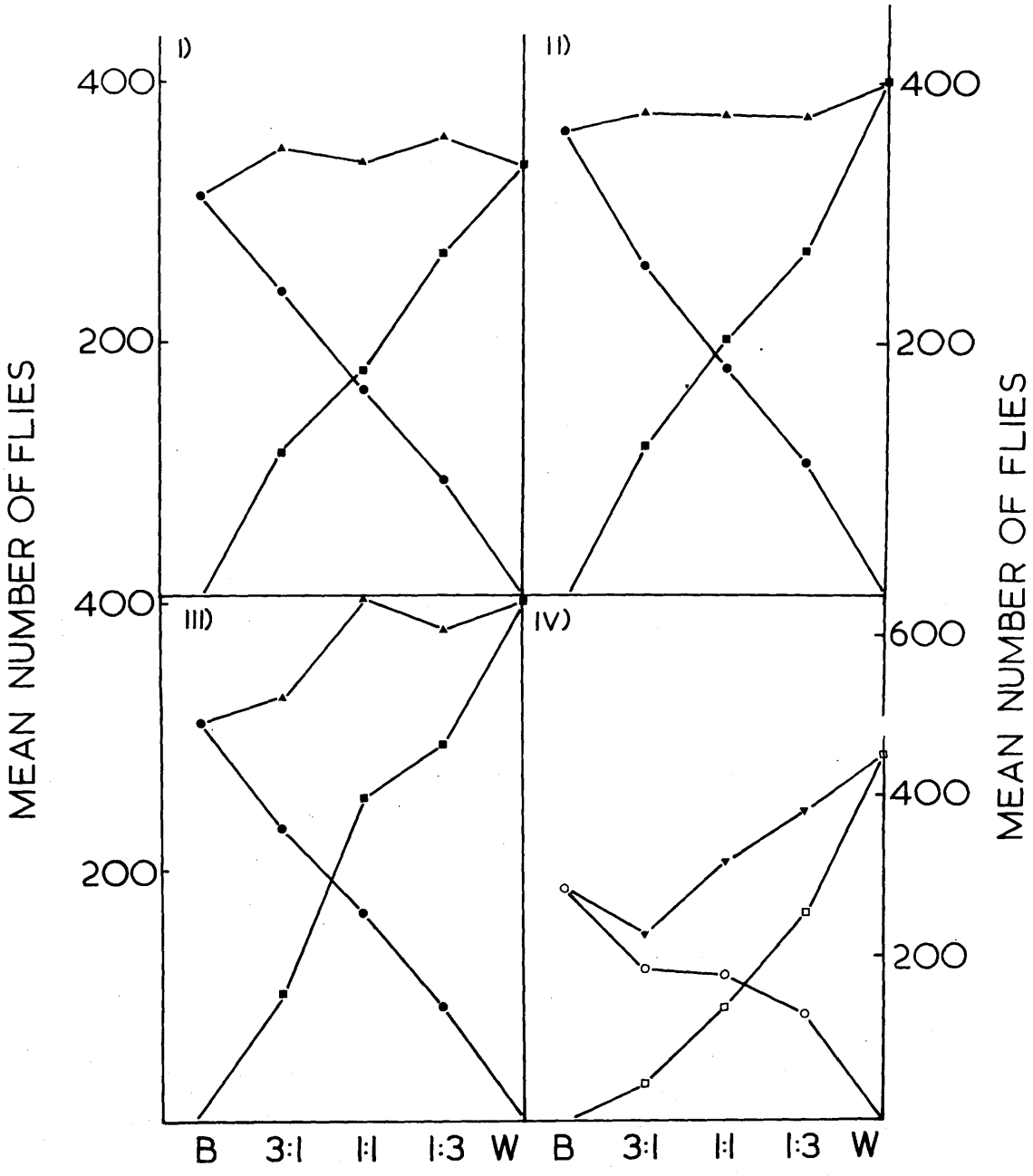

I)
II)
III)
IV)
400
200
400
200
400
200
600
400
200
MEAN NUMBER OF FLIES
MEAN NUMBER OF FLIES
B
3:1
1:1
1:3
W
B
3:1
1:1
1:3
W

FIGURE 26

Ratio diagrams of competition tests on

i) E6 in generation 12

ii) E6 in generation 15

iii) separated E6 in generation 15 and

iv) Stocks in generation 15.

All in Experiment 2.

Thin line represents the equilibrium line.

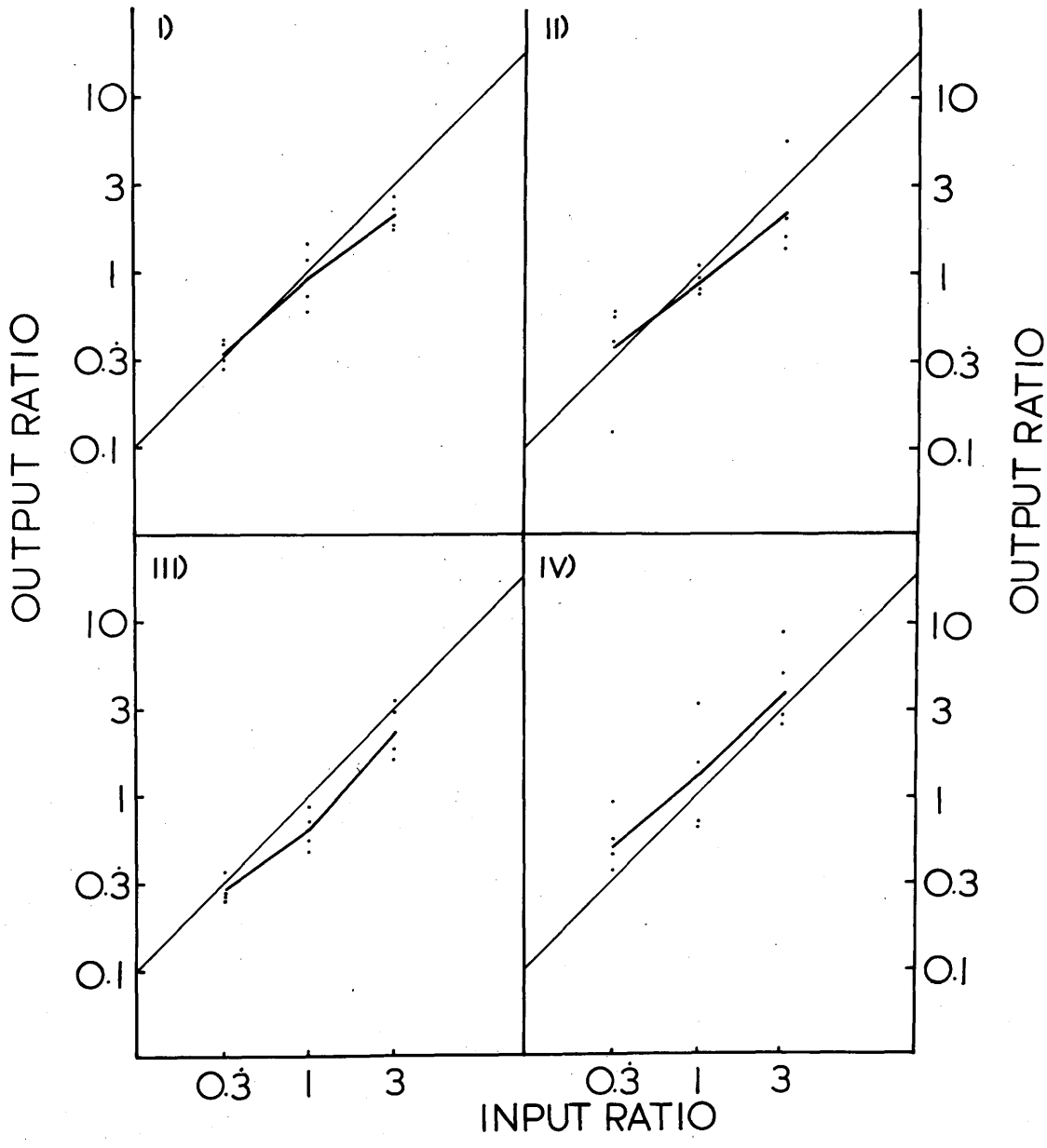

I)
II)
III)
IV)
10
3
1
0.3
0.1
OUTPUT RATIO
OUTPUT RATIO
0.3
1
3
INPUT RATIO

TABLE 19

(a) Values of $\frac{(\Sigma X)^2}{n}$, direction (d) and levels of significance (p), comparing observed and expected total yields at the three ratios in tests on E6, separated E6 and stocks during Experiment 2.

(b) Similar values for individual replicates of E6 for each ratio over the two generations.

(a)

		Input Ratio 3:1			1:1			1:3		
		$\frac{(\Sigma X)^2}{n}$	d	p	$\frac{(\Sigma X)^2}{n}$	d	p	$\frac{(\Sigma X)^2}{n}$	d	p
E6	Gen.12	8.76	+	1%	2.56	+	NS	14.06	+	.1%
	Gen.15	5.13	-	5%	0.71	-	NS	0.09	+	NS
	All Gens.	0.24	+	NS	0.29	+	NS	8.21	+	1%
Sep.E6	Gen.15	0.24	-	NS	32.55	+	.1%	0.76	-	NS
Stocks	Gen.15	8.24	-	1%	32.26	-	.1%	115.94	-	.1%

(b)

	Input Ratio 3:1			1:1			1:3		
	$\frac{(\Sigma X)^2}{n}$	d	p	$\frac{(\Sigma X)^2}{n}$	d	p	$\frac{(\Sigma X)^2}{n}$	d	p
E61	2.88	-	NS	1.04	+	NS	8.16	+	1%
E62	3.10	+	NS	2.16	+	NS	0.61	+	NS
E63	23.87	-	.1%	9.19	-	1%	2.98	-	NS
E64	33.70	+	.1%	2.60	+	NS	14.53	+	.1%

individual replicates E62, E63 and E64 showed a frequency dependent pattern while in E61 Bar performed poorly at the 1:3 ratio. Both ratio diagrams (Figure 26, i) and ii) show slopes of greater than one, though not significantly so, and both cross the equilibrium line.

The replacement series test results using the E6 lines where Bar and wild type had been separated for two generations are shown in Figure 25 iii). The total yields were higher than expected at the 1:1 ratio but not at the other two ratios (Table 19). The yield of Bar was not different from expected when it was the majority component (Table 20). It was lower than expected in the 1:1 mixture and higher when it was the majority component, although only one of the replicates was significant in the latter. The ratio diagram (Figure 26 iii) has a slope very close to one and does not cross the equilibrium line.

The replacement series graphs of the test with stock Bar and stock wild type carried out after 15 generations are shown on Figure 25 iv). Very low total yields are shown at all ratios (Table 19). Bar, as a majority component and in the equal mixture, was slightly but not significantly worse than expected (Table 20). As the minority component, it was better in two replicates, worse in one and not significantly different overall. Bar generally yielded higher than wild type accounting for the ratio diagram regression line being above the equilibrium line (Figure 26 iv). It does not cross this line and the slope is not significantly different from one.

TABLE 20

(a) Values of $\frac{(\Sigma\chi)^2}{n}$, direction (d) and levels of significance (p), comparing observed and expected numbers of Bar and wild type from the tests on E6, separated E6 and stocks in Experiment 2. (+ signifies more Bar, - less Bar than expected). Also the numbers of individual replicates showing significantly less than (sig$<$E), more than (sig$>$E) or not different from (NSD) expected values of Bar.

(b) Values of $\frac{(\Sigma\chi)^2}{n}$, direction and levels of significance for individual replicates of E6, pooling both tests.

(a)

	Input Ratio	$\frac{(\Sigma X)^2}{n}$	d	p	Number of Replicates: Sig <E	Sig >E	NSD
E6	3:1	13.47	-	.1%	2	0	2
Gen.12	1:1	0.87	+	NS	1	2	1
	1:3	6.38	+	1%	0	1	3
	3:1	20.12	-	.1%	3	1	0
Gen.15	1:1	0.07	-	NS	0	0	4
	1:3	20.25	+	.1%	1	3	0
	3:1	33.25	-	.1%	5	1	2
All Gens.	1:1	0.23	+	NS	1	2	5
	1:3	24.68	+	.1%	1	4	3
Sep.E6	3:1	0.65	+	NS	0	1	3
Gen.15	1:1	12.60	-	.1%	2	0	2
	1:3	8.88	+	1%	0	1	3
Stocks	3:1	0.03	-	NS	0	1	3
Gen.15	1:1	2.53	-	NS	2	1	1
	1:3	2.77	+	NS	1	2	1

(b)

	Input Ratio	$\frac{(\Sigma X)^2}{n}$	d	p	Number of generations: Sig <E	Sig >E	NSD
	3:1	0.59	+	NS	1	1	0
E61	1:1	4.09	+	5%	0	1	1
	1:3	12.01	-	.1%	1	0	1
	3:1	6.51	-	1%	1	0	1
E62	1:1	1.24	-	NS	1	0	1
	1:3	40.77	+	.1%	0	2	0
	3:1	26.57	-	.1%	2	0	0
E63	1:1	3.92	+	5%	0	1	1
	1:3	10.91	+	.1%	0	1	1
	3:1	6.13	-	1%	1	0	1
E64	1:1	2.18	-	NS	0	0	2
	1:3	13.78	+	.1%	0	1	1

The replacement series graphs and ratio diagrams of the tests on the E1 populations of Experiment 3 are shown on Figure 27 and 28, and the analysis in Tables 21, 22 and 23. Table 21 shows that, at the 3:1 ratio, total yields were higher than expected in generations 3 and 5, but lower in generation 1 and 7. At 1:1, yields were higher throughout but only significantly so in generations 3 and 5. At 1:3, yields were significantly lower than expected except in generation 5. There was very great variation in performance in the three mixtures between different replicates, with few consistently good or poor replicates. However, in the equal mixtures E13, E15, E17 and E18 were especially good performers and E19 and E10 poor ones. Table 22 shows the summary of the χ^2 analysis of Bar at different frequencies.

In the tests after 1 generation, Bar was better at the 1:1 ratio and worse as minority component, the ratio diagram being less than one (Figure 28). After 3 and 5 generations, Bar as majority was worse than expected and as minority was better than expected, both ratio diagrams having slopes of less than one, though not significantly so and as wild type was a far better performer than Bar, the equilibrium line was not crossed. After 7 generations, Bar yielded worse than expected at the 1:1 ratio and better as the minority component, the slope of the ratio diagram being close to one. Summing the values for all replicates in all tests, Bar was poorer when common and better when rare. At 3:1, 11 individual tests were worse than expected and 6 better; at 1:1, 7 were worse and 7 better, while at 1:3, 5 were worse and 11 were better, thus supporting the evidence that a degree of frequency dependent

FIGURE 27

Replacement series graphs of competition tests on E1 in generations 1, 3, 5 and 7 of Experiment 3.

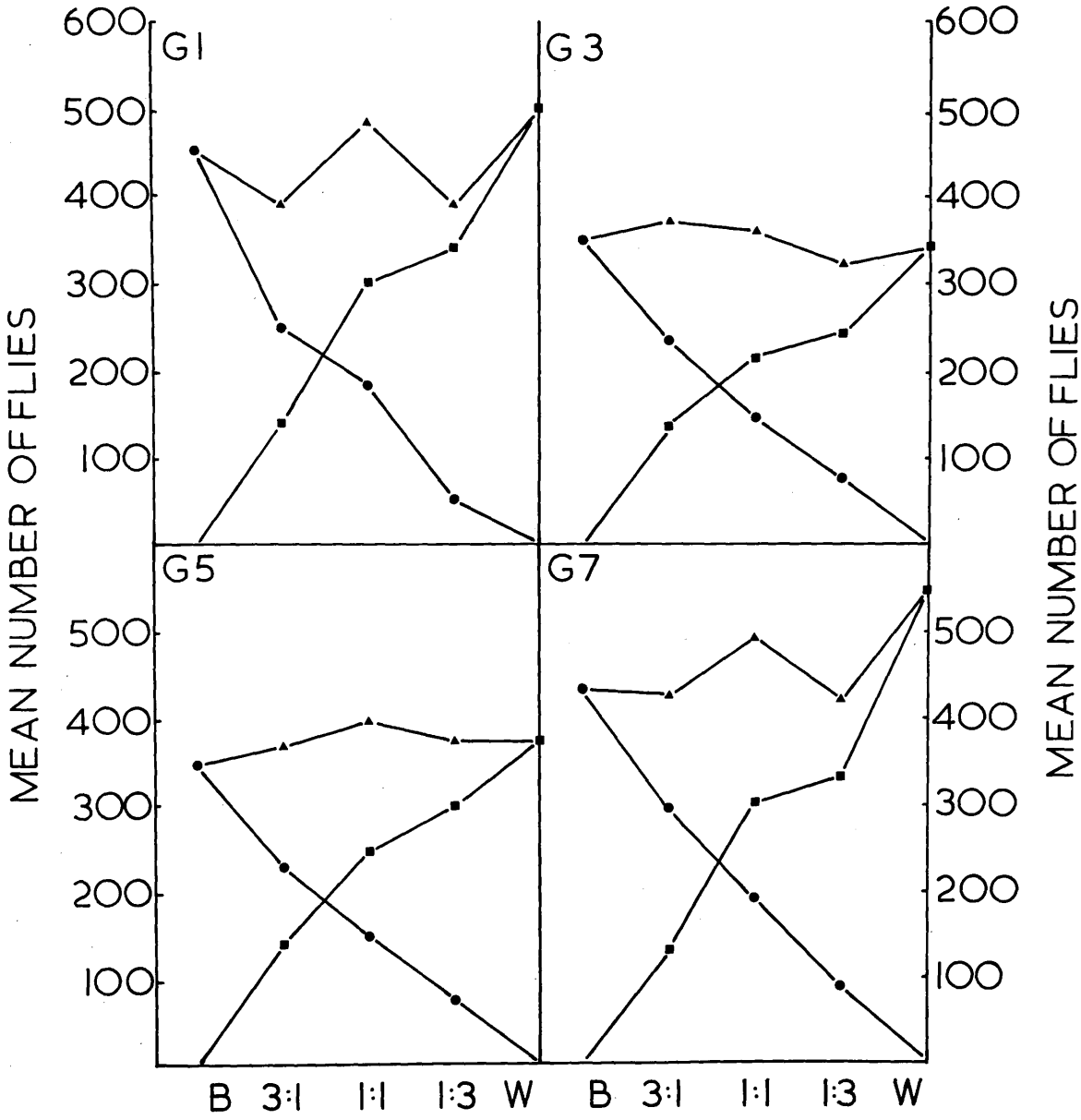

G1
G3
G5
G7
600
500
400
300
200
100
MEAN NUMBER OF FLIES
B
3:1
1:1
1:3
W

FIGURE 28

Ratio diagrams of competition tests on E1 in generations 1, 3, 5 and 7 of Experiment 3.

Thin line represents the equilibrium line.

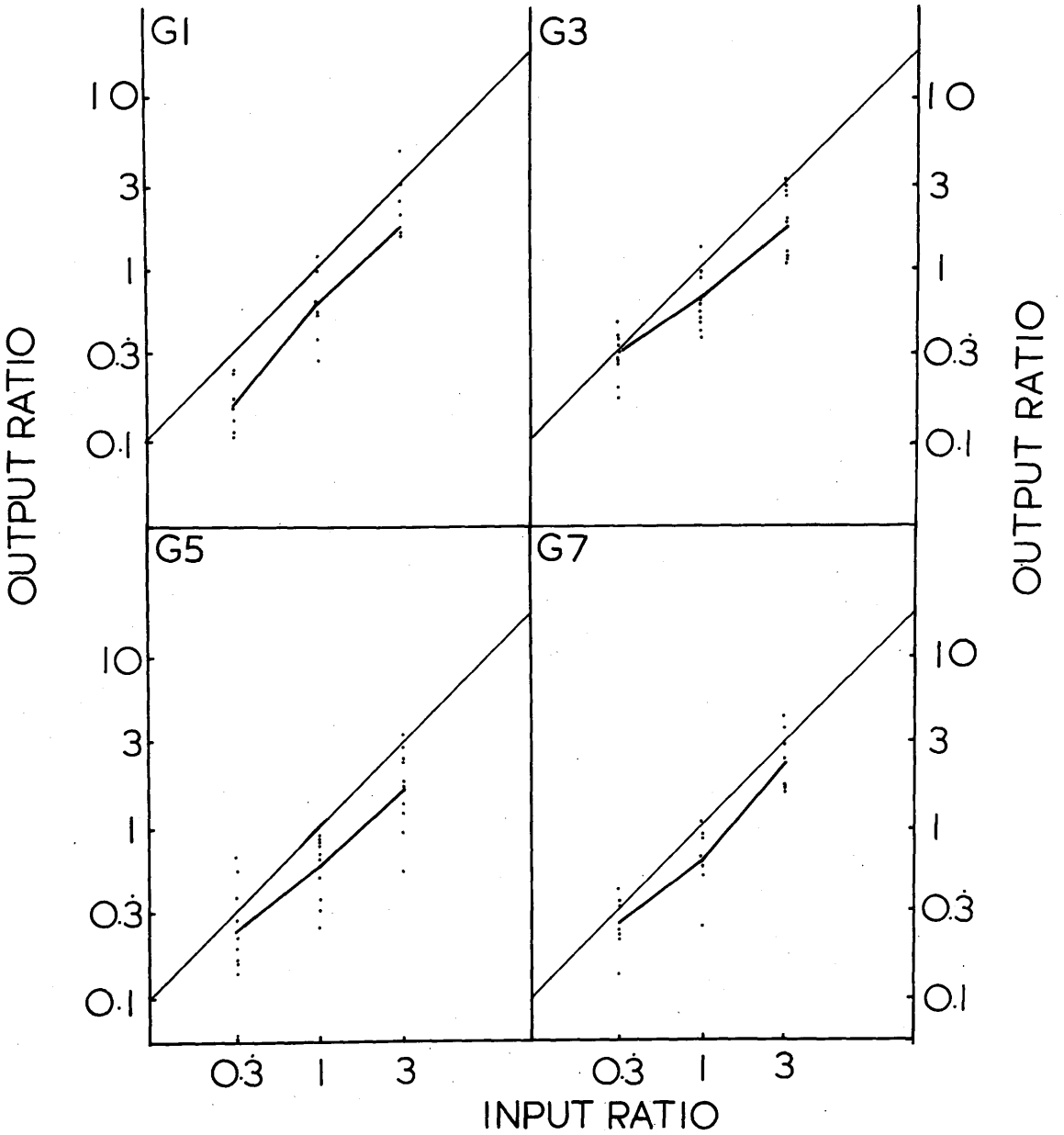

G1
G3
G5
G7
10
3
1
0.3
0.1
OUTPUT RATIO
INPUT RATIO
0.3
1
3

TABLE 21

(a) Values of $\frac{(\Sigma X)^2}{n}$, direction (d) and levels of significance (p), comparing observed and expected total yields (calculated from the pure culture yields) at all three ratios of the tests on E1 in Experiment 3. All replicates being pooled in each generation.

(b) Similar values for individual replicates, pooling tests.

(a)

	Input Ratio								
	3:1			1:1			1:3		
	$\frac{(\Sigma X)^2}{n}$	d	p	$\frac{(\Sigma X)^2}{n}$	d	p	$\frac{(\Sigma X)^2}{n}$	d	p
Gen.1	55.56	-	.1%	1.88	+	NS	117.44	-	.1%
Gen.3	16.32	+	.1%	6.23	+	5%	9.95	-	1%
Gen.5	9.97	+	1%	23.26	+	.1%	0.00	-	NS
Gen.7	19.80	-	.1%	0.57	+	NS	123.81	-	.1%
All Gens.	2.24	-	NS	24.34	+	.1%	142.90	-	.1%

(b)

	Input Ratio								
	3:1			1:1			1:3		
	$\frac{(\Sigma X)^2}{n}$	d	p	$\frac{(\Sigma X)^2}{n}$	d	p	$\frac{(\Sigma X)^2}{n}$	d	p
E11	0.97	+	NS	1.88	-	NS	4.50	-	5%
E12	11.38	-	.1%	0.02	+	NS	37.31	-	.1%
E13	1.80	-	NS	15.30	+	.1%	33.90	-	.1%
E14	5.48	+	5%	0.22	+	NS	62.17	-	.1%
E15	0.88	+	NS	35.22	+	.1%	11.08	+	.1%
E16	15.65	-	.1%	0.84	+	NS	81.88	-	.1%
E17	3.78	+	NS	26.69	+	.1%	1.30	-	NS
E18	15.36	+	.1%	37.45	+	.1%	2.38	-	NS
E19	19.73	+	.1%	14.97	-	.1%	2.51	-	NS
E10	1.15	-	NS	4.43	-	5%	5.02	-	5%
E116	2.20	-	NS	4.29	-	5%	1.14	-	NS
E1789	108.65	-	.1%	0.22	+	NS	21.25	-	.1%
E112	0.24	+	NS	0.82	-	NS	13.04	-	.1%
E190	23.31	-	.1%	1.90	+	NS	0.00	-	NS

TABLE 22

Values of $\frac{(\Sigma X)^2}{n}$, direction (d) and levels of significance (p), comparing observed and expected numbers of Bar and wild type from the tests on E1 in Experiment 3. (+ signifies more Bar, - less Bar than expected). Also the numbers of individual replicates showing significantly less than (sig<E), more than (sig>E), or not different from (NSD) expected values of Bar.

	Input Ratio	$\frac{(\Sigma X)^2}{n}$	d	p	Number of replicates: Sig <E	Sig >E	NSD
	3:1	2.96	+	NS	2	2	3
Gen. 1	1:1	12.89	+	.1%	2	3	2
	1:3	8.34	-	1%	3	2	2
	3:1	20.45	-	.1%	4	0	6
Gen. 3	1:1	2.83	-	NS	2	1	7
	1:3	48.14	+	.1%	0	4	6
	3:1	7.43	-	1%	4	3	3
Gen. 5	1:1	2.26	-	NS	2	1	7
	1:3	14.47	+	.1%	1	4	5
	3:1	2.20	+	NS	1	1	5
Gen. 7	1:1	7.76	-	1%	1	2	4
	1:3	8.04	+	1%	1	1	5
	3:1	6.14	-	5%	11	6	17
All Gens.	1:1	1.85	-	NS	7	7	20
	1:3	33.82	+	.1%	5	11	18

TABLE 23

Values of $\frac{(\Sigma X)^2}{n}$, direction (d) and levels of significance (p), comparing observed and expected numbers of Bar and wild type of individual replicates of E1 in Experiment 3, for each ratio over all tests. (+ signifies more Bar, - less Bar than expected). Also the numbers of tests where significantly less than (sig < E), more than (sig > E) or not different from (NSD) expected values of Bar.

	Input Ratio	$\frac{(\Sigma X)^2}{n}$	d	p	Number of generations: Sig <E	Sig >E	NSD
	3:1	4.09	+	5%	0	1	1
E11	1:1	5.58	-	5%	1	0	1
	1:3	0.52	+	NS	0	1	1
	3:1	26.00	-	.1%	2	0	1
E12	1:1	0.00	-	NS	1	0	2
	1:3	29.02	+	.1%	0	2	1
	3:1	21.66	+	.1%	1	1	1
E13	1:1	15.87	-	.1%	1	0	2
	1:3	0.98	-	NS	1	1	1
	3:1	0.89	+	NS	0	1	3
E14	1:1	0.19	-	NS	0	1	3
	1:3	0.46	-	NS	1	0	3
	3:1	1.51	-	NS	1	0	3
E15	1:1	4.82	+	5%	0	1	3
	1:3	0.67	-	NS	1	1	2
	3:1	8.20	+	1%	1	2	0
E16	1:1	5.02	-	5%	1	2	0
	1:3	1.69	+	NS	1	1	1
	3:1	19.66	-	.1%	2	0	1
E17	1:1	4.54	+	5%	0	1	2
	1:3	1.46	+	NS	0	0	3
	3:1	8.20	+	1%	0	1	2
E18	1:1	20.59	-	.1%	2	0	1
	1:3	7.71	+	1%	0	1	2
	3:1	29.72	-	.1%	2	0	0
E19	1:1	1.50	-	NS	0	0	2
	1:3	44.09	+	.1%	0	2	0
	3:1	4.94	-	5%	1	0	2
E10	1:1	0.26	+	NS	1	1	1
	1:3	15.19	+	.1%	0	1	2
	3:1	2.55	+	NS	0	0	1
E112	1:1	3.74	-	NS	0	0	1
	1:3	0.41	+	NS	0	0	1

TABLE 23 (continued)

	Input Ratio	$\frac{(\Sigma X)^2}{n}$	d	p	Number of generations Sig <E	Sig >E	NSD
	3:1	88.41	-	.1%	1	0	0
E116	1:1	93.07	+	.1%	0	1	0
	1:3	11.35	+	.1%	0	1	0
	3:1	1.91	+	NS	0	0	1
E1789	1:1	1.18	+	NS	0	0	1
	1:3	6.42	-	5%	1	0	0
	3:1	0.06	-	NS	0	0	1
E190	1:1	1.16	-	NS	0	0	1
	1:3	2.23	+	NS	0	0	1

selection was operating.

Table 23 shows the summary of the χ^2 analysis on separate replicates; this was complicated by the fact that a few tests used flies from different replicates and these are kept separate. There were considerable differences between replicates with Bar as the majority component (3:1) being significantly better in 4 of the replicates and worse in 4, at 1:1 Bar was better in 2 and worse in 4 while as the minority component (1:3) it was better in 4 replicates.

The replacement series graphs and ratio diagrams of the tests on the stocks in Experiment 3 are shown on Figures 29 and 30 and the analysis in Tables 24, 25 and 26.

Unlike the tests with the selected populations, the total yields of the stock mixtures were higher than expected from pure culture yield at all frequencies (Table 24). The only time when less than expected yields were shown was in the 1:3 mixture in generation 3. Testing the replicates individually, a few pairs of stocks performing particularly well were shown, especially S5, S7, S9 and SO, while S4 was consistently poor.

Table 25 shows the summary of χ^2 analysis of Bar at different frequencies.

In the test at generation 1, Bar was better at the 1:1 ratio though worse at the 3:1 mixture. In generations 3 and 5 as in the selected populations, Bar performed well when rare and badly when common, while in generation 7, none of the frequencies were different from expected. Pooling all replicates and all tests, Bar was worse than expected as the majority component but better than expected in the equal mixtures or as the minority component. A comparison of the ratio diagrams of selected and stock tests reveals no significant differences

FIGURE 29

Replacement series graphs of competition tests on Stocks in generations 1, 3, 5 and 7 of Experiment 3

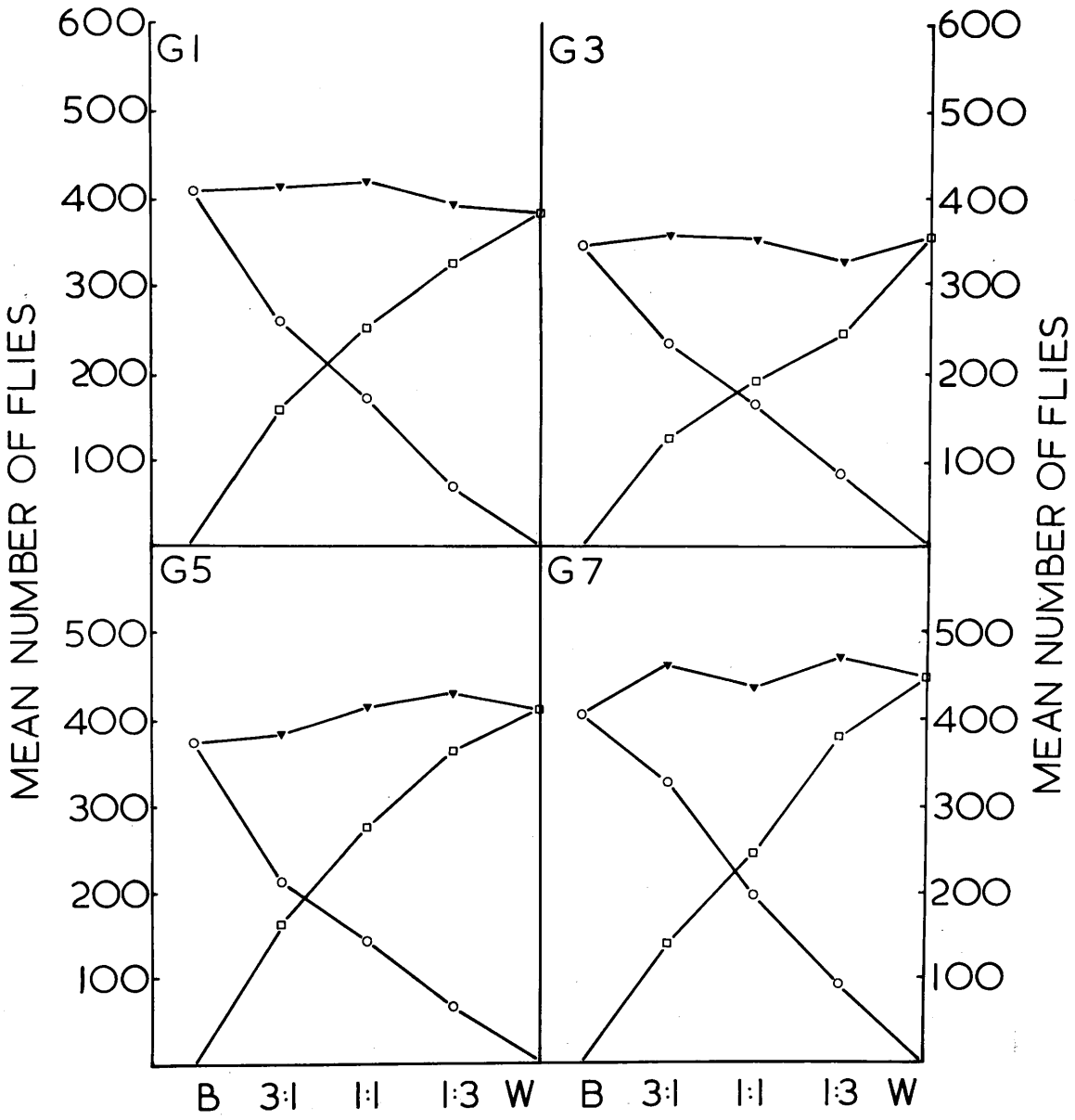

G1
G3
G5
G7
600
500
400
300
200
100
MEAN NUMBER OF FLIES
B
3:1
1:1
1:3
W

FIGURE 30

Ratio diagrams of competition tests on Stocks in generations 1, 3, 5 and 7 of Experiment 3.
Thin line represents the equilibrium line.

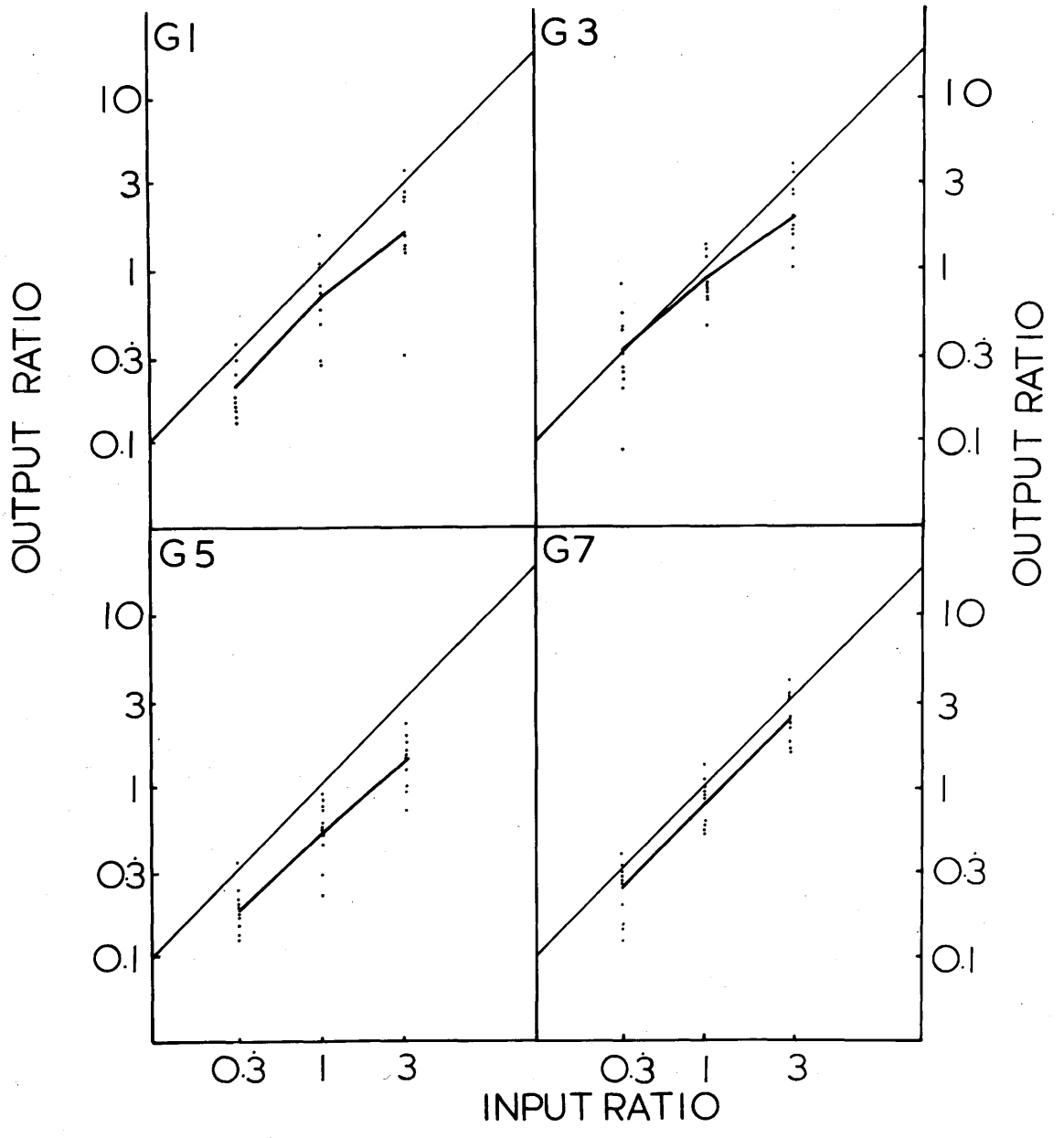

G1
G3
G5
G7
10
3
1
0.3
0.1
OUTPUT RATIO
0.3
1
3
INPUT RATIO

TABLE 24

(a) Values of $\frac{(\Sigma X)^2}{n}$, direction (d) and levels of significance (p), comparing observed and expected total yields (calculated from the pure culture yields) at all three ratios of the tests on stocks in Experiment 3. All replicates being pooled in each generation.

(b) Similar values for individual replicates, pooling tests.

(a)

	Input Ratio								
	3:1			1:1			1:3		
	$\frac{(\Sigma X)^2}{n}$	d	p	$\frac{(\Sigma X)^2}{n}$	d	p	$\frac{(\Sigma X)^2}{n}$	d	p
Gen. 1	5.74	+	5%	25.17	+	.1%	7.56	+	1%
Gen. 3	5.79	+	5%	0.20	+	NS	16.98	-	.1%
Gen. 5	1.07	-	NS	19.66	+	.1%	26.86	+	.1%
Gen. 7	92.96	+	.1%	8.56	+	1%	43.97	+	.1%
All Gens.	45.22	+	.1%	40.49	+	.1%	23.39	+	.1%

(b)

	Input Ratio								
	3:1			1:1			1:3		
	$\frac{(\Sigma X)^2}{n}$	d	p	$\frac{(\Sigma X)^2}{n}$	d	p	$\frac{(\Sigma X)^2}{n}$	d	p
S1	10.73	-	.1%	16.32	-	.1%	3.20	-	NS
S2	0.07	+	NS	0.06	+	NS	21.42	-	.1%
S3	19.38	-	.1%	41.18	-	.1%	17.10	+	.1%
S4	7.78	-	1%	25.20	-	.1%	6.89	-	1%
S5	123.10	+	.1%	212.58	+	.1%	110.25	+	.1%
S6	1.24	-	NS	4.67	+	5%	5.78	+	5%
S7	11.87	+	.1%	28.57	+	.1%	29.11	+	.1%
S8	41.28	+	.1%	69.72	+	.1%	0.18	-	NS
S9	84.69	+	.1%	2.29	+	NS	6.54	+	1%
S0	23.18	+	.1%	11.49	+	.1%	20.28	+	.1%
S90	0.25	-	NS	0.70	+	NS	2.67	+	NS

TABLE 25

Values of $\frac{(\Sigma X)^2}{n}$, direction (d) and levels of significance (p), comparing observed and expected numbers of Bar and wild type from the tests on stocks in Experiment 3. (+ signifies more Bar, - less Bar than expected). Also the numbers of individual replicates showing significantly less than (Sig < E), more than (Sig > E), or not different from (NSD) expected values of Bar.

	Input Ratio	$\frac{(\Sigma X)^2}{n}$	d	p	Number of replicates		
					Sig < E	Sig > E	NSD
	3:1	7.24	-	1%	2	1	6
Gen. 1	1:1	10.82	+	.1%	1	2	6
	1:3	0.55	-	NS	1	1	7
	3:1	40.40	-	.1%	5	1	4
Gen. 3	1:1	2.21	+	NS	2	3	5
	1:3	28.63	+	.1%	2	5	3
	3:1	16.54	-	.1%	4	1	5
Gen. 5	1:1	1.54	+	NS	2	2	5
	1:3	7.11	+	1%	2	4	4
	3:1	1.20	+	NS	1	2	7
Gen. 7	1:1	0.93	+	NS	1	1	8
	1:3	2.89	-	NS	2	2	6
	3:1	36.17	-	.1%	12	5	22
All Gens.	1:1	11.68	+	.1%	6	8	24
	1:3	8.08	+	1%	7	12	20

between their slopes in any of the tests. Overall 12 individual replicates gave lower than expected numbers of Bar when it was common and 5 higher, while at 1:1, 6 were lower and 8 higher, and when rare 7 were worse than expected and 12 higher than expected.

Table 26 shows the variation between different replicates of performance of Bar in the different frequencies.

Table 27 brings together the results from all the replacement series tests.

TABLE 26

Values of $\frac{(\Sigma\chi)^2}{n}$, direction (d) and levels of significance (p), comparing observed and expected numbers of Bar and wild type, of individual replicates of stocks in Experiment 3, for each ratio over all tests. (+ signifies more Bar, - less Bar than expected). Also the numbers of tests where significantly less than (Sig < E), more than (Sig > E) or not different from (NSD) expected values of Bar.

	Input Ratio	$\frac{(\Sigma x)^2}{n}$	d	p	Number of generations Sig < E	Sig > E	NSD
	3:1	25.71	-	.1%	2	0	2
S1	1:1	1.82	+	NS	0	0	4
	1:3	12.85	+	.1%	0	1	3
	3:1	12.50	-	.1%	2	0	2
S2	1:1	3.52	+	NS	0	0	4
	1:3	1.37	+	NS	0	1	3
	3:1	10.92	-	.1%	2	1	1
S3	1:1	23.42	+	.1%	0	2	2
	1:3	1.61	-	NS	1	1	2
	3:1	16.65	+	.1%	1	2	1
S4	1:1	10.79	-	.1%	2	0	2
	1:3	0.00	+	NS	1	2	1
	3:1	7.70	+	1%	0	1	3
S5	1:1	2.48	+	NS	0	0	4
	1:3	18.92	-	.1%	2	0	2
	3:1	13.00	-	.1%	2	1	1
S6	1:1	0.00	-	NS	0	0	4
	1:3	14.33	+	.1%	1	2	1
	3:1	1.82	-	NS	0	0	4
S7	1:1	13.03	-	.1%	2	0	2
	1:3	36.48	+	.1%	0	3	1
	3:1	5.52	-	5%	1	0	3
S8	1:1	3.82	+	NS	1	2	1
	1:3	0.03	-	NS	1	1	2
	3:1	0.00	-	NS	0	0	3
S9	1:1	5.96	+	5%	0	1	2
	1:3	5.91	-	5%	1	0	2
	3:1	48.72	-	.1%	2	0	1
S0	1:1	11.76	+	.1%	1	2	0
	1:3	10.57	+	.1%	0	1	2
	3:1	0.48	-	NS	0	0	1
S90	1:1	3.19	+	NS	0	0	1
	1:3	1.91	-	NS	0	0	1

TABLE 27

A summary of the results obtained from the replacement series tests, giving the generation numbers where the minority genotype (Bar or wild type) was favoured or disfavoured. ($\frac{(\Sigma\chi)^2}{n}$ value after pooling all replicates from each generation is significant).

			The generations where minority genotype is		
			FAVOURED		DIS-FAVOURED
		Tests carried out in generations	Bar	Wild type	Wild Bar type
E1	Exp. 1	12	12	12	
	Exp. 2	3, 6, 9, 14, 15	9, 15	6, 9, 15	
	Exp. 3	1, 3, 5, 7	3, 5, 7	3, 5	1
Stocks	Exp. 2	15			
	Exp. 3	1, 3, 5, 7	3, 5	1, 3, 5	
E6	Exp. 2	12, 15	12, 15	12, 15	
Sep.E6	Exp. 2	15	15		

Discussion

The experiments described here were designed to investigate the evolutionary changes that may result in populations due to selective interactions between populations in competition. Particular attention was paid to the changes in the intensity of the competitive interactions themselves. On the basis of previous studies, two significant changes might be expected. Firstly, an improvement of the competitive ability of the competing populations (Moore, 1952; Van Delden, 1971; Seaton and Antonovics, 1967). Secondly, there may be niche divergence and a greater level of ecological exploitation (Seaton and Antonovics, 1967, but for a counter argument, see Ayala, 1969b).

The term competitive ability has been used in a rather broad and hence ambiguous context previously, to the extent that Seaton and Antonovics (1967) avoided its use altogether. If carefully defined, however, it may still be a useful term, and in this thesis it describes both the performance of a particular component in a mixture and its effect on its competitor. It therefore encompasses the two measurements, numbers of flies of that particular genotype, and the proportion of the total which that genotype represents.

It can be seen from the graphs (Figures 1 - 5) of total numbers of stock Bar and wild type that Bar performed as well in pure cultures as wild type. In mixed cultures, however, Bar was generally a poor competitor. In Experiment 1 and 2, the total yield of the mixtures was little different from the yield of the pure cultures. This suggests that wild type took a disproportionate share of the total resources, performing

well at the expense of Bar. In Experiment 3 the mixed cultures (E1 only) generally yielded higher than the pure cultures so that wild type may have performed better than Bar by utilising somewhat different resources and not entirely by utilising common resources more efficiently. There is a great deal of fluctuation in the proportion of Bar in the mixed cultures throughout each experiment. In addition to chance variation in numbers, Bar and wild type may be differently affected by certain environmental conditions, as well as possibly changing genetically from generation to generation.

The measurements of the rate of emergence of mixed and pure cultures in Experiment 2 show that changes may have taken place in the Bar and wild type populations as a result of their being grown in competition. Stock Bar emerged later than stock wild type in all but generation 3. This may have been because Bar larvae eat more slowly than wild type larvae as suggested by Bakker (1961). The results for the E1 populations show that E1 Bar and E1 wild type from generations 6 and 9 were later in emerging than stock Bar and stock wild type respectively, but were earlier in generations 12 and 15. The emergence rates of E2 Bar and E2 wild type were more similar. This was most likely because of the gene flow between the two genotypes. E2 Bar was earlier than Stock Bar throughout, while E2 wild type was later in generations 6 and 9 but earlier in generations 15. E5 and E6 populations were generally later than their respective stocks in generations 9 and 12 but earlier in generation 15. Therefore, towards the end of the experiment, the selected populations did emerge earlier than the stock populations. In the interbreeding populations, this may have been largely due to increased variability resulting from gene

flow between the two genotypes. That the E1 lines in the absence of this gene flow also showed more rapid emergence however, suggests that some changes may have taken place through selection as well. Rate of emergence is likely to be an extremely important facet of fitness and competitive ability, especially in nature where food is much more likely to deteriorate or dry up.

The results of the experiments on the competitive ability of stock and selected populations are, at least partly, contrary to the expectation of improved competitive ability through selection and to the results of Seaton and Antonovics (1967). In all three experiments, E1 Bar became a worse competitor than stock Bar. It performed better against selected wild type in generation 15 of Experiment 1, and generation 4 of Experiment 3, but on all other occasions it was a worse competitor than stock Bar. E1 wild type was also worse than stock wild type in Experiment 1, but was no worse, and in two tests better, than stock wild type in Experiment 2. In Experiment 3, it was worse in generation 8, but no worse in the other three tests.

Three factors could have contributed to this very distinct and perhaps surprising change in the performance of E1 Bar; single generation carryover effects of wild type on Bar, inbreeding depression, or some selective changes brought about as a result of competition.

In the E2 tests, the selected lines were generally better than their respective stocks, with the exception of E2 wild type in generation 15 of Experiment 1, and generation 7 of Experiment 2, and of E2 Bar in generation 15 of Experiment 2.

In many of these tests, the E2 populations were very much superior in competitive ability to the stocks. In the E5 populations, originated from E1 in Experiment 2, a very rapid and significant change took place in three generations after interbreeding was started. Both E5 Bar and E5 wild type were much better than stock Bar and stock wild type, and this was also true in generations 13 and 14. Both E6 Bar and E6 wild type retained their good competitive abilities after 2 and 5 generations of isolated competition, although they were perhaps less good in the latter test.

It is possible that wild type might affect Bar when it is grown together with it in such a way that its performance in a subsequent test is affected. For instance, Bar emerging from medium containing wild type larvae might be less fecund in terms of egg laying. This would seriously affect the competitive ability of such selected flies. If all selected populations of Bar showed a poorer performance in such tests this might be an important factor; however, only the E1 mixtures showed reduced competitive ability, while the E2, E5 and E6 mixtures improved. Thus, a carry-over effect of wild type on Bar seems unlikely to account for the behaviour of the selected populations.

There is abundant evidence that inbred populations are less fit in pure culture than outbred populations, and the poor performance of inbred populations is shown up even more when they are competed with outbred populations. Gale (1964) found that an inbred population of Oregon wild type was poorer than either dumpy or vestigial (the latter is usually a very unfit mutant). Dyer (1971) has shown that although the F1 of two

inbred populations show poor performance initially (he believes due to upset of integrated gene pools) in later generations they perform very much better than the original inbred populations.

Perhaps the most significant experiments with regard to the effect of inbreeding on competitive ability of populations are those of Latter and Robertson (1962). They found that competitive ability fell very rapidly as populations became inbred. Full-sib lines showed a loss of 50% in competitive ability after only 4 generations, and of 80% and 90% after 7 and 10 generations. They also measured competitive ability of populations initiated each generation from ten flies of each sex. The competitive index, a measure of competitive ability against a constant stock, was found to fall nearly 20% after 5 generations and 40% after 15 generations. The inbreeding coefficients after these times were 20% and 50%.

Two separate problems need to be considered in these particular experiments with regard to inbreeding. First the extent to which populations used in the experiments were initially inbred and second, the rate of inbreeding throughout the experiments. The Bangor wild type and the Edinburgh Bar stock, used in the first two experiments, were probably quite inbred as they had been maintained as fairly small populations for many generations and may have been subjected to bottle necks in this time (personal communications - Maxwell and Spiers). The Kaduna wild type and Bar stock used in the third experiment was definitely outbred however. That E1 Bar became worse in the latter as well as in the former suggests that the initial level of inbreeding may not have been as

important in the E1 mixtures as the inbreeding which took place in the course of the experiment.

Latter and Robertson (1962) showed that the rate of inbreeding and consequent loss of competitive ability is quite rapid when 10 pairs of flies are used to initiate each generation. Although Bar and wild type would be becoming inbred in the E1 lines, they would be also becoming inbred, although at a slower rate, in the stocks, which were being initiated from 20 pairs of flies. It thus seems likely that at least some of the poor competitive performance of the E1 lines could be accounted for by inbreeding through the course of the experiment, especially in the first and last experiment. In Experiment 2, as there was interbreeding between replicates, the effective population size was higher than 20 so that the rate of inbreeding would have been much less. Despite this, Bar was already a poor competitor after only two generations of selection. A further anomaly to this simple explanation of loss of competitive ability due to inbreeding is presented by the fact that, although Bar very rapidly became a worse competitor, wild type, in most cases, did not. Further experiments described by Latter and Robertson (1962) indicate a very likely reason for this. In addition to measuring the loss of competitive ability in a population repeatedly initiated with 10 pairs of flies, they also maintained populations, again using 10 pairs per generation, which were subjected to directional selection; for high or low chaetae number, and high or low body size. They found that, in these lines, the drop in competitive ability was much more rapid and in the low lines was extremely rapid. Thus, the combination of strong directional selection and inbreeding seems to increase the rate of

inbreeding depression and consequent reduction in competitive ability. It seems possible then that the E1 Bar, as well as becoming inbred through small population size was also being subjected to strong selection. E1 wild type, on the other hand, was not being subjected to such strong selection. This factor is one which has probably been underestimated in similar experiments, although Robertson (1971) has drawn attention to it with regard to Thoday's work (see Thoday and Gibson, 1970). It is important to point out that in Latter and Robertson's experiments, and in most other experiments involving disruptive selection, the selective pressures imposed on the populations were artificial. In the experiments described in this thesis the selective pressures were a result of the interactions between two populations and not the experimenter. The importance of selection in leading to increased inbreeding depression has probably not been considered previously in this type of experiment.

The very good competitive ability of the selected lines which had undergone some degree of interbreeding (between Bar and wild type) was partly or almost entirely due to the increased outbred nature of these populations. However, they may also have been affected by selection for increased competitive ability and it is possible that these now more outbred populations would have shown a greater amount of niche divergence than the reproductively isolated populations. Evidence for this is seen in the replacement series tests on the E6 populations, where a clear frequency dependent effect was observed.

The evolution of niche divergence is another possible outcome of competition between populations. It should be evidenced by greater overall resource utilisation and hence greater yield of mixtures, and frequency dependent effects seen in replacement series graphs and ratio diagrams.

Most theoretical models of competition predict that mixtures of two components will yield higher than would be expected from the yields of each component separately if the resources utilised by the different components are not identical. In this way, the overall level of ecological exploitation is increased. This was shown in the experiments of Seaton and Antonovics (1967) and also by mixtures of different genotypes of barley by Allard and Adams (1968). Ayala's (1969b) conclusion that the principle of competitive exclusion must be invalidated as he found apparent equilibrium between two species of Drosophila and yet a reduced mixture yield shows how important this idea is. However, as argued by Antonovics and Ford (1972) reduction in yield of mixed cultures merely indicates that interference plays a large part in the interactions of the components, but does not preclude that co-existence can occur as some degree of niche divergence may also be present.

Differences in total yields of pure and mixed cultures in the replacement series tests carried out here seem to be small and not very consistent. In the first two tests of Experiment 1 yields of mixtures were higher than expected while in the third test they were lower. Three out of the four replicates showed better yields in the 1:1 mixtures over the three tests. In Experiment 2, the unequal mixtures gave low yields while the

equal mixtures gave high yields twice and low yields once. Two replicates were poor and two were generally good. This difference between the replicates was despite gene flow between them. The E6 mixtures again showed some higher and some lower mixture yields, and have one good and one poor replicate. The single test with the stocks gave very low total yields in the mixtures although in some bottles there was very poor emergence due probably to some environmental effect.

Again in Experiment 3, some tests gave high mixed yields and some low. In the E1 tests at the 1:1 ratio, yields were generally higher than expected while in the other mixtures they were sometimes lower than expected. Four of the replicates were good and two were poor. In the tests using the stocks, the total yields of the mixed cultures were almost always higher than expected, four of the replicates yielding particularly well and one badly.

When yields are higher than expected, it is most likely that any inhibitory effects of the components on each other are more than compensated for by ecological avoidance between them. Either slightly different resources were utilised by the two competitors and these could either be completely separate or merely a difference in the spectrum of tolerance of certain environmental conditions, or some form of mutual facilitation could take place. An example of this would be the production of a metabolite by one which could be utilised for growth by the other.

It is strange that the tests with the stocks in Experiment 3 showed greater mixed yields than did the tests with the E1 lines. Differences may already have been present between wild type and Bar as they were previously grown together for

many generations and the larger population size of the stock lines and the relaxation of selection may have permitted the retention of greater genetic variability.

The presence of niche divergence can also be shown clearly by looking at the output frequencies when different frequencies of the two components are grown together. The single test in Experiment 1 where three frequencies were used showed a clear frequency dependent effect favouring the rare component. This is evident from both the χ^2 analysis and the regression line of the ratio diagram. In Experiment 2, frequency dependent patterns were present in generation 6 (although when Bar was rare it did not perform better than expected) and in generations 9 and 15. They were absent in generations 3 and 14. Three out of four replicates showed frequency dependent effects. The E6 tests showed particularly good frequency dependent effects in both tests, as did separated E6, while the stocks did not. In Experiment 3, the tests with the E1 lines showed frequency dependent selection in generations 3 and 5, less well in generation 7 and not at all in generation 1. With the stock lines, a frequency dependent pattern was shown in generations 3 and 5 but not in generations 1 and 7. There is overall a very great similarity between the results from the E1 and the stock tests in Experiment 3.

Thus, frequency dependent relationships do exist although they were not always shown. It is thus important to decide whether these relationships can be changed through natural selection. This can be examined best in Experiment 2, where the E1 tests show no such relationship after 3 generations yet do so after 6 generations and to a greater extent after 9 generations. The rather poor tests on the stocks did not

show any such relationships and it is unfortunate that more tests on the stocks were not carried out in this experiment. That the E6 tests showed perhaps even greater effects may have been due to the more outbred nature of the two genotypes, which should have provided both with more variability with which to undergo changes.

In Experiment 3, some changes had probably already taken place while Bar and wild type were grown together and interbreeding for about 30 generations before the start of the experiment. These were probably not increased through the course of the experiment although no frequency dependent pattern was shown after one generation while it was after three generations. The great similarity between the E1 and the stock tests in each generation and yet the differences between the different generations suggests that the frequency dependent relationship is affected by differences in environmental conditions.

The experiments carried out on extinction rate at the end of Experiment 3 may also provide information on differences between the two competitors. In the first generation of these tests, it was found that E1 Bar was a poor competitor compared with stock Bar, as had been shown by the previous competitive ability tests. However, it had also been shown, in the replacement series tests, that when Bar was rare it performed proportionately better than when it was common. This ought to have become evident as Bar was gradually eliminated from the mixtures. Unfortunately, there was so much variation between replicates that statistical comparisons between the different types of mixtures would be unlikely to reveal any significant

differences. A certain amount of information can be gained from the extinction rate graphs of selected Bar against stock wild type as in four of the replicates the frequency of Bar appears to level off at around 0.1 and 0.2. This 'stability' is not shown by any of the stock Bar/selected wild type tests and the extinction rate graphs appear to be more convex (suggesting an increasing rate of extinction) whereas those of the former are more concave. Although the average frequency of E1 Bar in its mixtures was well below that of stock Bar initially, it was very nearly the same after 5 generations.

This type of test may prove useful for testing differences between competing components in the future though there are a few particular drawbacks. The first is that they must be continued over many generations, probably more than 5. This means that changes will take place in the competitive performance of each competitor through selection as well as through change in frequency. Secondly, as one component is gradually reduced in frequency the numbers used in initiating future tests become smaller so that the importance of random changes and severe inbreeding make extinction increasingly likely.

Before discussing the broader implications of the results of these experiments, it is worth looking briefly at some of the theoretical models which have examined the problems of co-existence between different populations or segments of a population.

The models of relevance here can be divided into two kinds; those dealing with the origin and maintenance of polymorphisms, and those dealing with the outcome of competition between two or more species.

Levene (1953) was the first to consider theoretically the

possibility of polymorphism being maintained by each homozygote being favoured in one niche. Clarke and O'Donald (1964) also examine polymorphism where the heterozygote is inferior to the homozygotes, the latter being maintained by frequency dependent selection as they occupy different ecological niches. Stability appears possible over a wide range of conditions. Levins (1962) takes the model further and examines the conditions under which stable equilibrium can result.

From the standpoint of competition, the earliest thorough models are those of Gause (1934) and Volterra (1928). They show that population growth depends upon (1) innate rate of increase, (2) carrying capacity of the environment, (3) population size of each species and (4) some function of the effect of each species on the other. Where species are less affected by competition from individuals of the other species than by individuals of their own species, their continued coexistence is possible.

De Wit (1960) considers competitive interaction in terms of individuals crowding each other for space. Crowding coefficients of one species on the other are calculated. Where crowding coefficients are above unity facilitation or avoidance occurs. Schutz *et al.* (1968, 1969) extend this to genotypes of one species. Their models show that even in self fertilising populations, considerable genetic heterozygosity is maintained by selection of heterozygotes and less common homozygotes. Thus frequency dependent selection can be extended to interpopulation competition.

Levin (1971) examines interspecific competition and appreciates at the same time intraspecific heterogeneity.

He considers the situation where two species are sympatric and where each have two genotypes. All have different competition coefficients in terms of the other genotype of their own species and the two genotypes of the other species. Although this model is extremely complex, the overall likely outcome is that the genotype in each species which is least sensitive to the other genotypes (of both species) will persist, and if the optimal genotypes of each species are less affected by each other than by themselves, the two species may co-exist.

Clarke (1972) tries to encompass the idea of frequency dependent selection maintaining a polymorphism with the idea of competition between genotypes giving rise to co-existence only when intergenotypic interactions are less important than intragenotypic interactions. He examines the situation of density dependent selection in each of two alternative niches, and shows that factors increasing reproductive capacity (often known as 'r' selection) may be important at low density, but that those maximising realisation of carrying capacity (often known as 'K' selection) are more important at higher densities.

The generality underlying all these models is that the coexistence of species or of morphs depends on frequency dependent selection where either type is favoured when it becomes rare. This can generally only be true when intra-population (intragenotypic) interactions are greater than interpopulation interactions. This can be complicated, however, by many other factors and it is of interest to find out whether the experiments described here are in accord with the models or whether they are invalidated through some limitation.

Although the problem of inbreeding in selected isolated populations may mask any sign of niche divergence when competitive ability is examined, it does not mean that niche divergence can be discounted in these lines. In fact, the results of the replacement series tests point very clearly to some ecological differences between the genotypes being present. The lines used in these experiments are inevitably only samples of a much larger population; however, this will also be true when two populations meet and also where a new morph has arisen in a population. Differences may be present initially due to sampling but these may be consolidated through selection. Any two samples from a single population are likely to be different, and clearly, so will samples from different populations.

Perhaps it is fairest to consider the two populations, Bar and wild type, as two separate species, each of which contains many different genotypes. This would make Levin's model particularly applicable. Of the possible genotypes shown by the two 'species' some will be represented in both, making them sub-optimal in fitness as they will be in direct conflict, while others will be shown by only one and will not compete directly with the other 'species'. The latter should be favoured by natural selection and should become more common. It is possible, in fact probable, that one 'species' will be superior to the other; in these experiments, wild type is superior to Bar. It is therefore possible that when the same genotypes are shown by both that those in Bar, the weaker competitor, will be lost. Thus the genetic variability of Bar would be reduced by selection as well as by inbreeding. This would account for Bar becoming worse as a competitor in

the second experiment where inbreeding should have been reduced to a minimum. Bar may thus have become specialised to occupy a small part of the environment. Possible evidence for this is shown by the extinction rate experiment where, in some of the replicates, selected Bar, when it is competing with stock wild type, drops rapidly in frequency until at a particular frequency it levels out and seems to reach an equilibrium.

There are several further limitations in this type of experiment. Firstly, as no equilibrium is reached during the experiment, and frequency dependent selection is inferred from only three frequencies, stability cannot really be confirmed as it would have been outside these frequencies. More experiments of the extinction rate type would be particularly valuable although these are subject to the limitations previously mentioned. Secondly, as a small number of parents was used relative to the number of offspring, it is possible that 'r' selection is more important than 'K' selection. There may be strong selection for rapid and prolific egg laying and rapid development but little selection for occupying different ecological niches. The results of the emergence rate experiments suggest that in time, emergence rate of both selected Bar and wild type might be speeded up. This may also involve ecological divergence.

The environment used in these experiments, being rather uniform, is probably not very suited to ecological divergence. Further experiments of this kind might introduce more heterogeneity, although the fact that frequency dependent effects were shown suggests some resource partitioning, perhaps to the

extremes of tolerance of continuously varying factors, such as oxygen, pH or waste products.

Thus, despite limitations, these experiments contribute to our knowledge of intergenotypic competition, as they show that ecological differences may be present between genotypes, that further changes may take place through selection and that increased inbreeding depression and consequent reduction in genetic variability may occur in the weaker competitor, possibly due to favouring of only a very few of the genotypes within that population.

DISRUPTIVE SELECTION IN TWO EXTREME ENVIRONMENTS

Introduction

Disruptive selection experiments carried out on *Drosophila* for sterno-pleural chaetae by Thoday (1958, 1959) and Thoday and Gibson (1962 and 1970), for cubitus interruptus by Scharloo *et al.* (1964 and 1967a, b) and for escape behaviour by Grant and Mettler (1969) have resulted in increased variance, sometimes to the extent of a bimodal distribution. On two occasions, (Thoday and Gibson, 1962 and Cogne and Grant, 1972) reproductive isolation apparently evolved between two segments of the population. There has, however, been some dispute about the possibility of the population from which Thoday's experiments were initiated having originated from two distinct populations between which there might already have been some degree of reproductive isolation (Scharloo, 1971 and Thoday, 1971).

In nature, disruptive selection operates when a single population is subjected to two or more opposing selective pressures. Examples from predator-prey interactions are those of Batesian mimicry (e.g. Clarke and Sheppard, 1962) and apostatic selection (Clarke, 1969). A similar phenomenon occurs when two adjacent populations are selected for more than one optimum. A good example, where actual selective values have been calculated, is that of heavy metal tolerance in the grasses *Agrostis tenuis* and *Anthoxanthum odoratum* (see Antonovics, Bradshaw and Turner for review, 1971). Another well studied example where there appears to be opposing selection in neighbouring populations is shown by the 'area effects' of the land snails, *Cepaea nemoralis*, (Cain and

Currey, 1963) and Partula (Clarke and Murray, 1969). The frequencies of particular genes change very rapidly over relatively short distances, although unlike the metal mine situation the selective reasons for these changes are less apparent.

Laboratory simulations of sympatric or parapatric divergence would be more convincing if some environmental factor other than the experimenter was to enforce the selection and several workers have looked at systems where adaptation is to two contrasting habitats. Robertson (1966) produced strains of Drosophila melanogaster adapted to medium containing EDTA. By connecting, with glass tubes, cages containing normal and EDTA medium, thus permitting gene flow, he found that differences between the EDTA strain and the normal strain were reduced but not eliminated. He also tested for assortative mating and found no evidence of increased homogametic matings. Pimentel, Smith and Soans (1967) gave house flies, Musca domestica, a choice of fish flavoured or banana flavoured medium selecting those which laid their eggs on the minority medium (1 out of 10 vials) at opposite ends of the cage. Thus strong selection pressures were operating in favour of two different neighbouring environments and some gene flow was allowed between them. Strong preferences were soon developed for fish and banana medium where these were the rare foods. These preferences were partly genetically controlled since flies grown on a different medium for one generation still showed strong preferences for the medium of their grandparents. Watson (personal communication) grew Drosophila melanogaster in beakers with a choice of normal and peppermint medium. There was no indication of the flies becoming adapted to peppermint

medium although they did evolve greater habitat preference so as to lay eggs on the medium from which they had emerged.

The purpose of the experiments described in this part of the thesis was to investigate the effect of subjecting a single population to a choice of two contrasting environments.

Methods

The two environments used in the experiment were media containing hydrochloric acid and sodium hydroxide. Suitable concentrations were determined in preliminary experiments (See Results section). Medium was made in exactly the same way as in the previous experiment, with the appropriate amount of acid or base being added just before pouring into bottles. The medium was seeded with six drops of yeast suspension.

A range of concentrations of hydrochloric acid and sodium hydroxide were used to determine a suitable concentration where yield of flies is depressed yet where some flies still emerge. Three series of tests were carried out with a different range in each. In the first 16, 20, 24, 28 and 32 mls of N NaOH and 1.5, 2.0, 2.5, 3.0 and 3.5 mls of N HCl were added to 250 mls of medium. In the second 5, 10, 15 and 20 mls of 5N NaOH and 4, 6, 8, 10 and 12 mls of N HCl were used, while in the third 6, 7, 8 and 9 mls of 5N NaOH and 11, 13, 15 and 17 mls of N HCl were used.

The flies used in the experiments were from the Kaduna stock from the Institute of Animal Genetics in Edinburgh.

Four replicates of pure acid (labelled A^p), pure base (B^p) and mixed (A^m and B^m) cultures were initiated in ½ pint milk bottles. In the mixed cultures, bottles (one of each type)

were laid on their sides and connected by transparent plastic tube 6" long and ½" internal diameter. This was to allow flies free access to both types of medium when they were laying eggs. Twenty males and twenty virgin females from each type of medium (A^m and B^m) were introduced into the plastic tube through a slit which was then covered with sellotape. Females were allowed to mate at random and to lay their eggs on either medium. Egg laying was allowed to continue for 7 days after which parents were removed and the connected bottles were separated so that flies emerging from the acid and base parts of the mixed environment could be kept separate from each other. Twenty mated females were used to initiate each generation of the pure cultures. If the medium showed signs of drying out, as it did in some generations, further dilute yeast suspension was added. Development usually took longer than on normal medium and new generations were initiated at 21 day intervals.

After 1, 12 and 15 generations of selection, tests were carried out to measure the performance of the different populations on acid, base and normal medium. In generations 12 and 15, the performance of normal flies, taken from the stock wild type of the competition experiments, was tested on acid, base and normal medium as controls. Two females from each replicate were placed in vials containing each of the kinds of medium. Numbers of flies emerging were counted after 16 days and again after 21 days. The numbers from each culture emerging from each type of medium were compared with 't' tests.

After 15 generations, experiments testing the mate choice of females from each culture were carried out. Single females were placed in a vial containing medium and yeast and were

presented with two males, one from each type of medium. To identify the males a small proportion of the wing of one of them was clipped. To overcome any bias against clipped males, equal numbers of mating trios were set up with as many of one type of male clipped as with the other kind clipped. Twenty-four such vials were set up at a time and when mating took place the male involved was noted and the vial removed. A group was given one hour in which to mate and those which had not mated in this time were discarded. Usually more than half of the females had mated in this time.

Results

Preliminary experiments

The numbers of flies emerging from each of the three replicates at each concentration of NaOH are given in Table 28 and from the HCl in Table 29.

TABLE 28

The numbers of flies counted from bottles containing medium with a range of concentrations of sodium hydroxide

Concentration	Replicate			
N NaOH	1	2	3	Total
16 mls	282	267	103	652
20 mls	105	114	41	260
24 mls	31	267	238	536
28 mls	81	262	86	429
32 mls	266	327	254	847
5N NaOH	1	2	3	Total
5 mls	408	136	104	648
10 mls	0	0	0	0
15 mls	0	0	0	0
20 mls	0	0	0	0
6 mls	610	755	478	1843
7 mls	287	441	350	1078
8 mls	646	491	492	1629
9 mls	232	294	0	526

TABLE 29

The numbers of flies counted from bottles containing medium with a range of concentrations of hydrochloric acid

Concentration	Replicate			
N HCl	1	2	3	Total
1.5 mls	223	277	290	790
2.0 mls	240	276	245	761
2.5 mls	268	65	210	543
3.0 mls	212	263	198	673
3.5 mls	262	273	148	683
4 mls	317	327	377	1021
6 mls	149	488	239	876
8 mls	503	342	260	1105
10 mls	380	385	303	1068
12 mls	236	275	216	727
11 mls	345	427	394	1166
13 mls	254	213	227	694
15 mls	223	263	351	837
17 mls	191	245	226	662

The results indicate that intermediate concentrations of both NaOH and HCl give the highest yields of flies. This might be due to several environmental factors such as slightly different amounts of medium being used in the different tests or to slight differences in the dryness of the medium. Cultures on NaOH seem to either give high yields or to fail completely. It seems probable that as soon as the surface of the medium has been broken up and made more liquid by the first

larvae it becomes easier for other larvae to establish themselves. The waste products of the first larvae might also make the medium less alkaline. From these results, the concentrations chosen for the main experiments were 9 mls of 5N NaOH and 17 mls of N HCl.

Performance of selected and unselected flies on different media

Table 30 shows the performance of flies from acid or base cultures on vials of acid or base medium after one generation.

TABLE 30

The numbers of flies emerging from vials containing acid or base medium, whose parents had grown for one generation on acid or base medium

n is the number of vials in each group

	Acid medium			Base medium		
Parents	No.of flies	n	$\sigma/\sqrt{n}$	No.of flies	n	$\sigma/\sqrt{n}$
Acid	62.2	22	2.9	70.8	18	5.4
Base	50.4	9	6.9	49.4	11	7.1

Flies which had grown on base medium gave lower yields on base medium than flies which had grown on acid medium ($p<0.05$). They also gave lower yields than acid flies on acid medium but this difference is not significant. Paradoxically then acid flies are better on base medium, while base flies are as good on acid medium as on base medium.

Table 31 shows the performance of acid, base and normal flies on acid, base and normal medium after eleven generations.

TABLE 31

The number of flies emerging from vials containing acid, base and normal medium, whose parents had grown on acid or base medium for eleven generations or on normal medium

n is the number of vials in each group

	Acid medium			Base medium			Normal medium		
Parents	No.of flies	n	$\sigma/\sqrt{n}$	No.of flies	n	$\sigma/\sqrt{n}$	No.of flies	n	$\sigma/\sqrt{n}$
A^m	70.1	8	3.2	78.4	8	4.8	116	8	7.3
A^p	36.1	8	1.3	60.1	8	4.6	110.8	8	4.4
ALL A	53.1	16	4.7	69.3	16	4.0	113.4	16	4.2
B^m	32.3	6	8.6	68.3	6	4.8	116.8	6	8.8
B^p	52.6	8	7.2	63.3	8	3.9	125.5	8	2.9
ALL B	43.9	14	6.0	65.4	14	3.0	121.8	14	4.1
Normal	10.2	6	3.4	26.8	9	5.8	33.3	9	8.4

The normal flies are much worse on both acid and base but are also very poor on normal medium. All groups yield best on normal medium and worst on acid medium;in all cases except A^m (flies from the acid bottle of the mixed cultures)on acid and base these differences are significant. A^m yields higher than A^p on both acid and base. Acid flies give higher yields than base flies on acid medium though none of these differences is significant. Also A^m gives a higher yield on base medium than B^m while B^p yields higher than A^p on base.

Table 32 shows the results obtained from the tests carried out after fifteen generations of selection.

TABLE 32

The numbers of flies emerging from vials containing acid, base and normal medium, whose parents had grown on acid or base medium for fifteen generations or on normal medium

n is the number of vials in each group

	Acid medium			Base medium			Normal medium		
Parents	No.of flies	n	$\sigma/\sqrt{n}$	No.of flies	n	$\sigma/\sqrt{n}$	No.of flies	n	$\sigma/\sqrt{n}$
A^m	153.3	8	12.9	99.6	8	8.1	99.8	8	8.7
A^p	144.3	8	11.4	119.9	8	7.8	130.9	8	13.7
ALL A	148.8	16	7.8	109.8	16	6.1	115.3	16	8.9
B^m	109.9	8	14.1	120.4	7	11.1	106.4	8	13.7
B^p	139.3	4	10.6	145.3	4	10.2	113.7	3	29.5
ALL B	119.7	12	10.7	129.5	11	8.6	108.4	11	11.6
Normal	117.3	6	8.1	104.2	6	5.6	126.3	6	34.4

It can be seen that acid flies do better on acid medium than on base medium (1% significant for A^m, not significant for A^p). Base flies yield higher on base medium than on acid medium but this difference is not significant. Base flies do better than acid flies on base medium and acid flies do better on acid medium than do base flies though neither of these is significant. It is particularly interesting that base flies perform worse on normal medium than on base medium (5%), and acid flies are worse on normal medium than on acid (1%). This is true in both pure and mixed cultures. Normal flies do better on normal medium than on either acid or base medium though this is not significant. Thus improved yield on their own type of medium has evolved in both the pure cultures which were

subjected to directional selection and in the mixed cultures which were subjected to disruptive selection, and hence under the influence of gene flow.

Mating preference tests

Table 33 summarises the results obtained from the tests of mating preferences of female flies.

TABLE 33

Numbers of matings between single females and a choice of males, one acid and one base

* indicates which male was wing clipped

	males					
females	*A	B	A	*B	all A	all B
A^m	12	18	20	2	32	20
B^m	14	20	25	9	39	29
A^p	11	22	6	7	17	29
B^p	13	21	17	11	30	32
all A	23	40	26	9	49	49
all B	27	41	42	20	69	61
all	50	81	68	29	118	110

The overall results show 110 like matings and 118 unlike matings, a difference which is not significant ($\chi^2 = 0.28$). A comparison of the choice of all acid females and base females is also not significant ($\chi^2 = 0.22$), neither are the comparisons of A^m and B^m females or A^p and B^p females.

A comparison of performance of clipped and unclipped males is very significant however ($\chi^2 = 21.48$) with clipped males much worse than unclipped males. However, if the data for

acid and base males is separated, acid males do not seem affected by clipping (χ^2 = 2.74) while base males are seriously affected (χ^2 = 24.58).

Discussion

The results obtained in the three tests of the different populations on the three types of medium are somewhat inconsistent. The tests after a single generation of selection show no adaptation of either acid or base populations to their own media. Neither do the tests after eleven generations show any strong adaptation to either acid or base medium. However, both acid and base flies perform better on all types of medium than do normal flies. As many of these vials showed signs of drying out, it seems likely that the acid and base cultures, rather than or as well as adapting to acid and base media, were also becoming adapted to dehydrated medium. Both hydrochloric acid and sodium hydroxide would be likely to have considerable dehydrating properties.

However, after 15 generations of selection, when the tests were carried out under more optimal conditions (damp cotton wool was placed over the vials and there was no sign of the medium drying out) there is strong evidence that acid flies were adapted to acid medium and base flies to base medium. What is particularly significant is that this is true in both the mixed cultures and the pure cultures. Thus disruptive selection has resulted in a single population becoming adapted to two alternative unpleasant environments. The acid and base populations of both mixed and pure cultures now give higher yields on their own medium than on normal. As normal flies perform best on normal medium, it suggests that the original

populations, from which acid and base adapted flies evolved were best suited to this medium.

The good performance of the selected populations on their own medium could be due to either a greater tendency to lay eggs on this medium or to increased survival of larvae. The former could be investigated by egg laying experiments similar to those of Watson (personal communication), and the latter by putting a known number of larvae on each type of medium. Neither of these tests was carried out here, but should be planned in future experiments of this type.

Thus both mixed and pure cultures are clearly adapted to their own type of medium while at the same time they are probably adapted to withstand dehydration. It is highly likely that in the wild, food used by this species does tend to dry out and larvae may have to withstand dehydration. Perhaps selection for this is relaxed in the laboratory where medium is usually optimally moist. Fruit flies probably also experience a fairly large range of pHs in the wild so that they may well be physiologically capable of withstanding this, and also possess the genetic variability to adapt to particular pHs. Again selection could be greatly relaxed in the laboratory.

Future experiments would necessitate more control over the wetness of the medium and also in maintaining the same pH, although the effect of the larvae on the medium pH may be a significant part of their adaptation to acid or base media. Scharloo (personal communication) is carrying out similar experiments in population cages with acid and base media but where the pH is kept constant by using a buffer. Future experiments might also gradually increase the concentration

of acid and base in the media in later generations to see if selection could force the populations to become even more different.

Despite the evidence of divergence between acid and base sections of the mixed population, there is no evidence of any assortative mating having evolved between acid and base flies. There are several possible reasons for this. Both types of fly perform well on the 'wrong' kind of medium, so that selection is probably less severe than in many disruptive selection experiments. In addition, reproductive isolation has only evolved in a few of the disruptive selection experiments on Drosophila. It also depends to what extent the better performance of the acid flies on acid medium and the base flies on base medium is due to greater egg laying or to better larval survival.

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APPENDIX

Replacement Series Graphs for Individual Replicates

FIGURE 1

Replacement series graphs for individual replicates of E1 in generations 8, 12 and 15 of Experiment 1.

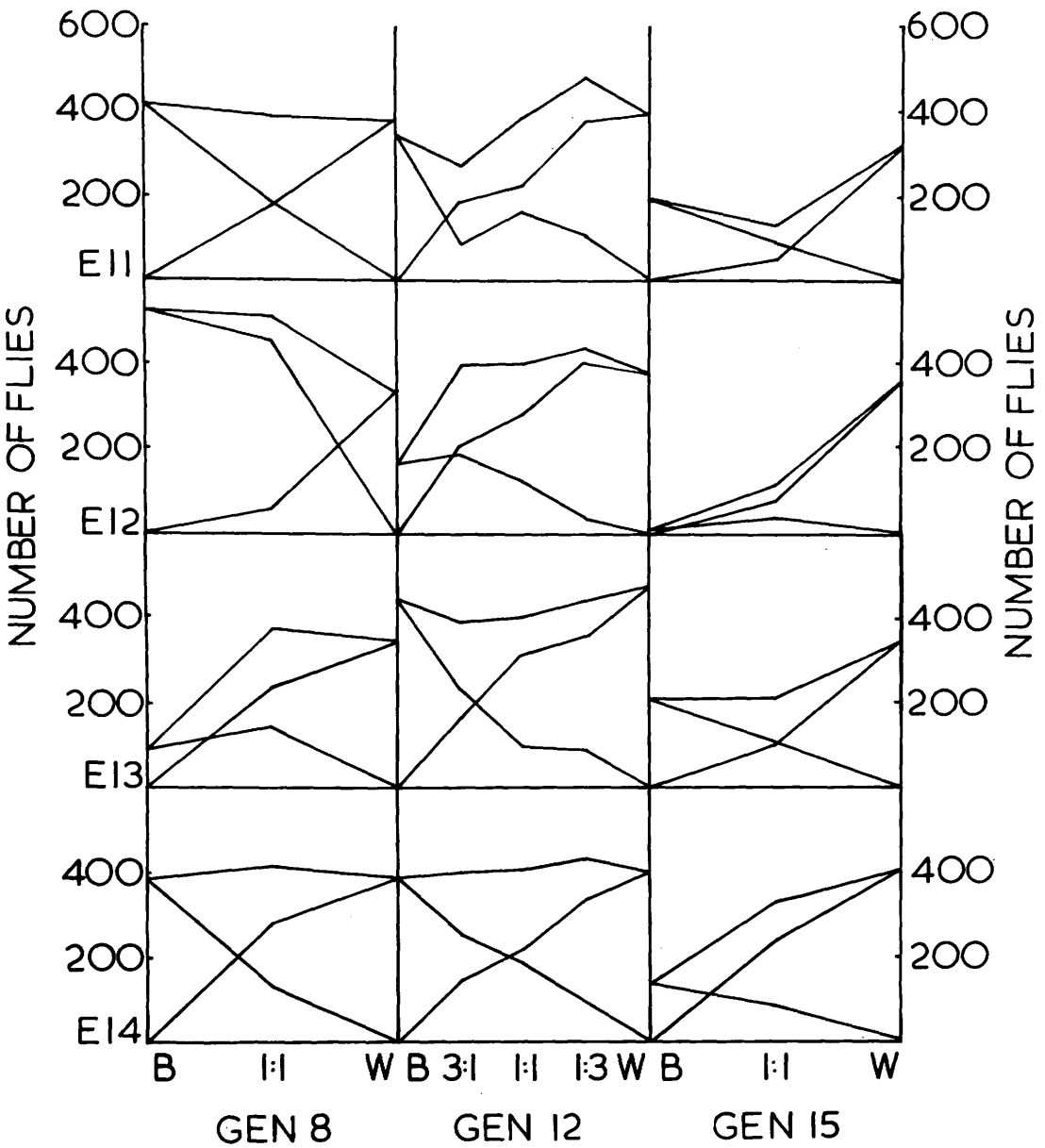

600
400
200
E11
400
200
E12
400
200
E13
400
200
E14
NUMBER OF FLIES
B
1:1
W
B
3:1
1:1
1:3
W
B
1:1
W
GEN 8
GEN 12
GEN 15

FIGURE 2

Replacement series graphs for individual replicates of E1 in generations 3, 6, 9, 14 and 15 of Experiment 2.

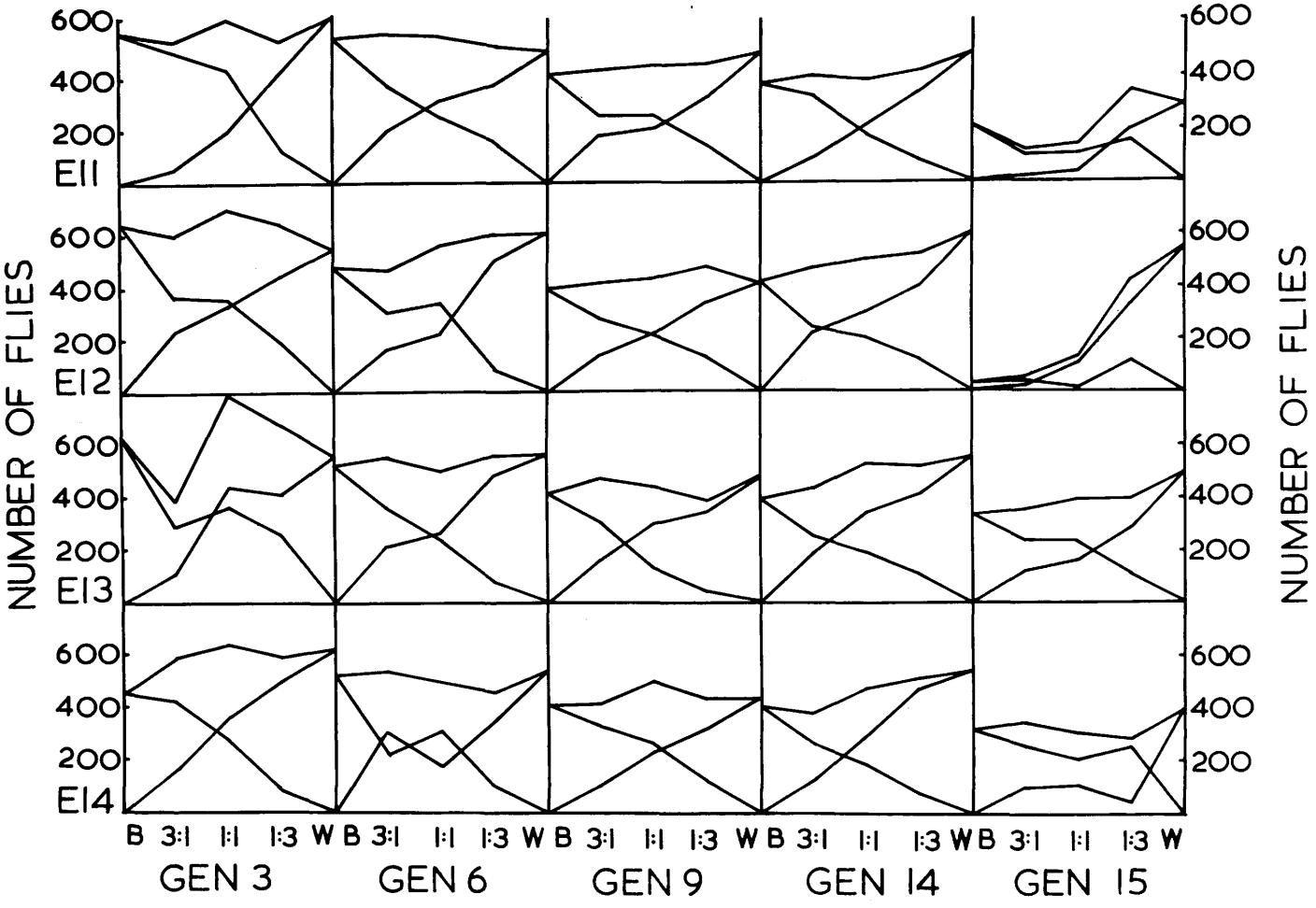

NUMBER OF FLIES
600
400
200
E11
E12
E13
E14
B 3:1 1:1 1:3 W
GEN 3
GEN 6
GEN 9
GEN 14
GEN 15

FIGURE 3

Replacement series graphs for individual replicates of E6 in generations 12 and 15, sep. E6 and Stocks in generation 15 of Experiment 2.

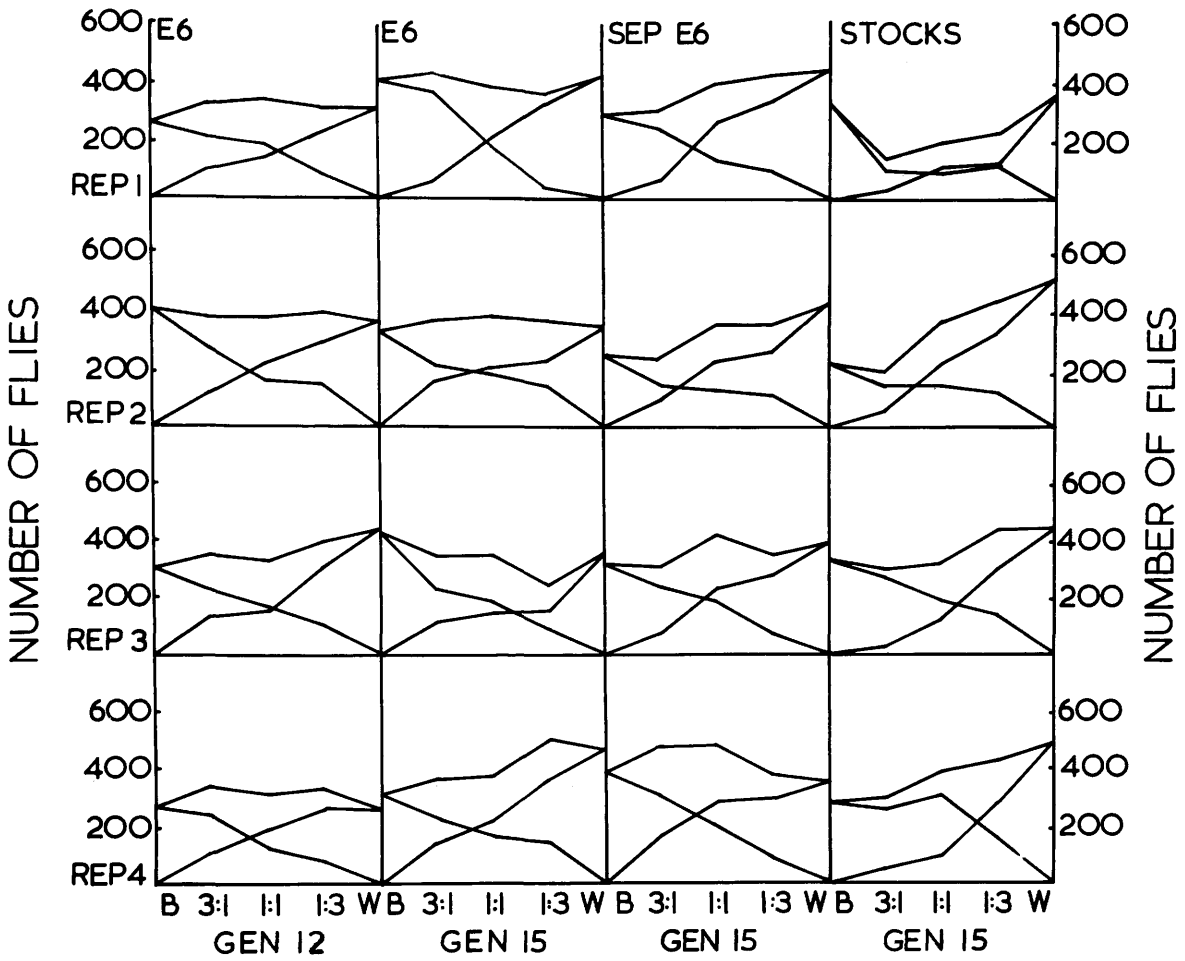

E6
E6
SEP E6
STOCKS
600
400
200
REP 1
REP 2
REP 3
REP 4
NUMBER OF FLIES
B 3:1 1:1 1:3 W
GEN 12
GEN 15
GEN 15
GEN 15

FIGURE 4

Replacement series graphs for individual replicates 1 to 5 of E1 in generations 1, 3, 5 and 7 of Experiment 3.

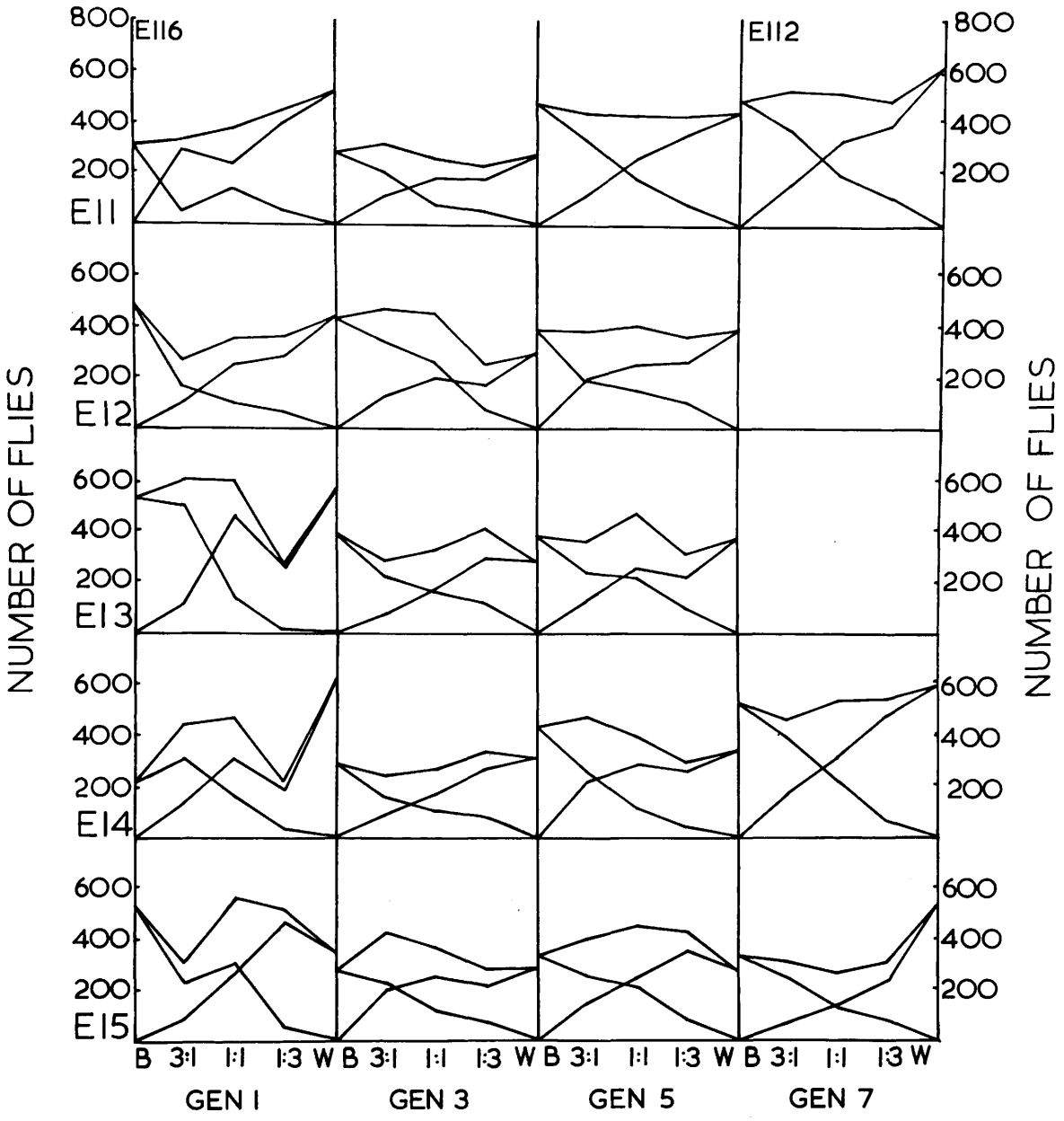

E116
E112
E11
E12
E13
E14
E15
800
600
400
200
NUMBER OF FLIES
B 3:1 1:1 1:3 W
GEN 1
GEN 3
GEN 5
GEN 7

FIGURE 5

Replacement series graphs for individual replicates 6 to 0 of E1 in generations 1, 3, 5 and 7 of Experiment 3.

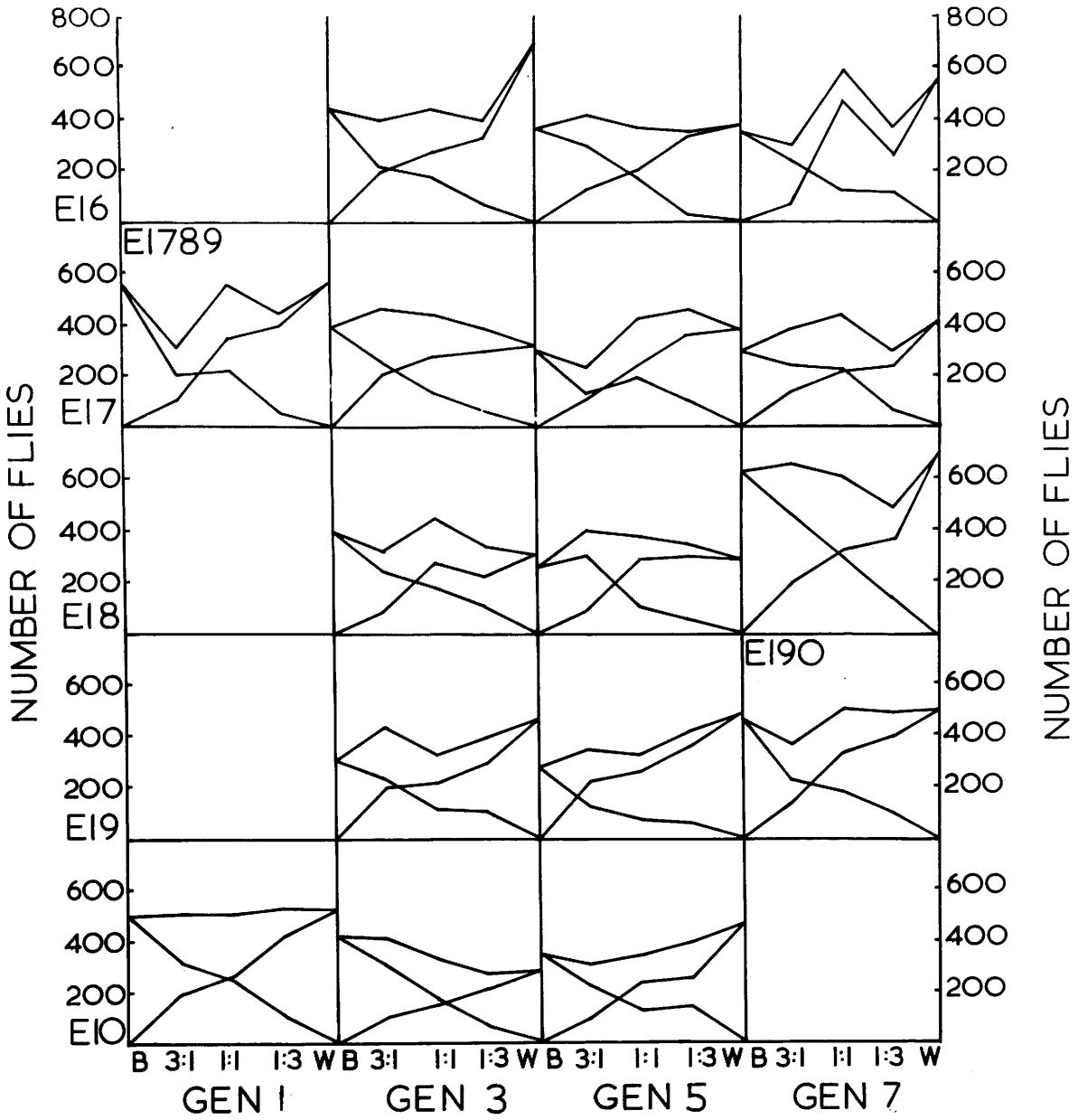

NUMBER OF FLIES
NUMBER OF FLIES
800
600
400
200
E16
E1789
600
400
200
E17
600
400
200
E18
E190
600
400
200
E19
600
400
200
E10
B 3:1 1:1 1:3 W B 3:1 1:1 1:3 W B 3:1 1:1 1:3 W B 3:1 1:1 1:3 W
GEN 1
GEN 3
GEN 5
GEN 7

FIGURE 6

Replacement series graphs for individual replicates 1 to 5 of stocks in generation 1, 3, 5 and 7 of Experiment 3.

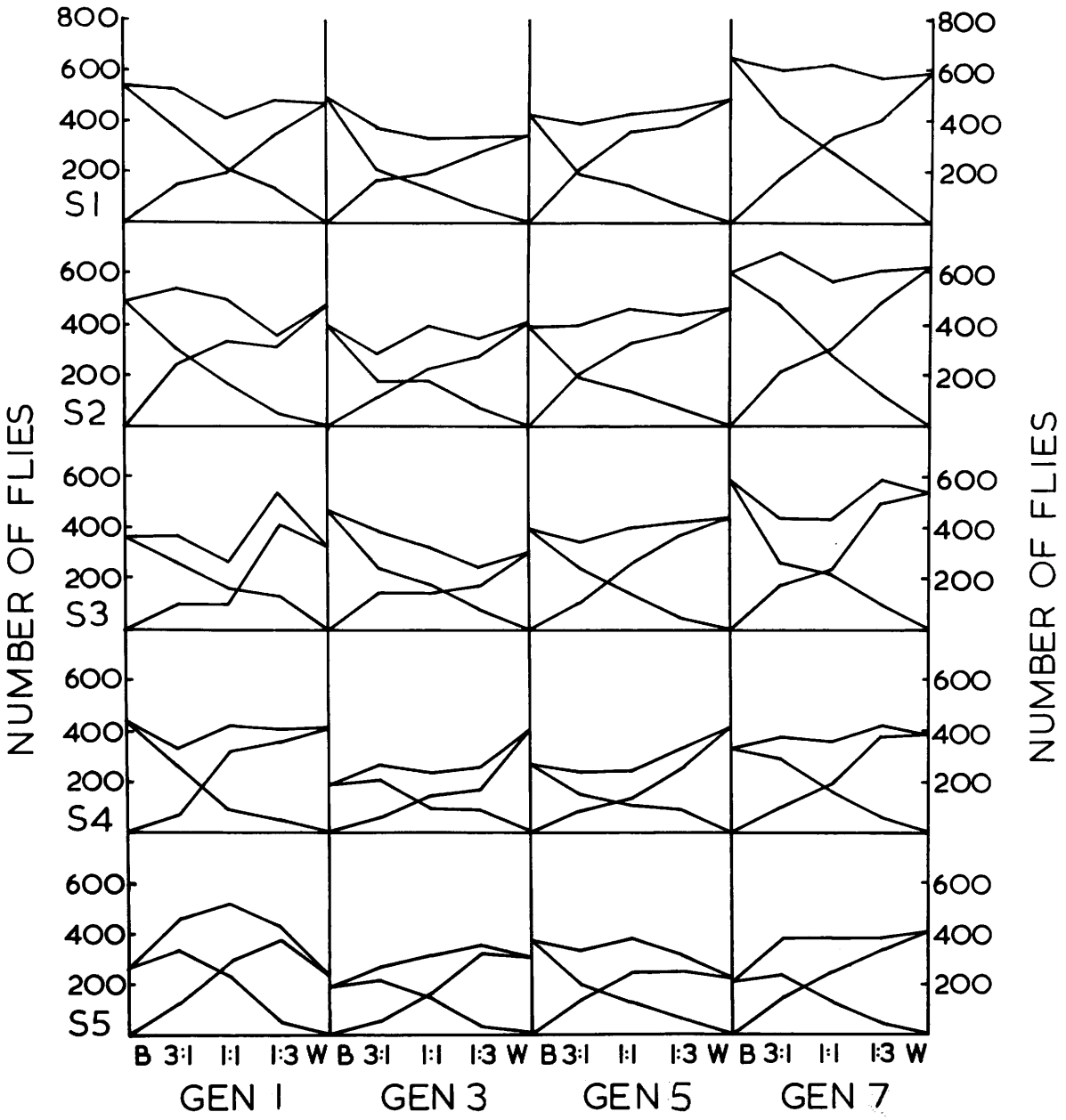
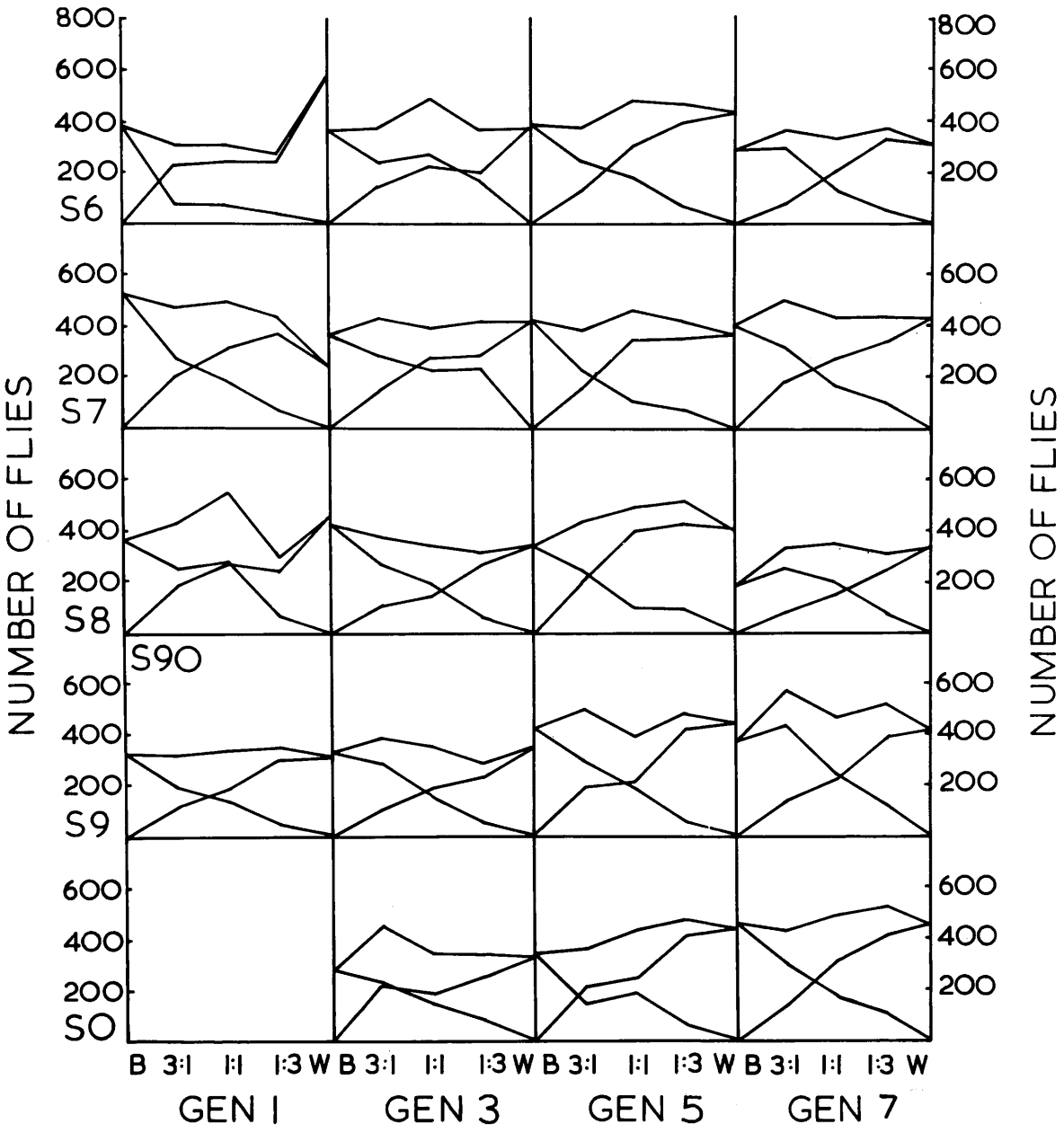

NUMBER OF FLIES
NUMBER OF FLIES
800
600
400
200
S1
S2
S3
S4
S5
B 3:1 1:1 1:3 W
GEN 1
GEN 3
GEN 5
GEN 7

FIGURE 7

Replacement series graphs for individual replicates 6 to 0 of stocks in generations 1, 3, 5 and 7 of Experiment 3.


800
600
400
200
S6
600
400
200
S7
600
400
200
S8
S90
600
400
200
S9
600
400
200
SO
NUMBER OF FLIES
NUMBER OF FLIES
B 3:1 1:1 1:3 W B 3:1 1:1 1:3 W B 3:1 1:1 1:3 W B 3:1 1:1 1:3 W
GEN 1
GEN 3
GEN 5
GEN 7